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SHORT COMMUNICATION

Structure-activity studies for the teratogenic effects of disazo dyes

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ADMINISTRATION of trypan blue to pregnant rats leads to the appearance at term of offspring exhibiting a variety of congenital malformations. The defects induced are similar in nature to common spontaneous malformations (e.g. anencephaly, hydrocephalus, spina bifida) and consequently the dye is widely used today in experimental work on the genesis of deformities. Investigations into the relationship between chemical structure and teratogenic activity of dyes of the trypan blue group have been made (Gillman, Gilbert, Spence & Gillman, 1951; Wilson, 1955; Beaudoin & Pickering, 1960) which indicate that apart from trypan blue, only an isomeric dye (azovan blue) has significant activity. Two aspects of this earlier work suggest the need for a re-evaluation of the problem, namely the widespread use of dyestuffs of unconfirmed identity and purity and the restriction of biological testing to a single dose level. Our experience has shown that these may constitute serious sources of error (Lloyd & Beck, 1963; Beck & Lloyd, 1964).

METHODS AND RESULTS

Commercial samples of Niagara blue 2B, Niagara blue 4B, Afridol blue and azovan blue (Evans blue) were confirmed as authentic (see Lloyd & Beck, 1964a for formulae and method), then freed from salt and converted into the free acid form as has been described previously (Lloyd & Beck, 1964b). Inbred Wistar rats were injected subcutaneously at 8·5 days pregnancy (the time of maximum sensitivity to trypan blue; Wilson, Beaudoin & Free, 1959) with 1% aqueous solutions of the dyes at doses from 50 to 200 mg/kg. At 20·5 days the mothers were killed, the foetal resorption sites counted and the live foetuses examined for evidence of external malformation. The results show that, in addition to trypan blue, Niagara blue 2B and Afridol blue are potent teratogens in the rat, the difference being that the first is optimally active at 50 mg/kg and the other two at 150 mg/kg. Neither Niagara blue 4B nor azovan blue has more than marginal teratogenic activity, although both caused foetal death at high doses.

As a possible aid to explaining these results a study was made of the plasma levels of the dyes at various times after subcutaneous injection of 50 mg/kg. To date, these experiments have been made on the dyes trypan blue, Niagara blue 4B, Afridol blue and azovan blue, and the most striking feature observed has been the behaviour of trypan blue, which

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reaches and maintains plasma levels of 15–18 mg %, some 3–4 times those maintained by the other dyes. Differences between the remaining dyes are also apparent, azovan blue reaching the blood only slowly (maximum concentration at ca. 36 hr) but Niagara blue 4B very quickly (maximum at 4 hr). These findings suggest an explanation for the differences in teratogenic behaviour of the dyes. Trypan blue at 50 mg/kg and Afridol blue at 150 mg/kg are teratogens of similar potency, and the plasma dye concentration experiment suggests that injection at these doses would result in similar levels being reached. This was confirmed by determining plasma levels in a further series of rats injected with Afridol blue at 150 mg/kg, and it would appear, therefore, that both dyes are teratogenic if plasma concentrations of 15–18 mg % are maintained for some hours on about the 9th day of gestation. With Niagara blue 4B the observed plasma levels lead to the expectation that teratogenicity would be manifest at doses of 150–200 mg/kg, but at these doses the dye is toxic to the mother, resulting in a high mortality before term (Beck & Lloyd, 1965). In surviving mothers a high resorption rate masks the teratogenic effects of the dye, the resorptions being due to a direct toxic effect upon the embryo (Beck & Lloyd, 1965), no doubt related to the maternal toxicity. Azovan blue reaches the bloodstream much more slowly than the other dyes, suggesting that, to obtain 15 mg% on the 9th day of gestation, azovan blue would have to be administered at 100 mg/kg at 7·5 days. A series of rats was therefore subjected to this régime and a high teratogenic potency observed. Five mothers yielded 43 implantations of which 19 were resorbed and 7 (16·3%) exhibited external malformation.

DISCUSSION

The mechanism of teratogenic action of disazo dyes is at present unknown and a number of hypotheses have been advanced (see review by Beck & Lloyd, 1965). In discussing the question many authors have placed much emphasis upon differences in ability to elicit a variety of biological responses between "teratogenic" and "non-teratogenic" dyes. The present report shows that many disazo dyes are active teratogens; indeed no disazo dye has yet been investigated over a wide dosage range and at different stages of pregnancy and found to be without activity. The dosage levels at which activity is observed, and the degree of response, are both subject to wide variation. These differences are in part, at least, explained by differences between the dyes in the rate of release from the subcutaneous injection site and in the rate of removal from the circulation.

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