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Effect of aminoazo dyes on rat serum paraphenylene diamine oxidase activity

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HEPATOCARCINOGENIC substances including aminoazo dyes caused a temporary decrease in the activity of rat serum para-phenylenediamine oxidase (a copper-containing enzyme probably identical with caeruloplasmin) and in the level of serum copper when they were injected as solutions or suspensions in arachis oil intraperitoneally into male albino rats.[1] Woodhouse[2] confirmed this effect with the carcinogenic aminoazo dye 4-dimethylaminoazo-benzene (DAB).

Attention is now drawn to a similar suppressive effect which has been observed subsequently with the supposedly non-carcinogenic dye, 2-methyl-4-dimethylaminoazobenzene (2-MeDAB).

EXPERIMENTAL

Serum was obtained by tail-bleeding male albino rats (body weight ~ 200 g) 24 hr before and 24, 48, 72 and 120 hr after intraperitoneal injection of 16·5 mg of 2-MeDAB (or the molar equivalent of other azo dyes) in 0·6 ml of arachis oil per 100 g body weight. Control rats received arachis oil only. Sera were stored at −15° until required for para-phenylenediamine oxidase (PPDO) determination which was carried out according to the method of Ravin.[3]

RESULTS

As shown in the table, arachis oil injection was followed by a rise in serum PPDO which was sustained for at least 5 days after injection. The same effect was observed after injection of the non-carcinogenic dye 2-methoxy-4-dimethylaminoazobenzene (2-MeODAB) in arachis oil.

On the other hand, the strong carcinogen 3′-methyl-4-dimethylaminoazobenzene (3′-MeDAB) caused a pronounced drop in serum PPDO during the first 3 days post injection after which the enzyme activity began to increase gradually. With the carcinogen DAB of intermediate activity serum PPDO reached a minimum value at 48 hr after injection. Practically the same course of events was observed with the strong carcinogen 4′-fluoro-4-dimethylaminoazobenzene (4′-FDAB).

The non-carcinogen 2-MeDAB at first caused an increase in PPDO but this was followed by an abrupt decline to a minimum at 48 hr. Thereafter the recovery pattern of PPDO activity resembled that found with DAB or 4'-FDAB.

DISCUSSION

Evidently 2-MeDAB is able to imitate to some extent the suppressive action of hepatocarcinogenic azo dyes on rat serum PPDO activity. It may be presumed from our observations on the suppression of serum PPDO that hepatocarcinogens have the ability to interfere with copper metabolism in the liver. If this disturbance is important for liver carcinogenesis it must be asked why 2-MeDAB fails to act as a carcinogen.

TABLE 1. EFFECT OF AMINOAZO DYES ON THE PPDO ACTIVITY OF RAT SERUM

Dye injected in arachis oil	Relative carcinogenic activity[4]	No. of rats	Mean Serum PPDO values (E at 530 mμ) at				
			24 hr before injection	24	48	72	120
				hr after injection			
nil		3	0·442	0·444	0·485	0·485	0·557
2-MeODAB	0	4	0·411	0·409	0·483	0·532	0·532
3'-MeDAB	10–12	4	0·410	0·289	0·199	0·174	0·296
DAB	6	2	0·354	0·325	0·293	0·429	0·557
4'-FDAB	10–12	2	0·439	0·427	0·290	0·364	0·576
2-MeDAB	0	5	0·402	0·450	0·294	0·375	0·554

A possible explanation may be found in the work of Maini and Stich[5] who showed that liver carcinogens such as 3'-MeDAB caused nuclear damage and promoted liver cell proliferation. Although 2-MeDAB was able to produce as much nuclear damage as 3'-MeDAB it lacked the ability to promote cellular proliferation which appeared to be essential for the propagation of chromosomal abnormalities resulting eventually in the formation of tumour cell lines. It is now suggested that there may be a relationship between the nuclear damaging action of aminoazo dyes and their ability to interfere with liver copper metabolism in the cell nucleus. In this connection it is worth noting that dietary supplements of copper acetate greatly inhibited the production of liver tumours in rats by DAB.[6] It is also of interest that the non-carcinogen 2-MeDAB (in our view, possibly an incomplete carcinogen[7]) shares with the carcinogenic azo dyes the ability to bind to rat liver protein[8] whereas the non-carcinogen 2-MeDAB which does not depress serum PPDO fails to produce bound dye.[7] Thus protein binding as well as the nuclear damaging activity of aminozao dyes may be linked with disturbances in copper metabolism caused by these dyes.

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