Species Differences in the Metabolism of Diphenhydramine (Benadryl[1])[2] (35157)

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Early observations on the metabolic disposition of DPHM[4] by Glazko *et al.* (1–3) indicated that high concentrations of free drug were present in the lungs and spleen of the rat and guinea pig. Unchanged DPHM was identified in rat urine, and the major urinary excretion products were found to be nonbasic metabolites. DPHM was converted to nonbasic derivatives by incubating drug with tissue homogenates *in vitro* (3). Roozemond *et al.* (4) found that DPHM was readily demethylated by rat liver microsome preparation *in vitro*. Hespe *et al.* (5) reported that N-demethylation and O-dealkylation took place with the *o*-tolyl analog of DPHM. More recently, Drach and Howell (6) obtained evidence for N-dealkylation, N-oxide formation, and oxidative deamination of DPHM in rhesus monkeys, with the resulting DPMA being conjugated with glutamine. The present report is concerned with additional observations in the Rhesus monkey and other species of laboratory animals.

Materials and Methods. Tritium-labeled DPHM·HCl prepared by Blackburn and Ober (7) was diluted with unlabeled drug to a sp act of 0.4 to 1.1 mCi/mmole, and administered in an aqueous solution. Plasma was obtained by centrifugation of heparinized blood samples. Tritium determinations were made by the oxygen-flask combustion and liquid scintillation counting technics described by Ober *et al.* (8). DPHM was determined by double extraction from plasma with heptane (9) followed by the fluorescent dye-salt procedure with Tinopal GS described by Glazko *et al.* (10). Interference from primary and secondary amines was eliminated by adding 0.10 ml of acetic anhydride to the heptane extract and shaking it for 15 min at room temperature, before returning the drug to acid.

Radioactive metabolites in plasma or urine were characterized by direct thin-layer chromatography on silica gel plates, using the solvent systems described previously (6). TLC was performed on $CHCl_3$ extracts of plasma, by extracting repeatedly under acidic and basic conditions, with 91–95% recovery of the radioactivity in the combined extract.

The glycine conjugate of DPMA from dog urine was isolated and identified using procedures similar to those described previously for the isolation and identification of the glutamine conjugate of DPMA in monkey urine (6).

The protein binding of DPHM and DPMA was studied by equilibrium dialysis technics (11) using a 4% solution of human serum albumin. One ml of albumin solution was dialyzed against 19 ml of 0.85% saline containing the noted concentrations of DPHM and DPMA. Dialysis was continued at 37° to equilibrium: 20 hr for DPHM, 14 hr for DPMA. All solutions were adjusted to pH 7.0. The concentrations of total and free ^{3}H-DPHM were determined by liquid scintillation counting. DPMA was assayed by a modification of the colorimetric procedure originally developed by Dill *et al.* (12) for diphenylhydantoin.

[1] Benadryl is the Parke-Davis trade name for diphenhydramine hydrochloride.

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[4] Abbreviations used: DPHM, diphenhydramine; DPMA, diphenylmethoxyacetic acid; DPMA-Gln and DPMA-Gly, the glutamine and glycine conjugates of DPMA; TLC, thin-layer chromatography; iv, intravenous.

Results and Discussion. The plasma levels following 10 mg/kg iv doses of tritium-labeled DPHM·HCl to rhesus monkeys are shown in Fig. 1. Low levels of unchanged drug (ca. 3 μg/ml) were observed by fluorimetric assay 1 min after dosing; these declined rapidly with an estimated half-life of about 1 hr. However, the tritium assays indicated a gradual *rise* over a period of approximately 4 hr, representing drug levels about 4-fold greater than the 1 min postdose levels. The tritium concentration in the plasma then declined with an estimated half-life of 10 to 12 hr. A similar pattern of plasma levels was seen in two pregnant female rhesus monkeys near term as well as in the normal male and female monkeys. The route of administration also appeared to have little effect. Following 3 and 10 mg/kg *peroral doses* of ^{3}H-DPHM·HCl to rhesus monkeys, the same pattern was observed as in the iv series. Peak plasma levels (0.25–0.3 μg/ml) of the unchanged drug occurred between 1–2 hr after dosing and declined thereafter with an apparent half-life of about 1 hr. On the other hand, plasma levels of radioactivity reached a peak 4 hr after dosing, at 7.5 and 26 μg/ml, respectively, and then fell with a half-life of about 12 hr.

A metabolic instability of the label, resulting in the formation of ^3H_2O, might account for the rising plasma level of tritium. The radioactivity in plasma specimens which were evaporated to dryness and run through oxygen-flask combustion procedures was equal to that found in specimens which were counted directly, indicating good stability of the tritium label. In addition, tritium assays of the blood cells showed good agreement with the levels of *unchanged drug* in plasma, indicating that the removal of drug from plasma was not due to accumulation in the cellular elements of blood.

Identification of the labeled material in the plasma was achieved initially using a 4-hr plasma specimen from a pregnant monkey which had received a 14 mg/kg iv dose of ^{3}H-DPHM·HCl. Ninety-one percent of the radioactivity recovered from the TLC plate had the same R_f as DPMA. The 1 min postdose sample showed no DPMA present. Similarly, in a 5-min specimen from an iv-dosed dog, only 10% of the radioactivity corresponded to DPMA, whereas more than 96% of the label was identified as DPMA in the 4- and 24-hr plasma samples. As expected, unchanged drug was detected only in the early plasma samples, along with small amounts of the N-dealkylated and N-oxide metabolites.

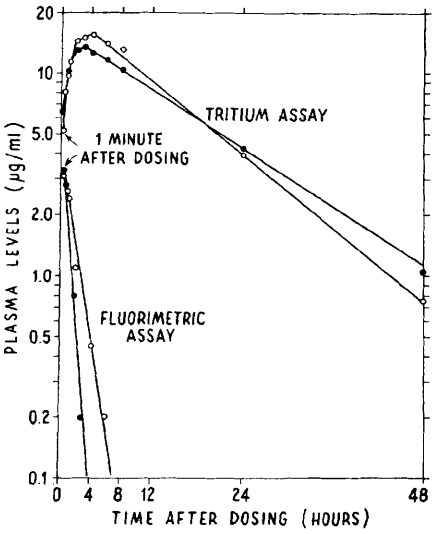

FIG. 1. Plasma levels in rhesus monkeys following iv administration of ^{3}H-DPHM • HCl in aqueous solution. Ten mg/kg doses were injected into the left saphenous vein of a 4.2 kg male (●); and a 3.3 kg female (○). Blood samples (0–8 hr) were obtained by cannulation of the right saphenous vein; the remaining samples were drawn from a cephalic vein. Animals were fasted overnight before dosing and for 8 hr afterwards. Water was available *ad libitum*.

Since the plasma half-life of DPMA appeared to be unusually long in comparison with DPHM, the protein binding characteristics of these compounds were examined with human serum albumin. A substantially greater amount of DPMA was bound compared to the amount of DPHM (Fig. 2). The number of binding sites per molecule of albumin was estimated at 0.5 for DPHM and 3 for DPMA. The corresponding apparent dissociation constants were calculated to be 1.1×10^{-3} *M* for DPHM and 2.6×10^{-5} *M* for DPMA. Thus the binding affinity was about 40-fold greater for the metabolite than for unchanged drug.

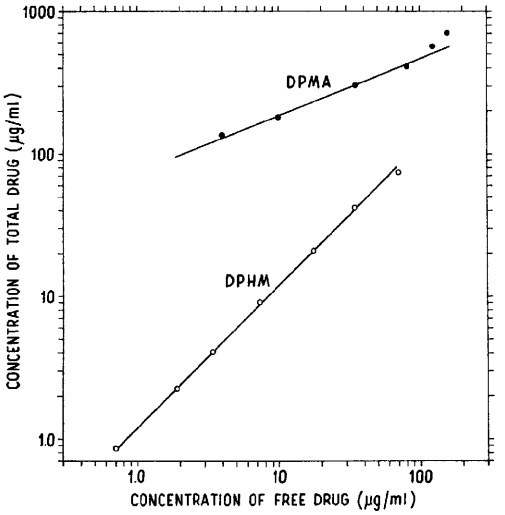

FIG. 2. Binding of DPHM and DPMA to human serum albumin. The dissociation constants and number of binding sites were calculated from these data using the double reciprocal plot method described by Goldstein (11.) Slopes and intercepts were determined by linear regression analysis.

The metabolic disposition of DPHM also was examined in other species of laboratory animals. Intravenous administration of ^{3}H-DPHM·HCl to male beagle dogs produced the results shown in Fig. 3. Divergent plasma levels for radioactivity and for unchanged drug were noted in this species. The peak levels of tritium occurred later than in the monkey, and had a longer apparent half-life. Other species which exhibited this effect following iv doses included the rabbit, mouse, and guinea pig. However, the time required to attain peak levels of tritium activity and the plasma half-life varied among the species examined. Data are summarized in Table I.

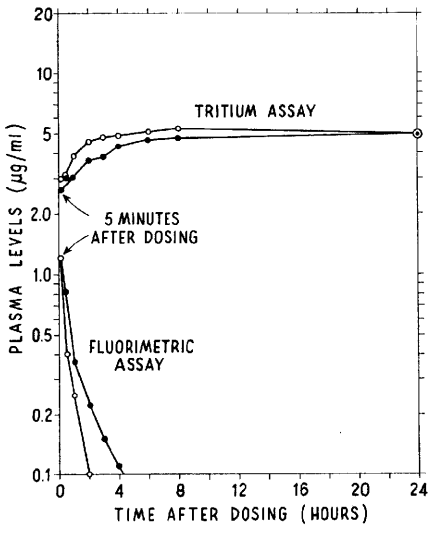

FIG. 3. Plasma levels in beagle dogs following iv administration of ^{3}H-DPHM • HCl in aqueous solution. Five mg/kg doses were injected into the left jugular vein of 11.9 kg (●); and 15.5 kg (○) male dogs. Blood samples were drawn from the right jugular vein. Animals were fasted overnight before dosing. Water was available *ad libitum*.

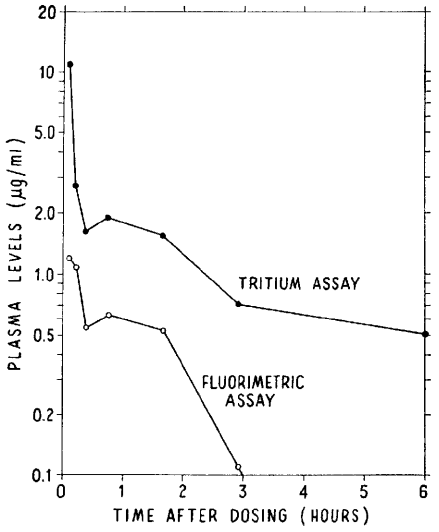

FIG. 4. Plasma levels in rats following a single, iv administration of ^{3}H-DPHM • HCl in aqueous solution. Seven mg/kg doses were injected into a lateral tail vein of 200 g Holtzman, male, albino rats. Sequential blood samples were obtained from the opposite lateral tail vein of each rat. The animals were fasted overnight before dosing. Water was available *ad libitum*. Values are the average of determinations in three animals.

Of the six animal species studied, only the rat did not exhibit divergent plasma levels (Fig. 4). Following iv administration of the labeled drug to this species, plasma levels of radioactivity and unchanged drug fell simultaneously with an estimated half-life of about 1 hr, although the ^{3}H levels were significantly higher than the assays for unchanged drug.

We previously reported partial identification of urinary metabolites from these species

TABLE I. Clearance of Unchanged Drug and Total Tritium Activity from the Plasma of Various Species of Animals.

Species[d]	Dose (mg/kg)	Route	Time of peak plasma tritium level (hr postdose)	Approx biological half-life (hr)	
				DPHM	Tritium[a]
Monkeys (2)	10	iv	3–4	1	10–12
Monkey (pregnant)	10	iv	4	2	10
Monkey	10	oral	4	1	12
Monkey	3	oral	4	1	12
Dogs (2)	5	iv	8, 24	1	>16
Rabbits (2)	3	iv	1, >6	0.3	—[b]
Guinea pigs (7)[c]	5	iv	1	1	2.5
Mice (15)[c]	3	iv	0.5–2	0.1	1.5
Rats (3)	7	iv	<0.1	1	1

[a] Identified as ^{3}H-DPMA in the monkey and dog.

[b] Not calculated. Experiment terminated 6 hr after dosing, while plasma levels in 1 animal were still rising.

[c] Blood obtained by sacrificing animals at time point of interest. Blood specimens from other species obtained by sequential sampling. Strains of animals used: albino mice and guinea pigs, New Zealand white rabbits, others listed in figure legends.

[d] Number of animals indicated in parentheses.

(13). DPMA and its *glutamine* conjugate accounted for the majority of the monkey urinary metabolites. Examination of pooled urine from the dogs used in this study indicated that the metabolites were similar to those found in the monkey, but DPMA was present mainly in the form of its *glycine* conjugate. In contrast, no free or conjugated DPMA could be detected in the urine of rats (Table II). The sequence of events in the metabolic disposition of this drug thus appears to involve very rapid removal of a portion of the dose from the blood stream by tissue elements. Preliminary observations in mice indicated initial drug accumulation in the kidneys and heart. In most species of laboratory animals the drug was metabolized by oxidative deamination, with some of the resulting DPMA being returned to the circulating blood. This metabolite was firmly bound to plasma protein, producing a rise in plasma tritium levels. The greatest part of the DPMA eventually was conjugated with glutamine or glycine and excreted by the

TABLE II. Metabolites in the Urine of Laboratory Animals After Dosing with ^{3}H-DPHM·HCl.

Metabolite	Percentage of total urinary metabolites			
	Monkeys (4)		Dogs (2)	Rats (10)
	Mean	Range	Mean	Mean
Unchanged DPHM	6	2–10	8	2
N-Demethyl-DPHM	9	5–11	2	Trace
N,*N*-didemethyl-DPHM	8	3–13	3	Trace
DPHM-*N*-oxide	12	7–15	25	16
DPMA	12	4–20	9	—
DPMA-Gln	48	35–59	—	—
DPMA-Gly	—	—	33	—
Uncharacterized conjugate(s)[a]	5	0–14	20	80

[a] Hydrolyzed with Glusulase (Endo Lab., Inc., Garden City, N. Y.). Dog conjugate yielded one unidentified product; rat conjugates gave several uncharacterized products.

kidneys. The rat did not show a rising plasma level of radioactive metabolite, and the presence of DPMA could not be demonstrated in the urine.

Summary. Tritium-labeled diphenhydramine given iv to rhesus monkeys produced a rapid drop in plasma levels of unchanged drug, but the total tritium activity rose slowly over a 4-hr period to levels severalfold greater than those observed 1 min after dosing. This was attributed to rapid removal of drug by the tissues, followed by a slow return of metabolites to the plasma. The major metabolite, strongly bound to plasma protein, was identified as diphenylmethoxyacetic acid. Similar observations were made in the dog, rabbit, guinea pig, and mouse; but not in the rat, which did not form diphenylmethoxyacetic acid. Species differences were noted in the conjugation of this metabolite with glutamine in the monkey, and with glycine in the dog.

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