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SHORT COMMUNICATION

Metabolism of some dimethylaminoazobenzene derivatives

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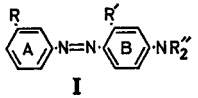
RECENT knowledge about the carcinogenic action of 4-dimethylamino-azobenzenes on the liver of the rat has been summarised by Arcos & Arcos (1962), but the metabolism of these compounds has received little attention. Stevenson, Dobriner & Rhoads (1942) showed that the rat metabolised 4-dimethylaminoazobenzene (DAB) to *p*-aminophenol and *p*-phenylenediamine, which were then excreted as conjugates. The sequence in which the oxidation of the phenyl ring, reduction of the azo link, and demethylation of the tertiary nitrogen occurs, still remains uncertain.

No quantitative work has been done on the excretion of the dye metabolites although Berenbom & White (1951) examined the distribution of isotopic nitrogen in the tissues and excreta of rats dosed with each of the three possible ^{15}N-labelled dyes: no estimation of individual metabolites was included. We have measured from urine the amount of each aromatic ring arising from the metabolic fission of four fat soluble azo dyes, DAB (I; R=R′=H; R″=Me), 3′-methyl-DAB (I; R=R″=Me; R′=H), 2-methyl-DAB (I; R=H; R′=R″=Me) and 4-aminoazobenzene (I; R=R′=R″=H). We have also examined the excretion of *p*-phenylenediamine.



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Of these compounds, the first two are carcinogenic to rat liver (Miller, Miller, Kline & Rusch, 1948) and the other two are non-carcinogenic (Miller, Sapp & Miller, 1949).

The first three dyes were each labelled with tritium in one ring and carbon-14 in the other; 4-aminoazobenzene was labelled with carbon-14 in both rings. Aniline and *m*-toluidine were tritiated by the Wilzbach (1957) procedure. Tritiated *NN*-dimethyl-*m*-toluidine was prepared by methylation of tritiated *m*-toluidine with trimethyl phosphate. A similar procedure gave dimethylaniline-^{14}C. The distribution of tritium in the tritiated molecules was determined in order to correct for loss due to metabolic reactions (Table 1).

The labelled dye (10 mg) and *m*-toluidine (5 mg) dissolved in arachis oil and *p*-phenylenediamine (5 mg) dissolved in water were given to rats by intraperitoneal injection. Urine was collected over 24, 48 and 72 hr. Known amounts of acetates of the expected metabolites (see Table 2)

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TABLE 1. DISTRIBUTION OF ACTIVITY IN TRITIATED AMINES

Compound	% activity in ring positions			
	*o**	*m**	*p*	methyl
Aniline	8·8	26·4	29·8	—
m-Toluidine ..	—	—	25·9	0·7

* activity found for 1 position

were added to each sample and the urine was hydrolysed with acid. The hydrolysed urine was extracted with ether and the metabolites separated on a silica gel column; suitable derivatives were purified to constant specific activity. Counting was in a Packard Tricarb liquid scintillation counter, model 314.

The results in Table 2 show a distinct difference in the metabolism of each aromatic compound derived from fission of the azo link. With DAB it can be seen that there is a clear difference in the amount of *p*-phenylenediamine excreted compared to *p*-aminophenol; a similar though less

TABLE 2. EXCRETION OF DYE METABOLITES AS CUMULATIVE % OF DOSE ADMINISTERED (INDIVIDUAL RESULTS)

Dye and metabolite	Time, hr		
	24	48	72
DAB			
p-Aminophenol	35, 64, 73	71, —, 71	67, 67, 73
p-Phenylenediamine	5, 9, 13	7, 7, 10	10, 10, 11
3′-Methyl-DAB			
4-Amino-2-methylphenol	25, —, 32	23, 28, —	23, —, 32
p-Phenylenediamine	18, 11, 18	18, 19, 27	18, 19, 20
2-Methyl-DAB			
p-Aminophenol	37, 47, 47	36, 71, 75	80, 80, 83
2,5-Diaminotoluene	13, 15, 15	30, 37, 43	70, 86, —
4-Aminoazobenzene			
p-Aminophenol	76, —, 103	91, 89, 106	81, —, —
p-Phenylenediamine	28, 17, 15	18, 17, 19	20, 19, 19
p-Phenylenediamine	40, —, 58	39, 50, 59	51, 59, 61

marked difference occurs with *p*-phenylenediamine and the accompanying 4-amino-2-methylphenol derived from 3′-methyl-DAB. A second interesting point is that in both dyes the excretion of the metabolites assayed reached a maximum at about 24 hr; thereafter no significant amounts were excreted.

For the non-carcinogenic dye 2-methyl-DAB there is a significant increase in the amount of metabolite from the aromatic ring to which the basic group is attached in the original dye. However, the excretion of *p*-phenylenediamine from 4-aminoazobenzene is only about the same as that from 3′-methyl-DAB, though greater than that from DAB itself. The amount of *p*-aminophenol arising from the ring A is approximately equal to the amount excreted from the carcinogenic dyes.

p-Phenylenediamine separately administered is excreted to a much greater extent than when derived from the dyes. With the exception of 3′-methyl-DAB, the excretion of ring A from the dyes does not differ

significantly from the amounts excreted when aniline is administered (Parke, 1960). With 3'-methyl-DAB it may be that this ring is metabolised by a route other than hydroxylation.

A possible explanation for the results obtained is that the dyes are bound in the tissues, possibly by a reaction between the amino-group and cell constituents. The bound dye is then reduced and ring A rapidly metabolised and excreted. The bound ring is retained and excreted much more slowly. It may be that the bound ring is important in the carcinogenic process. We are examining this problem further.

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The paper was presented by PROFESSOR WRIGHT.