THE EXCRETION OF SOME AZO DYES IN RAT BILE

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A quantitative comparison of the biliary excretion of sixteen water soluble sulphonated azo dyes has been made to ascertain if any relation between the excretion pattern and molecular structure exists.

After the discovery of the carcinogenicity of the fat soluble dyes 4-dimethylaminoazobenzene (D.A.B.) and 3′-methyl-4-dimethylaminoazobenzene (3′Me-D.A.B.) (reviewed by Miller and Miller, 1953) a number of countries have introduced regulations to control the use of food colours. Dyes which have been proved to cause tumours or to show acute toxicity have not been approved and where little information about toxicity has been available, the chemical nature of the molecule has been used as a guide to its safety.

For azo dyes, the tendency has been to approve those which are highly sulphonated since aromatic sulphonic acids are normally excreted unchanged (Williams, 1959) and because of their highly polar nature they are not so readily absorbed from the alimentary canal (Brodie and Hogben, 1957). Preference is also given to those dyes which are sulphonated in aromatic groups on both sides of the azo linkage so that, should reductive fission of the azo linkage occur, the sulphonated fragments will be readily eliminated. There is however little exact knowledge of the fate of azo dyes in animals, except for a preliminary report by Mellinger and Radomski (1959).

Observations in these laboratories on the metabolism of foreign substances (Cox and Wright, 1959; Slaytor, Pennefather and Wright, 1959) have shown the value of examining their qualitative and quantitative excretion in the bile after intravenous injection. In general, substances of relatively high polarity are poorly absorbed from the gut and excreted rapidly, and usually unchanged, in the bile; those which are readily absorbed are slowly excreted and then mainly as metabolites. We have now examined the biliary excretion of sixteen azo dyes sulphonated on at least one side of the azo linkage with the object of estimating the stability and persistence of the dye in the animal and also to obtain information which might reveal a relation between the chemical structure of the dye and its excretion and metabolism.

Experimental

Materials. All the dyes were commercial samples with the exception of 3′-sulpho-4-dimethylaminoazobenzene, which was prepared by standard methods. Most were specially pure samples for use in foodstuffs, but each dye was recrystallised from water, or more generally, water-ethanol mixtures. Each dye gave one spot only when chromatographed on paper with the solvent systems pentanol : ethanol : ammonia : water (40 : 40 : 10 :

20) and ethyl methyl ketone: 4N acetic acid: water (120:40:25) (Verma and Das, 1956).

3'-Sulpho-4-dimethylaminoazobenzene. This dye was prepared by coupling diazotised metanilic acid with dimethylaniline according to the conditions described for the preparation of methyl orange (Vogel, 1956). It was recrystallised as the sodium salt monohydrate from hot water. Found: C, 48·6; H, 4·4; N, 12·4 per cent. $C_{14}H_{14}N_3O_3SNa.H_2O$ requires C, 48·8; H, 4·7; N, 12·2 per cent.

Biliary excretion. Albino rats (300 to 400 g. weight) were anaesthetised with urethane and the bile ducts cannulated with the shaft of a hypodermic needle attached to a length of polythene tubing. The dose of each dye injected in aqueous solution into a femoral vein was molecularly equivalent to 1 mg. of 4-dimethylaminoazobenzene per kg. Bile was collected

TABLE I

BILIARY EXCRETION OF WATER SOLUBLE SULPHONATED AZO DYES FROM RATS

Name	Colour Index No.	Average excreted per cent (4 exp.)	Excretion range
Azobenzenes			
Methyl orange	13025	55	38–59
3'-Sulpho-4-dimethylaminoazobenzene	—	27	15–40
Fast yellow	13015	10	2–16
Phenylazonaphthalenes			
Naphthalene fast orange 2GS	15510	46	25–60
Red 10BS	17200	12	5–20
Geranine 2GS	18050	64	60–70
Ponceau RS	16150	15	10–20
Orange GCN	15980	23	10–40
Sunset yellow	15985	22	20–30
Scarlet GN	14815	0	—
Ponceau SX	14700	48	30–60
Azonaphthalenes			
Carmoisine	14720	38	30–40
Brilliant scarlet	16255	34	30–45
Amaranth	16185	53	43–79
Phenylazopyrazoles			
Tartrazine	19140	1	0·2
Lissamine fast yellow 2G	18965	96	95–100

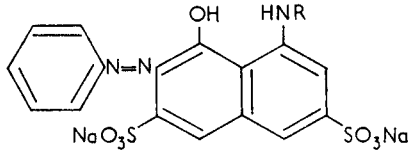
for 6 hr. and the whole sample used for analysis. To the bile was added 40 per cent zinc sulphate solution (3 ml.) followed by 11·2 per cent of potassium hydroxide (3 ml.). The solution was filtered and the precipitate washed with warm water until no more colour was eluted. The combined filtrates and washings were then adjusted to a suitable volume (50–100 ml.) and the optical density measured at the wavelength of maximum absorption. The dye concentration was read from a standard curve prepared with the original dye injected and the total amount of dye excreted then calculated. The identity of the dye was confirmed by spectra and comparative paper chromatography using the systems described above.

Recovery Experiments. Using the above methods of extraction and spectrophotometric analysis the recovery of each dye was not less than 95 per cent of the amount (0·5 mg.) added to the bile used as blank.

RESULTS AND DISCUSSION

The results of the excretory experiments are shown in Table I. The biliary excretion of the dyes studied does not show a regular pattern.

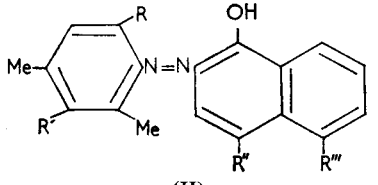
There does not appear to be any relation between the extent of excretion of unchanged dyes and the polarity of the molecule probably because polarity differences in highly sulphonated molecules are of little significance in relation to metabolism. Thus Red 10BS (I, R=H) is excreted only to the extent of 12 per cent whereas its *N*-acetyl derivative (Geranine



(I)

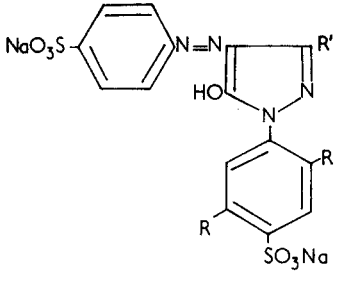
2GS) (I, R=COMe) which is less polar and less water soluble is excreted approximately 60 per cent unchanged.

It might be expected that if reductive fission of the azo linkage is responsible for low excretory rates of the unchanged dye, groups such as a hydroxyl ortho to the azo linkage could affect the stability of the molecule. However, Scarlet GN (II, R=R'''=SO_3Na; R'=R''=H) is not excreted



(II)

at all whereas its isomer Ponceau SX (II, R=R'''=H; R'=R''=SO_3Na) is excreted unchanged to about 50 per cent. Tartrazine (III, R=H; R'=COONa) which has a hydroxyl group ortho to the azo linkage is



(III)

almost completely absent from the bile, whereas the closely related dichlorinated dye Lissamine fast Yellow 2G (III, R=Cl; R'=Me) is quantitatively excreted.

There is some evidence to indicate that when large doses of azo dyes cause liver tumours the intact dye or some slight modification of it is the active carcinogen (Miller and Miller, 1953). It might be that dyes, such

as Tartrazine, which appear to be without any toxic or carcinogenic properties, are harmless because they are completely metabolised. It could also be inferred that dyes which are excreted quantitatively in the bile are less likely to be harmful than those which are retained. Further study of the metabolism of those dyes which are only partially excreted in bile is needed to determine whether the dye is retained unchanged or whether it is metabolised. The nature of any metabolites as well as the extent of urinary excretion must also be studied and work with these objects in view is proceeding in this laboratory.

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