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Investigations on the Disposition of Oral Doses of Some Water-insoluble Pigments

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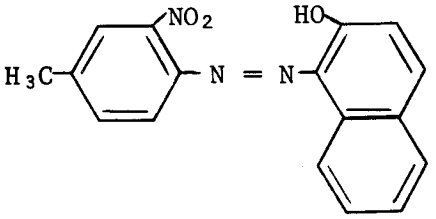
For organic molecules used as pigments, insolubility in a range of solvents, especially water, is considered a highly desirable property, associated with prolonged color fastness and with resistance to fading and biological degradation. Since many pigments are structurally related to known carcinogens, it is of interest to determine how these insoluble compounds are handled by higher animals. We have investigated the disposition of three insoluble pigments, administered to rats as oral doses, to determine if appreciable absorption and/or metabolism occurs in intact animals.

C. I. Pigment Red #3 (Red #3, Fig. 1) and C. I. Pigment Red #23 (Red #23) were selected for study because of significant potential for human exposure through production and through end use in paints, inks, dyes, cements, and art materials and because of structural similarities to known phenylazonaphthol carcinogens such as Ponceau 3R and Acid Red #26 (Helmes et al. 1982). C. I. Pigment Yellow #74 (Yellow #74) was selected for similar reasons and because reductive cleavage of the azo linkage would yield a nitro-substituted, aromatic ring related to the carcinogen o-anisidine (Helmes et al. 1982).

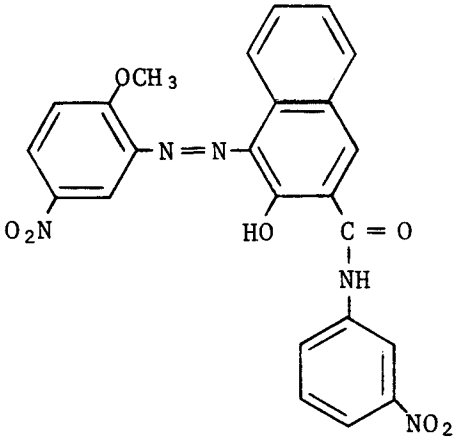
MATERIALS AND METHODS

Red #3 and Red #23 were obtained from American Cyanamid, Wayne, NJ, and Yellow #74 from Radian Corp., Austin, TX. Dosing suspensions of the three pigments were prepared in corn oil. Spectrophotometric assays were performed to determine the concentration of the dosing solutions and to assure that the preparations were homogeneous and stable during the time of dosing. For such assays, the pigments were dissolved in methylene chloride. Doses administered were determined to be as follows: Red #3, 11.8 mg/kg; Red #23, 5.2 mg/kg; and Yellow #74, 12.6 mg/kg. The lower dose for Red #23 was necessitated by its extreme insolubility in methylene chloride.

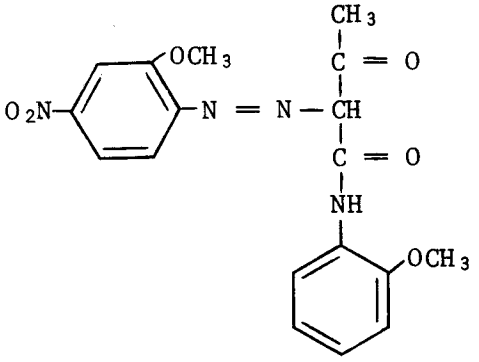
Chemical purity was assessed by high-pressure liquid chromatographic anal with a Waters series ALC 200 instrument equipped with a model 440 absorbance detector and with a column of Spherisorb ODS, 10-micron particle size. Detection was at 254 nm. Eluting solvents were acetonitrile:water (7:3, v/v) for Red #3 and Yellow #74 and acetonitrile:water (17:3, v/v) for Red #23. Purities were determined to be as follows: Red #3, 94.7%; Red #23, 84.6%; and Yellow #74, 89.1%. Recoveries were calculated on the basis of the amount of authentic compound administered.



C.I. Pigment Red #3



C.I. Pigment Red #23



C.I. Pigment Yellow #74

Figure 1. Structures of pigments studied.

Table 1. Disposition of Pigments

Pigment	Time after Dosing (hr)	Disposition: Gut Contents	Feces	Tissues Collected	Total
		(% of Dose)[a]			
Red #3	1	99.3±5.8	<0.1	1.8±0.3	101±5.5
	4	89.1±10.1	<0.1	2.0±0.7	91.0±9.5
	24	16.8±19.3	32.9±9.4	0.3±0.0	50.0±10.1
	48	-[b]	72.4±10.1	-[b]	72.4±10.1
Red #23	1	84.8±13.3	<0.1	1.5±0.2	86.3±13.5
	4	85.9±2.8	<0.1	0.8±0.3	86.7±2.5
	24	16.1±7.4	45.8±14.0	0.1±0.1	87.8±3.1
	48	-[b]	93.0±16.8	-[b]	93.0±16.8
Yellow #74	1	111±2	<0.1	2.6±0.4	114±2
	4	103±1	<0.1	2.1±0.3	105±1[c]
	24	25.5±16.1	70.4±11.5	0.1±0.8	95.9±7.1
	48	2.1±1.4	85.9±22.3	-[b]	88.2±21.7

[a]The values represent the mean % of dose ± standard deviation for three rats.

[b]Not measured.

[c]There are values for only two rats in this group.

Animals used in these studies were male Fischer 344 rats, 7-8 weeks old, purchased from Charles River Laboratories, Portage, MI, and weighing 146-180 g. They were dosed by oral gavage in amounts of about 1 ml. During the experimental period, each rat was housed individually in a stainless steel metabolism cage.

At 1, 4, 24, and 48 hr after dosing, 3 rats in each group were killed by an overdose of ether. Feces and urine were collected from each surviving animal at 1, 4, 24 and 48 hr. Stomach contents, intestinal contents, and various tissues were collected from each animal sacrificed at 1, 4, and 24 hr. Except for rats dosed with Yellow #74, only feces was collected at 48 hr. Samples were stored frozen until analyzed. For analysis, the samples, except urine, were homogenized in 4 volumes of saline. All samples were extracted with methylene chloride until no color was visible in the extract. HPLC analysis confirmed that no appreciable amount of extractable pigment was present in the final extract.

RESULTS AND DISCUSSION

At 1 hr and 4 hr after dosing with each of the compounds, most of the administered material was present in the gut contents; and none was in the feces (Table 1). At 24 hr and 48 hr, however, most was in the feces. Only those tissues directly in contact with the compounds contained detectable amounts. None was found in samples of plasma, whole blood, liver, kidneys, or lungs, even after administration of doses ten times larger (not shown). Accordingly, the small quantities of compounds present in samples of tissues that were in direct contact with the chemicals can be attributed to mechanical adherance to the tissues rather than to absorption.

Small amounts (less than 1%) of the compounds were occasionally detected in the 24-hr and 48-hr urine samples. These amounts were probably due to contamination of urine by feces, since none could be detected in the blood or kidneys. (For purposes of summation, the amounts present in urine samples were included in the values for feces.)

Recoveries of the pigments were nearly complete, except that at 24 hr and 48 hr after dosing, the amount of Red #3 recovered was clearly lower than the amount administered. Red #23 and Yellow #74 were apparently not altered in the gastrointestinal tract of rats. Failure to account for all of the administered dose of Red #3 suggested that it may be degraded by intestinal bacteria or complexed in an inextractable form during passage through the gastrointestinal tract. Studies regarding possible absorption of metabolites of Red #3 will require radioactively labeled material.

The data are too variable to conclude that these pigments were not absorbed at all from the gastrointestinal tract, but they do indicate that absorption of even these rather low doses, if it occurred, was minimal and that effective exposure to these compounds was not achieved.

Acknowledgements.

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REFERENCES

Helmes CT, Fung VA, Lewin B, McCaleb KE, Malko S, and Pawlovich, AM. (1982) J Environ Sci Health A17, 75-128.

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