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Dermal Absorption of Benzidine Derivatives in Rats

P. V. Shah* and F. E. Guthrie

Toxicology Program, North Carolina State University, Raleigh, NC 27650

Benzidine and benzidine-based dyes are known to be carcinogenic in small animal tests, mutagenic in short-term bioassays, and implicative in epidemiological tests regarding hazards to human health (I.A.R.C., 1982). Following governmental action indicating that benzidine derivatives may cause bladder cancer (FEDERAL REGISTER, 1974), the use of these compounds has been severely curtailed or production phased out (NIOSH, 1980). Hazards are primarily from inhalation, oral ingestion and dermal exposure during dye manufacture, textile dyeing, printing paper, and leather industries. Metabolism of benzidine-based dyes to benzidine is recognized as the step necessary to produce the ultimate carcinogen (FOUTS et al., 1957).

Although dermal entry has been shown (MEIGS et al., 1951, MEIGS et al., 1954, VON EHRLICHER, 1958, SCIARINI AND MEIGS, 1961, and CASE, 1965), no specific study of dermal penetration has been made.

MATERIALS AND METHODS

Adult male Fisher 344 rats weighing 225-250 gr were purchased from Charles River breeding farms. Following delivery, rats were caged in polystyrene cages (3-4/cage), provided with Purina Dog Chow and water ad libitum, and held for 72-96 hr before treatment. A 5-6 sq cm area of the mid-back region was carefully shaved (electric hair clippers) 24 hr before application of an acetone solution (0.2 ml) of ^{14}C compound containing 1 mg/kg of total dose. A plastic collar was attached to prevent licking or abrasion of the treated area following evaporation of solvent. Rats were then placed in all-glass metabolism cages as described previously (SHAH AND GUTHRIE, 1983) and held for 1, 8, and 24 hr intervals. Treated rats (3/replicate) were killed and radioactivity determined in the tissues and organs noted in Tables 1-3. Whole organs or excretory products were

* Present address: Reproductive Toxicology Branch, U.S.E.P.A., Research Triangle Park, North Carolina 27711.

digested except as noted. A 4-5 sq cm section of the skin at the application site was removed to assay radioactivity which had not penetrated beyond this site. Tissue aliquots were combusted in a Harvey Biological Oxidizer equipped with a CO_2 trapping device containing 15 ml of Carbon-14 Scintillation fluid®. The carcass of the rat was cut into small pieces, which were plunged into liquid nitrogen and blended in a Waring Blender containing liquid nitrogen. The resultant powder was resuspended in water, and the final volume was recorded. Aliquots of the homogenates were oxidized as above. As preliminary experiments did not detect radioactivity in the gaseous phase, this sample was not included with data.

Radioactivity was determined by a Packard Tricarb Scintillation Spectrometer. Quenching was corrected by internal standardization, and samples oxidized in the Harvey Oxidizer were corrected to total percent recovery for each time period.

The specific activities of ^{14}C-labeled compounds were as follows: benzidine (uniformly ring labeled) 13.39 mCi/mmol, 3-3'-dichlorobenzidine (uniformly ring labeled) 4.09 mCi/mmol, and 3,3-dimethoxybenzidine (uniformly ring labeled) 8.97 mCi/mmol. The radiochemical purity of these compounds was reconfirmed by appropriate TLC systems. Recovery of radioactivity varied between 85-97% of the applied dose.

RESULTS AND DISCUSSION

Recovery of radioactivity from a number of tissues and organs as well as from the site of dermal application for benzidine and two cogeners is shown in Tables 1-3.

A number of tissue assays showed < 0.1% of radioactivity for all compounds at all time intervals [heart, spleen, kidney, fat, bladder, ear, bone marrow, brain, muscle, and (usually) stomach]. The blood levels of radioactivity were between 0.1-0.5% during all intervals and were relatively consistent among the compounds. Radioactivity in lungs was slightly higher (but < 0.5%) for the 8 and 24 hr assays of benzidine and dichlorobenzidine in contrast to dimethoxybenzidine which was consistently below 0.1%. Radioactivity in the liver was usually above 0.1% but below 2% except for dichlorobenzidine which was consistently higher than the other derivatives (although below 5%). Stomach and intestinal assays were somewhat variable, consistently lower for dimethoxybenzidine, and the intestine occasionally exceeded 10% of the radioactivity for the other compounds. As expected, radioactivity in urine and feces was in trace quantities at the early time intervals steadily increasing to 10-20% at the 24 hr assay. Radioactivity from benzidine was consistently higher in the urine-feces samples than the other derivatives. The quantity in carcass

TABLE 1

Recovery of Radioactivity in Tissue and Organs of Rats Following Dermal Application of Benzidine

	% Recovery of Radioactivity After:					
	1 hr		8 hrs		24 hrs	
Sample	%	± SD	%	± SD	%	± SD
Blood[a]	0.23	0.28	0.34	0.09	0.74	0.59
Heart	0.01	0.01	0.02	0.01	0.01	0.01
Lung	0.09	0.08	0.22	0.04	0.18	0.15
Spleen	0.01	<0.01	0.01	<0.01	0.01	0.01
Kidney	0.02	0.02	0.04	0.02	0.03	0.03
Fat[b]	<0.01	<0.01	<0.01	<0.01	ND[f]	ND
Bladder	<0.01	<0.01	0.01	<0.01	0.02	0.02
Ear[c]	0.01	0.01	0.02	0.02	0.02	0.01
B. M.[b,d]	<0.01	<0.01	0.01	0.01	<0.01	<0.01
Brain	0.01	0.01	0.01	<0.01	0.01	0.01
Muscle[d]	0.01	0.01	0.03	0.02	0.01	0.01
Liver	1.46	2.02	0.96	0.76	0.71	0.45
Stomach	0.53	0.88	0.35	0.17	0.08	0.06
Intestine	1.04	0.62	13.98	7.35	1.31	0.55
Carcass	1.89	0.73	4.13	1.59	6.94	1.69
Urine	0.08	0.09	4.14	1.34	22.79	9.05
Feces	0.01	0.02	0.65	0.94	18.70	3.41
Appl.[e]	94.59	4.57	75.09	8.62	48.64	10.55

[a] 65 ml/kg body weight

[b] % recovered from 100 mg sample

[c] Digested both ears

[d] B. M. = bone marrow

[e] Recovered from site of application

[f] Not detected

TABLE 2

Recovery of Radioactivity in Tissues and Organs of Rats Following Dermal Application of 3,3'-Dichlorobenzidine[a]

	% Recovery of Radioactivity After:					
	1 hr		8 hrs		24 hrs	
Sample	%	± SD	%	± SD	%	± SD
Blood	0.52	0.54	0.82	0.30	0.75	0.14
Heart	0.03	0.02	0.04	0.01	0.03	<0.01
Lung	0.19	0.06	0.46	0.16	0.45	0.09
Spleen	0.02	0.01	0.03	0.01	0.02	<0.01
Kidney	0.02	0.03	0.03	0.02	0.04	0.04
Fat	ND	ND	<0.01	<0.01	ND	ND
Bladder	<0.01	<0.01	0.02	0.01	0.01	<0.01
Ear	0.01	0.01	0.03	0.02	0.02	<0.01
B. M.	<0.01	<0.01	0.01	0.01	<0.01	<0.01
Brain	0.01	<0.01	<0.01	<0.01	0.01	0.01
Muscle	0.02	0.02	0.02	<0.01	<0.02	<0.01
Liver	1.17	0.61	3.61	1.22	4.09	0.36
Stomach	0.04	0.03	0.35	0.17	0.20	0.12
Intestine	0.63	0.74	11.27	5.19	5.66	0.74
Carcass	3.37	1.07	2.79	0.41	10.00	3.88
Urine	0.06	0.07	2.76	1.24	8.44	0.47
Feces	<0.01	<0.01	0.98	1.10	19.33	7.88
Appl.	93.86	2.11	76.78	8.64	50.92	3.87

[a]Subscripts same as in Table 1.

TABLE 3

Recovery of Radioactivity in Tissues and Organs of Rats[a] Following Dermal Application of 3,3'-Dimethoxybenzidine

	% Recovery of Radioactivity After:					
	1 hr		8 hrs		24 hrs	
Sample	%	± SD	%	± SD	%	± SD
Blood	0.46	0.69	0.17	0.08	0.08	0.01
Heart	0.02	0.02	0.01	<0.01	<0.01	<0.01
Lung	0.08	0.11	0.03	0.01	0.01	0.01
Spleen	0.01	0.01	0.01	0.01	<0.01	<0.01
Kidney	0.02	0.02	0.01	<0.01	0.01	<0.01
Fat	<0.01	<0.01	<0.01	<0.01	ND	ND
Bladder	<0.01	<0.01	0.01	0.01	0.01	<0.01
Ear	0.03	0.02	0.01	0.01	0.02	0.01
B. M.	0.01	0.02	0.01	0.01	<0.01	<0.01
Brain	0.01	0.02	0.01	<0.01	<0.01	<0.01
Muscle	0.01	0.02	0.01	<0.01	<0.01	<0.01
Liver	0.39	0.55	0.23	0.04	0.40	0.08
Stomach	0.29	0.49	0.08	0.05	0.05	0.01
Intestine	0.58	0.86	1.46	0.65	2.30	0.86
Carcass	1.36	0.48	0.75	0.28	4.20	2.77
Urine	0.03	0.02	1.58	0.43	12.08	7.05
Feces	0.11	0.19	0.07	0.08	9.21	3.28
Appl.	96.58	3.31	95.57	1.02	71.61	9.22

[a]Subscripts same as in Table 1.

was fairly low (< 5%) throughout except at the later time intervals for dichlorobenzidine where it approached 10%.

The amount disappearing from the site of application (presumed penetration) was the same for benzidine and its dichloro derivative, reaching about 50% in 24 hr. The dimethoxy derivative was absorbed less rapidly, and only 29% had disappeared from the application site in 24 hr. The $t_{\frac{1}{2}}$ disappearance values reflect these latter conclusions and were 22.6, 24.1 and 52.9 hr for benzidine and the dichloro and dimethoxy derivatives, respectively. It should be noted that virtually all of the radioactivity which disappeared from the site of application was found in the tissues and excreta.

These results indicate that benzidine and its cogeners are readily absorbed through mammalian skin. Therefore, skin may be a major portal of entry and possibly the most important entry for all environmental and occupational exposures as has been suggested by MEIGS et al. (1951).

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