Comparative Studies on Distribution, Excretion, and Metabolism of Hydroxyzine-^{3}H and Its Methiodide-^{14}C in Rats

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Abstract □ The comparative excretion, distribution, and metabolism of hydroxyzine-^{3}H hydrochloride and hydroxyzine methiodide-^{14}C were studied in rats after intraperitoneal administration. Both compounds were excreted mainly within the 1st day and mainly in feces *via* the bile. Hydroxyzine was rapidly distributed to the organs, with the highest specific activity found in the lungs, followed by fat, liver, spleen, and kidneys. It was completely metabolized in the bile and urine mainly to the glucuronides of the diphenylmethane derivatives. Based on differential solvent extraction and TLC, five metabolites, *p*-chloro-*p'*-hydroxybenzophenone, *p*-chlorobenzhydrol, *p*-chlorobenzophenone, piperazine, and 2-[2-(1-piperazinyl)ethoxy]ethanol, were identified. The distribution pattern of hydroxyzine methiodide was basically similar to that of hydroxyzine, except that it was restricted to fewer organs with lower specific activity. It was excreted mainly unchanged as studied by paper chromatography.

Keyphrases □ Hydroxyzine and methiodide, radiolabeled—comparative studies on distribution, excretion, and metabolism, rats □ Biotransformation—radiolabeled hydroxyzine hydrochloride and methiodide, comparative studies on excretion, distribution, and metabolism, rats □ Metabolism—radiolabeled hydroxyzine hydrochloride and methiodide, comparative study, rats

The central nervous system-depressant effect of hydroxyzine (I) provides the basis for its wide clinical application as a minor tranquilizer in recent years. Many reports have appeared attesting to its efficacy in the treatment of minor emotional disturbances such as anxiety, tension, fear, and agitation. The drug has been evaluated particularly for its ability to make disturbed patients more approachable and amenable to long-term psychotherapy (1–4). The efficacy of I as an adjunct to preoperative and postoperative sedative and analgesic medication has been well established (5–8). Its antihistaminic property, for which it was originally designed, has been recently reappraised (9, 10). However, little is known concerning the metabolic behavior and fate of this drug *in vivo*. Therefore, it is necessary to study the distribution, excretion, and metabolism of this drug. Since quaternization of the side-chain nitrogen often modifies the pharmacological properties of a drug, hydroxyzine methiodide (II) was included for comparison.

EXPERIMENTAL[1]

Preparation of Hydroxyzine Methiodide (II) and Hydroxyzine Methiodide-^{14}C (II-^{14}C)—Compound II was prepared by adding methyl iodide in acetone to the free base of I (11). The product was recrystallized from acetone–ether or ethanol to a white crystalline powder, mp 181–182°, UV_{max}: 214 and 228 nm.

Anal.—Calc. for $C_{22}N_{30}ClIN_2O_2$: C, 51.13; H, 5.85; N, 5.42. Found: C, 51.33; H, 5.91; N, 5.27.

Compound II-^{14}C was prepared according to the procedure described for II with a slight modification. The free base (0.9 mmole) of I was dissolved in acetone (1.5 ml) and pipetted into the upper part of the breakseal tube containing 1 mCi (0.6 mmole) of methyl iodide-^{14}C. The capillary seal was broken to allow 1 ml of the solution to enter the container under negative pressure. The tube was stoppered immediately and allowed to stand overnight at room temperature. A suitable amount of ether was added to the reaction mixture and left standing for 15 min for complete crystallization. Then the crystals were collected and washed with ether. The radiochemical purity of the product was checked by employing paper chromatography or TLC coupled with autoradiography and radiochromatographic scanning[2]. Only one spot with the same R_f value as authentic II in various solvent systems was obtained. Therefore, the product was radiochemically pure. The specific activity of the product was 3.36 μCi/mg.

Table I—R_f Values (TLC) of Hydroxyzine and Related Compounds

Compound	Solvent System[a] A	B	C	D	E	F
Hydroxyzine (I)	0.41	0.80	0.68	0.65	0.30	0
p-Chlorobenzophenone (IV)	0.69	0.90	0.81	0.76	0.83	0.80
p-Chloro-*p'*-hydroxybenzophenone (V)	0.04	0.85	0.80	0.74	0.80	0.03
p-Chlorobenzhydrol (VI)	0.52	0.86	0.81	0.78	0.84	0.41
Piperazine (VII)	0.05	0.20	0.31	0.06	0.04	0.06
2-[2-(1-Piperazinyl)-ethoxy]ethanol (VIII)	0.08	0.23	0.37	0.18	0.11	0.04

[a] A, cyclohexane–benzene–diethylamine (75:15:10); B, methanol–acetone (1:1); C, 95% ethanol; D, *n*-butanol–ethanol–water (5:2:2); E, water-saturated isoamyl alcohol containing 15% acetic acid; and F, chloroform–cyclohexane (1:1).

Tritiation and Purification of Hydroxyzine-^{3}H Hydrochloride (I-^{3}H)—Compound I-^{3}H was prepared commercially[3] according to the Wilzbach (12) tritium gas-exposure method, in which 1 g of I was exposed to 3 Ci of tritium gas at 250 mm Hg at 26° for 2 weeks. The product was purified by dissolving in methanol–chloroform (10:1) and evaporating to dryness *in vacuo* to remove labile tritium. The same procedure was repeated once and the residue was taken up in hot methanol. The I-^{3}H thus obtained gave only one spot with an R_f value corresponding to that of authentic I in Solvent Systems A–D (Table I) and was found to be 98% pure as determined by the TLC–zonal scraping–liquid scintillation counting to be described later.

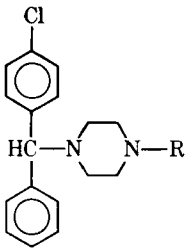
Preparation of Hydroxyzine *N*-Oxide (III)—Since it was speculated that III might play a role in the metabolism of I, synthesis of III was carried out. Compound I (3 g) was dissolved in water (50 ml), adjusted to pH 10, and extracted three times with ether. The combined ether extracts were evaporated to dryness *in vacuo* and the residue was dissolved in ethanol (10 ml). To this solution, 5 ml of 10% hydrogen peroxide was added and the mixture was stirred at room temperature for 2 days. After the ethanol was removed *in vacuo*, the residue was dissolved in water and extracted with chloroform. The chloroform extract was evaporated to dryness and the residue was recrystallized from acetone to white crystals, mp 189-190°, UV_{max}: 211 and 231 nm.

Anal.—Calc. for $C_{21}H_{27}ClN_2O_3$: C, 64.52; H, 6.96; N, 7.17.

[1] Melting point was measured on a Fisher-Johns melting-point apparatus. Elemental analysis was carried out by Galbraith Laboratory, Knoxville, Tenn. The UV spectrum was recorded in a Perkin-Elmer spectrophotometer model 202, using ethanol as a solvent.

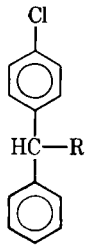
[2] Actigraph III, Nuclear-Chicago.

[3] New England Nuclear Corp.

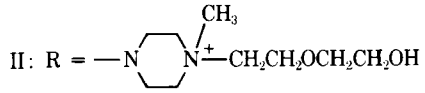


hydroxyzine I: R = $CH_2CH_2OCH_2CH_2OH$

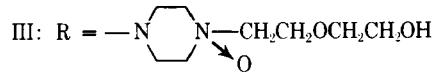
norchlorcyclizine IX: R = H



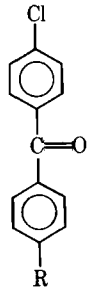
hydroxyzine methiodide



hydroxyzine *N*-oxide

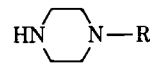


p-chlorobenzhydrol VI: R = OH



p-chlorobenzophenone IV: R = H

p-chloro-*p'*-hydroxybenzophenone V: R = OH



piperazine VII: R = H

2-[2-(1-piperazinyl)ethoxy]ethanol

VIII: R = $CH_2CH_2OCH_2CH_2OH$

Chemical structure of hydroxyzine and related compounds

Found: C, 64.41; H, 7.09; N, 7.24.

Lipid–Water Partition of Labeled Compounds—To find an optimal condition of extraction for metabolic study, a lipid–water partition study was carried out using the labeled compounds. Compounds I-^{3}H and II-^{14}C were dissolved in water and diluted with a series of buffers (pH 2.0–10.0). Each buffered solution was equilibrated with an equal volume of organic solvent. The radioactivity of the aqueous phase was compared with that of the original buffered solution. The result indicated that I-^{3}H was extractable in organic solvent only at alkaline pH, while II-^{14}C was only slightly soluble in organic solvents at all pH levels.

Animals and Method of Administration—Male albino rats of a Sprague–Dawley strain, weighing 170–250 g, were used. The animals were conditioned to the new environment and provided with food and water for 5 days. Compound I-^{3}H was dissolved in saline and Compound II-^{14}C was dissolved in 30% dimethyl sulfoxide in a concentration of 1.5–2.0 mg/ml. Rats were lightly anesthetized with ether and injected with 5–10 mg/kg ip of I-^{3}H, which is equivalent to a human dosage.

Table II—Urinary, Fecal, and Biliary Excretion of Hydroxyzine-^{3}H and Hydroxyzine Methiodide-^{14}C in Rats

Excretion	Time	Cumulative Percent of Administered Radioactivity (dpm ± *SE*)	
		Hydroxyzine-^{3}H Hydrochloride	Hydroxyzine Methiodide-^{14}C
Urinary	1 day	12.0 ± 0.8[a]	6.8 ± 0.4[b]
	2 day	14.3 ± 0.9	7.4 ± 0.4
	3 day	14.8 ± 0.9	7.7 ± 0.4
	4 day	15.2 ± 0.9	—
	5 day	15.5 ± 1.0	7.8 ± 0.1
Fecal	1 day	27.6 ± 4.6[a]	34.9 ± 3.5[b]
	2 day	32.2 ± 4.2	39.6 ± 2.5
	3 day	33.4 ± 4.1	40.0 ± 2.5
	4 day	34.3 ± 4.1	—
	5 day	34.9 ± 4.0	40.4 ± 2.3
Biliary	0.5 hr	19.8 ± 0.9[c]	1.8 ± 0.2[c]
	1 hr	34.9 ± 0.6	5.5 ± 0.7
	2 hr	44.3 ± 1.2	13.0 ± 0.9
	4 hr	49.9 ± 1.0	26.6 ± 1.7
	8 hr	53.7 ± 0.9	34.0 ± 4.2

[a] $n = 6$. [b] $n = 5$. [c] $n = 3$.

Urinary and Fecal Excretion—After the administration of the solutions, the animals were housed individually in metabolic cages and allowed food and water *ad libitum*. Urine and feces specimens were collected separately at 24-hr intervals for 5 days. The collected specimens were stored in a deep freeze until analyzed.

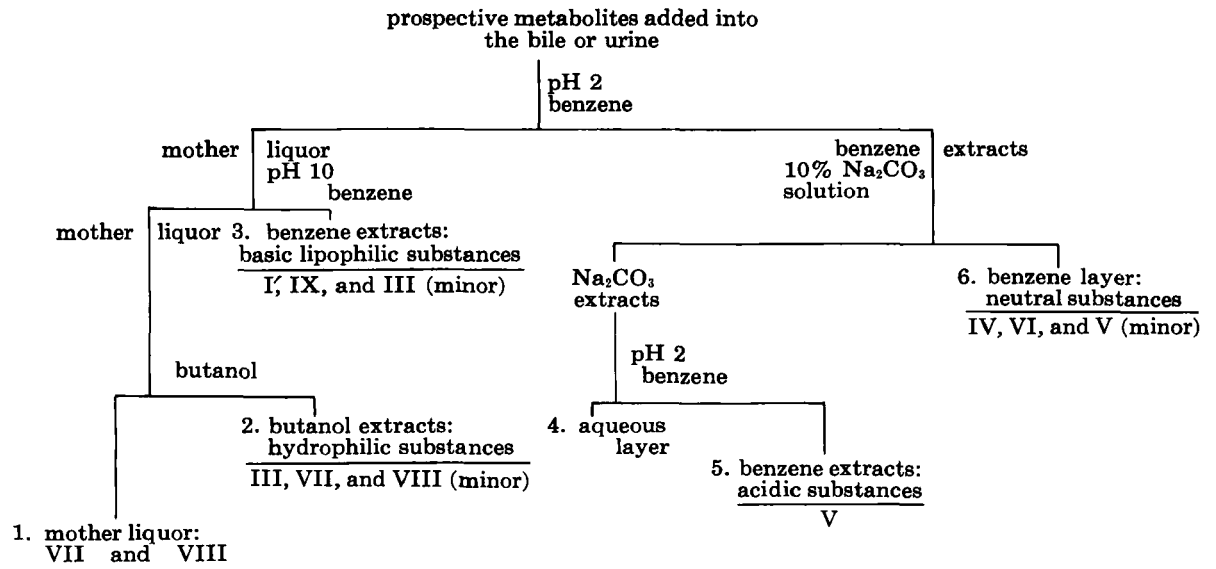
Pulmonary Excretion—This study was conducted to test the possible pulmonary excretion of $^{14}CO_2$ derived from *N*-demethylation of II-^{14}C. After intraperitoneal administration, the animals were maintained in a modified metabolic jar (13) for 24 hr with a constant flow of air current which was passed through two gas-washing bottles, each containing 150 ml of 30% NaOH. The carbon dioxide trapped in the gas-washing bottles was precipitated with 12% $BaCl_2$ solution containing a small amount of ammonium chloride (14). The barium carbonate thus formed was centrifuged, washed several times, and dried. The dried barium carbonate was ground to a fine powder, an aliquot of 50 mg was placed in a planchet, and the activity was recorded[4].

Biliary Excretion—Three rats were anesthetized with sodium pentobarbital (25 mg/kg sc). An abdominal incision was made and the bile duct was cannulated with a polyethylene tube (PE-50). Then 0.5 ml of the test solution was immediately dropped into the abdominal cavity, and the incision was closed by suture. Bile specimens were collected at the intervals of 0.5, 1, 2, 4, and 8 hr.

Tissue Distribution—The animals were administered intraperitoneally with the compound under study and were sacrificed at the following intervals: 0.5, 1, 2, 4, and 8 hr. Blood specimen was collected in a test tube containing 1 ml of 5% edetate (EDTA) and centrifuged at 2000 rpm for 10 min to separate the plasma. Tissues and organs were removed, rinsed thoroughly with water, and blotted on a filter paper. The fresh specimens were weighed and prepared for assay. The total blood volume was taken as 8% (v/w; hematocrit, 0.45); the weights of the muscle, bone, fat, and skin were calculated as 45, 22, 3, and 3%, respectively, of the body weight of the animal.

Radioactivity Counting—A liquid scintillation spectrometer[5] was used to record the activity. An aliquot of 100 μl of the urine and 20 μl of the diluted bile specimens were counted directly in 10 ml of Bray's scintillator (15). The bone, skin, and feces were prepared according to the method of Mahin and Lofberg (16). Specimens of bone, skin (100–200 mg), and feces (100 mg) were placed in a counting vial containing 0.2 ml of 60% perchloric acid and 0.4 ml of 30% hydrogen peroxide. The vial was tightly screw capped and the content was heated to 70–80° for 1–2 hr with occasional shaking. After the vial was cooled to room temperature, 5 ml of 2-ethoxyethanol[6] and 10 ml of scintillating solution containing 0.6%

[4] Geiger-Muller counter, Tracer-Lab, TGC-2.
[5] Corumatic 100, Tracerlab.
[6] Cellosolve.



Scheme I—Differential solvent fractionation of prospective metabolites added into the bile or urine according to the method of Soudijn and Van Wijngaarden (18)

2,5-diphenyloxazole in toluene were added and the activity was determined. The specimens (100–200 mg) of kidneys, lungs, spleen, heart, muscle, and fat were digested with 1.5–2.0 ml of hyamine hydroxide at 50–60° for from 12 hr to 2 days with constant shaking. Aqueous hydrogen peroxide was employed, if necessary, to bleach the specimens and 0.2 ml of concentrated hydrochloric acid was added to eliminate the phosphorescence (17) after the hyamine hydroxide digestion. Ten milliliters of Bray's scintillating solution was added and the activity was recorded. Control runs with the starting materials were conducted to correct for the loss of radioactivity due to the mechanical adsorption to the upper part of the vial and to the cap lining. An internal standard was used to estimate the counting efficiency and to correct for the quenching of the samples.

Metabolic Study of II-^{14}C—An aliquot of urine and bile from rats treated with II-^{14}C was placed linearly on a strip of Whatman No. 3 chromatographic paper (4 × 30 cm) and was developed ascendingly in Solvent Systems D and G. The dried chromatogram was scanned in a radiochromatogram scanner[2]. The R_f value of the radioactive peaks was compared with that of reference materials, and the percent of the radioactivity of each peak was estimated.

***Metabolic Study of* I-^{3}H**—A differential solvent extraction procedure (18) was carried out on authentic specimens of prospective metabolites of hydroxyzine added individually to control bile and urine. The procedure (Scheme I) was as follows. An aliquot of bile or urine containing a reference compound was acidified with concentrated hydrochloric acid to pH 2 and extracted with benzene. The combined benzene extracts were extracted with 10% Na_2CO_3 solution, leaving behind the neutral substance in the organic layer (Fraction 6). The sodium carbonate extract was acidified with concentrated hydrochloric acid to pH 2, and the acidic compound was extracted with benzene (Fraction 5). The initial aqueous solution was alkalized with concentrated ammonium hydroxide to pH 10 and extracted with benzene (basic lipophilic substance, Fraction 3) followed by *n*-butanol (hydrophilic substance, Fraction 2), leaving behind the mother liquor (Fraction 1). Each fraction was chromatographed by TLC and scanned under a UV lamp or sprayed with an appropriate color reagent to establish the identity of each compound. The same procedure was applied to the bile and urine specimens of rats receiving II-^{3}H. Each fraction obtained by solvent extraction was further fractionated by TLC, and the R_f was compared with that of the reference compounds. The percent ratio of the subfractions was quantified by zonal scraping–liquid scintillation counting.

TLC—Silica gel[7] and silica gel[8] IB-F were used. An aliquot of 5–10 μl of the sample solution was spotted 1.5 cm from the bottom of a chromatographic sheet and developed in a solvent system to a height of 10–15 cm. The developed chromatograms were dried and the spots were detected under a UV lamp and subsequently sprayed with Dragendorff reagent or exposed to iodine vapor. The R_f values of hydroxyzine and the related compounds are shown in Table I.

Autoradiography and fluorography (19) were carried out to check the purity of the substrate and to detect the metabolites on the chromatogram. The TLC plates containing ^{14}C samples were exposed to medical X-ray film[9] for 1–3 days. Those TLC plates containing a ^{3}H sample were first sprayed[10] and then exposed to medical X-ray film[11] over dry ice for 1–7 days. After the exposure, the films were processed according to the manufacturer's recommendation.

Zonal Scraping–Liquid Scintillation Counting (20)—This method was adapted to quantitate the radioactive metabolites on the chromatograms. A chromatogram (5 cm in width) was divided into two equal parts of 2.5 cm each by drawing a perpendicular line in the center of the adsorbent layer. One-half of the plate was used for reference compound and the other for the sample solution. After development, the sheet was cut into two pieces and the plate containing the reference compound was scanned to detect the spot. Silica gel zones (0.5–1 cm) of the specimen on the other half of the sheet were scraped into counting vials containing 12 ml of a scintillation solution[12]. The vials were shaken thoroughly and placed in a liquid scintillation spectrometer for activity recording. The profile for the distribution of the radioactivity was constructed by taking the net count accumulated in all zones and the percent of the activity calculated.

RESULTS AND DISCUSSION

Urinary, Fecal, and Biliary Excretion—Results of the urinary, fecal, and biliary excretion of I-^{3}H and II-^{14}C are summarized in Table II. Apparently, fecal excretion was the major route for these two compounds. The radioactivity of II-^{14}C in the feces appeared to be higher than that of I-^{3}H; however, the difference was insignificant ($p < 0.05$). Compound I-^{3}H (16%) exhibited a higher urinary excretion than II-^{14}C (8%) in 5 days. The majority (40–42%) of the administered radioactivity (urine and feces combined) was excreted in the 1st day for both compounds, and a slow excretion ensued thereafter. The higher biliary excretion of I-^{3}H (54%) than of II-^{14}C (34%) in 8 hr ($p < 0.01$) was consistent with the fecal excretion.

Pulmonary Excretion—No radioactivity was detected in the

[7] Eastman chromogram 6060.
[8] Baker-flex.
[9] Kodak no-screen.
[10] Omnispray, New England Nuclear Corp.
[11] Kodak Royal Blue.
[12] Containing 4% Cab-O-Sil.

Table III—Comparative Distribution of Radioactivity in the Rat after Intraperitoneal Injection of Hydroxyzine-^{3}H Hydrochloride and Hydroxyzine Methiodide-^{14}C Expressed in Terms of Relative Specific Organ Activity[a] (Disintegrations per Minute per Gram Wet Organ) (That in the Blood of Hydroxyzine-^{3}H Hydrochloride-Injected Rat Served as 1.00)

	Hours after Intraperitoneal Administration									
	Hydroxyzine-^{3}H Hydrochloride					Hydroxyzine Methiodide-^{14}C				
Organ	0.5	1	2	4	8	0.5	1	2	4	8
Blood	1.00	1.12	1.21	0.93	0.72	0.18	0.33	0.15	0.11	0.04
Brain	2.47	1.47	1.47	1.27	0.56	0	0	0	0	0
Liver	10.8	9.76	6.81	5.37	3.58	19.8	16.8	12.3	10.2	2.40
Intestines	9.75	13.0	20.9	22.4	31.0	9.90	23.7	29.0	32.0	35.4
Kidneys	6.91	6.52	4.63	2.62	1.18	9.20	5.48	3.32	2.33	0.91
Lungs	11.4	11.5	9.20	5.22	2.25	0.67	0.32	0.43	0.59	0.57
Spleen	8.65	6.96	4.14	4.09	2.50	3.72	2.72	3.30	1.32	0.81
Heart	2.98	2.25	1.57	0.87	0.72	0	0	0	0	0
Stomach	5.08	3.07	2.20	1.69	1.53	2.47	2.77	3.42	1.49	0.87
Testis	1.01	1.50	1.77	1.87	0.69	1.06	1.06	2.92	1.50	0.31
Muscle	1.11	1.02	0.90	0.56	0.32	0	0	0	0	0
Fat	11.2	5.27	3.53	2.65	1.06	12.0	3.86	4.04	5.28	1.79
Bone	3.14	2.43	1.70	1.52	1.15	—[b]	—	—	—	—
Skin	2.03	1.79	1.82	1.29	1.01	—	—	—	—	—

[a] Represents the average of three experiments. [b] Data not available.

trapped carbon dioxide in the expired air by the animal injected with II-^{14}C. This indicated that no *N*-demethylation of the quaternary ammonium salt occurred *in vivo* in the rat, which is in contrast to the documented *N*-demethylation of certain secondary and tertiary amine derivatives (21, 22).

Distribution Study—The radioactivity in the organs was expressed in terms of relative specific organ activity (disintegrations per minute per gram wet organ) for easy comparison, using activity in blood as 1.00 (Table III). The radioactivity of I-^{3}H was absorbed rapidly, reaching a peak level within 0.5 hr in most organs excepting blood, intestines, and testis; this finding verified the previous claim for hydroxyzine as a fast-acting tranquilizer (1). The specific activity was highest in the lungs, followed by the fat, liver, spleen, and kidneys. The sharp increase in the intestinal activity and urinary level coincided with the rapid fall of activity in the liver and kidneys. About 50% of the radioactivity was found in the intestines at 8 hr.

Compound II-^{14}C was also rapidly absorbed, with a peak level in the liver, fat, kidneys, and spleen within 0.5 hr, in the blood at 1 hr, and in the stomach and testis at 2 hr. The distribution pattern was similar to, but quantitatively less than, that of I-^{3}H, and the activity in most organs declined rapidly. At the end of 8 hr, about 57% of the radioactivity was recovered in the intestines.

From Table III, it is obvious that hydroxyzine, which is mostly nonionized at physiological pH, was more readily absorbed from the peritoneal cavity, while hydroxyzine methiodide, which is highly ionized at all pH's, was less readily absorbed but more rapidly released from organs after being absorbed.

Metabolic Study of II-^{14}C—As shown in Table IV, II-^{14}C gave three metabolites in the urine and bile as studied by paper chromatography. The R_f values of the major metabolite (about 77% in the urine and 75% in the bile) matched that of the parent compound. Therefore, it is apparent that the quaternary salt of hydroxyzine was excreted mainly unchanged. The nature of the two minor metabolites was not investigated.

Metabolic Study of I-^{3}H—As indicated in Table II, biliary excretion was the major route of elimination of I-^{3}H, with the majority of the radioactivity excreted within the 1st hr. Therefore, the combined bile collected during the 1st hr was used in the metabolic study using differential solvent extraction and TLC.

Table IV—Metabolites of Hydroxyzine Methiodide-^{14}C in Urine and Bile after Intraperitoneal Injection

	R_f Values (Paper Chromatography)		
Solvent System	Urine	Bile	Hydroxyzine Methiodide
D, butanol–ethanol –water (5:2:2)	0.90 (22)[a]	0.07 (6)	
	0.34 (3)	0.33 (19)	
	0.81 (75)	0.83 (75)	0.83
G, butanol–acetic acid–water (12:3:5)	0.06 (19)	0.06 (9)	
	0.52 (2)	0.54 (16)	
	0.89 (79)	0.91 (75)	0.91

[a] Figures in parentheses represent the percent of the total radioactivity.

The metabolites were fractionated according to the differential solvent extraction procedure (18) in which authentic specimens of prospective metabolites added in the bile served as a control to give constructive information as to the nature of the metabolites in each fraction (Scheme I). The percent radioactivity of the fractionated bile obtained from I-^{3}H-injected rats is shown in *Table V*. Only about 2% of the radioactivity was found in Fraction 3 (basic lipophilic substances, I and IX). This indicated that more than 98% of I-^{3}H was metabolized and only a small amount of IX, if any, was formed. The major metabolites were hydrophilic in nature, as evidenced by the findings that about 85% of the radioactivity was located in Fractions 1 and 2. The rest of the radioactivity (about 13%) was distributed into Fractions 4, 5, and 6, which comprised the neutral (IV and VI) and acidic (V) substances.

Studies with fluorograms indicated four metabolites in the bile on the thin-layer chromatogram developed in Solvent System D. Solvent System D (Table V) appeared to be the most efficient solvent system for the separation of the metabolites of I, so it was used preferably to separate the metabolites in the bile on TLC[13]. The developed chromatograms were then subjected to zonal scraping–liquid scintillation counting to obtain the TLC radioactivity profile (Fig. 1). It is obvious that the more hydrophilic the metabolites, the lower the R_f values in this solvent system. All fractions except Fraction 4 were chromatographed in other solvent systems, and the R_f values of the radioactive peaks and the percent radioactivity were recorded (Table V).

By comparing the R_f values of the radioactive peaks in the profile of Fraction 6 (Table V) with that of the authentic specimens (Table I), 2.5, 5.8, and 1.2% of the radioactivity were found to be attributable to IV, V, and VI, respectively. The R_f values of Fraction 5 (Table V) resembled that of V. Since the activity in Fraction 4 was insignificant, it was not further investigated. The R_f value of Fraction 3 in Solvent System B (Table V) indicated that the radioactive peak (R_f 0.82) did not match either IX (R_f 0.55) or III (R_f 0.45, Table I). The profile of radioactive peaks in Solvent System A (R_f 0.12 and 0.67) also excluded the presence of unchanged hydroxyzine (R_f 0.41). The nature of these two metabolites was unknown.

Fraction 2 was chromatographed on silica gel sheets in Solvent System D. The silica gel zone with radioactivity corresponding to the main metabolite 2d was scraped off and extracted with methanol. Its profile in Solvent System B (Fig. 2A) indicated the presence of two metabolites, 2d-1 and 2d-2. No radioactivity was found at R_f values higher than 0.60.

A study indicated that about 12% of the radioactivity in the bile was extractable in chloroform, and an additional 27% was extract-

[13] Baker-flex silica gel sheets.

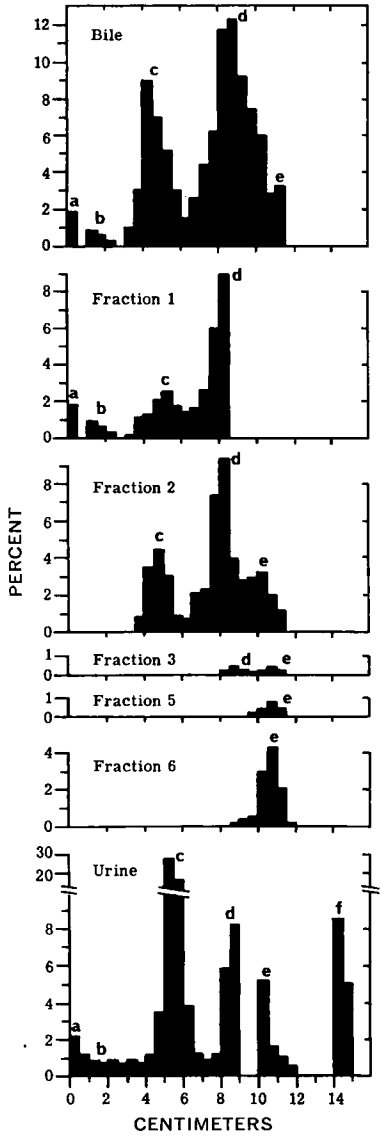


Figure 1—*TLC radioactivity profile in Solvent System D of fresh bile, fractionated bile (Fractions 1, 2, 3, 5, and 6), and the urine of the bile duct-cannulated rats injected intraperitoneally with hydroxyzine-^3H. Expressed in terms of percent radioactivity in the bile and urine, respectively.*

ed after the chloroform-extracted bile was incubated with β-glucuronidase[14] for 2 days. This finding was substantiated by the shift in the radioactivity profile of Fraction 2 in which the nonpolar subfraction, 2d-3, increased with a concurrent decrease in the polar subfractions, 2d-1 and 2d-2, after β-glucuronidase incubation. This finding indicated that the large fraction of the radioactivity was due to the glucuronide(s). The purified subfraction, 2d-3, gave a minor spot with R_f 0.56 and 0.45 in Solvent Systems A (Fig. 2C) and F (Table I), respectively, which corresponded to that of Compound VI in the same solvent systems. The majority of the radioactivity remained at the origin where Compound V was located (R_f 0.03–0.04). Therefore, the major metabolites of Fraction 2 were found to be the glucuronides of V (93%) and VI (7%). These findings coincided with the reported metabolic pathway of structurally related meclizine (23) and chlorcyclizine (24) in which formation of IV, V, VI, and VII was suggested. Metabolite 1d in Fraction 1 (Fig. 1) was similarly purified and incubated with or without β-glucuronidase according to the procedure described for 2d. Essentially the same result was obtained. Therefore, metabolite 1d was identified to be the same as Metabolite 2d.

[14] Sigma Chemical.

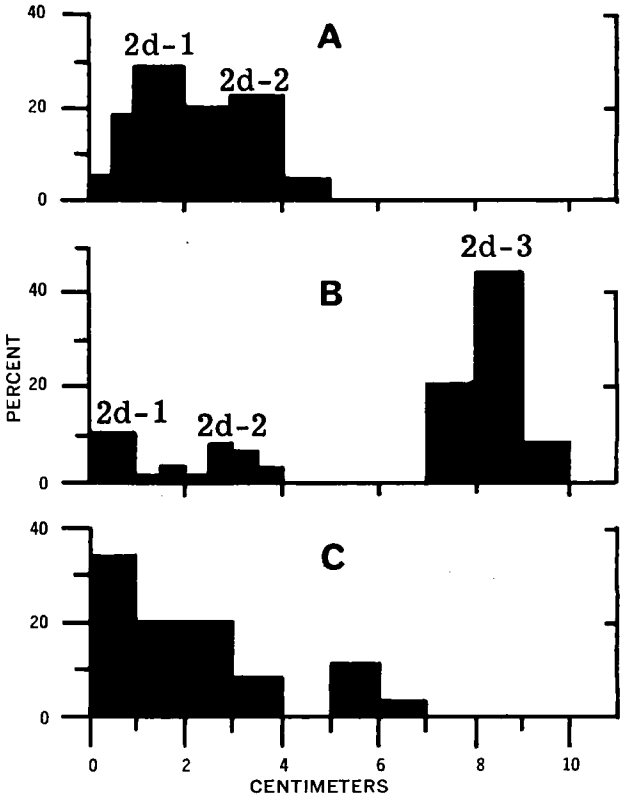


Figure 2—*TLC radioactivity profile in Solvent System B of Metabolite d in Fraction 2 (Fig. 1) after purification (A) and following incubation with β-glucuronidase (B); TLC radioactivity profile of purified 2d-3 in Solvent System A (C).*

When the profile of the 2-week-old bile in Solvent System D was compared with that of fresh specimens, it was apparent that the radioactive peak d decreased from about 60 to 40% with concurrent increase of peak e from 5 to 19%, while peak c did not change. This indicated that Metabolite c was stable in contrast to the unstable Metabolite d. Subfractions 1c and 2c were isolated from the silica gel sheets, combined together, and rechromatographed in Solvent Systems A, B, and F. More than 97% of the combined radioactivity was located at the origin. The chromatographic behavior of Metabolite c resembled that of VIII in Solvent Systems A and F but differed in D (R_f 0.34); therefore, the possibility that Metabolite c may be VIII was ruled out. The nature of Metabolite c remained unknown. Fractions 1a and 1b (Fraction 1, Fig. 1) were similarly isolated and rechromatographed in other solvent systems (Table V). By comparing the R_f values of the radioactive peaks with those of the reference compounds (Table I), 1a and 1b were identified to be VII and VIII, respectively. Thus, I, 5–10 mg/kg ip, was completely metabolized within 1 hr (Table V). Neither IX nor III was found to be the metabolites. The metabolites identified were V (7.2%) and its glucuronide (53.3%), VI (1.2%) and its glucuronide (4.1%), IV (2.5%), VII (2.2%), and VIII (3.3%), accounting for about 74% of the total radioactivity in the bile.

The urine specimens collected from the bladders of rats injected with I-^3H for the biliary metabolic study were chromatographed on silica gel sheets in Solvent System D to give the profile shown in Fig. 1. In comparison to the profile of bile, Metabolite c increased remarkably while d decreased appreciably, with about 14% of the metabolites appearing at R_f 0.95. As a result of the differential solvent extraction, Fractions 1, 2, and 3 contained about 72, 5, and 13%, respectively, of the radioactivity in the urine, while 13% was located in Fractions 4, 5, and 6. The profile in Solvent System A showed that only about 2% of the radioactivity was located at R_f 0.40 (I, 0.41; IX, 0.43). This finding indicated that more than 98% of I-^3H was metabolized and that the parent compound recovered or the amount of IX formed was not more than 2%. By comparing these data and the profile of the urine with that of the bile (Fig. 1 and Table V), inferentially about 7% of the radioactivity was at-

Table V—R_f Values (TLC) and Percent Radioactivity of Metabolites of Hydroxyzine-^{3}H in the Bile of Rats after Intraperitoneal Injection

Fraction Number	Radioactivity[a], %	R_f Value in Solvent System[b] A	B	D[c]	F	Metabolites[d]
1	34.6 ± 14.7[e]	Origin (34.6)[f]	Origin (34.6)	Origin (2.2) 0.12 (3.3) 0.35 (9.2) 0.55 (19.8)	Origin (34.6)	VII (2.2) VIII (3.3) VI–Gl. (1.4)[g] V–Gl. (18.4) Unknown (9.3)
2	50.2 ± 16.6	Origin (50.2)	0.15 (30.2) 0.34 (20.0)	0.32 (12.5) 0.55 (28.6) 0.68 (9.0)	Origin (50.2)	VI–Gl. (2.7) V–Gl. (34.9) Unknown (12.6)
3	1.9 ± 1.7	0.12 (1.6) 0.67 (0.3)	0.82 (1.9)	0.58 (1.2) 0.72 (0.7)	0.09 (1.6) 0.73 (0.3)	Probably spilled from Fraction 2
4	2.6 ± 0.4	—	—	—	—	Not investigated
5	1.3 ± 0.3	0.05 (1.3)	0.86 (1.3)	0.75 (1.3)	0.06 (1.3)	V (1.3)
6	9.4 ± 3.1	0.08 (5.5) 0.48 (1.0) 0.62 (2.9)	0.82 (9.4)	0.73 (9.4)	0.04 (6.0) 0.43 (1.3) 0.74 (2.1)	IV (2.5) VI (1.2) V (5.8)

[a] Expressed as the percent of total radioactivity in the bile. [b] See Table I. [c] Baker-flex silica gel IB-F sheets were used; solvent front 15 cm. For other solvent systems, Eastman chromagram sheets were used; solvent front 10 cm. [d] R_f values of prospective metabolites; see Table I. [e] n = 3. [f] Figures in the parentheses represent the percent radioactivity of the metabolite expressed in terms of the total radioactivity in the bile. [g] Gl. = glucuronide.

tributed to IV, V, and VI, about 15% to the glucuronides of the latter two compounds, and about 5% to VII and VIII.

SUMMARY

The excretion, distribution, and metabolism of hydroxyzine-^{3}H hydrochloride in the rat after intraperitoneal administration were compared with those of hydroxyzine methiodide-^{14}C. Both compounds were excreted largely within the 1st day and mainly in feces *via* the bile. Hydroxyzine-^{3}H was excreted more rapidly into the bile within 8 hr and exhibited a higher rate of urinary excretion within 5 days than the methiodide. Hydroxyzine-^{3}H was absorbed rapidly, with a peak level in most organs within 30 min, and decreased rapidly within 8 hr, confirming the previous claim for hydroxyzine as a fast-acting tranquilizer. The specific activity was the highest in the lungs, followed by the fat, liver, spleen, and kidneys; that in the blood, testis, and muscle was relatively low. The distribution pattern of hydroxyzine methiodide-^{14}C was basically similar to that of hydroxyzine-^{3}H. However, the distribution was more restricted, with insignificant radioactivity in the brain, heart, and muscle, and the specific activity in most organs was lower and declined more rapidly than hydroxyzine-^{3}H.

The metabolism of hydroxyzine methiodide-^{14}C, as studied by paper chromatography coupled with radiochromatographic scanning, indicated that it was excreted mainly unchanged in the urine (77%) and bile (75%) with two minor metabolites. The metabolism of hydroxyzine-^{3}H hydrochloride was studied by differential solvent fractionation and TLC, and this was found to be completely metabolized. Among the metabolites identified were *p*-chloro-*p'*-hydroxybenzophenone (7.2% in free form and 53.3% as glucuronide), *p*-chlorobenzhydrol (1.2% in free form and 4.1% as glucuronide), *p*-chlorobenzophenone (2.5%), piperazine (2.2%), and 2-[2-(1-piperazinyl)ethoxy]ethanol (3.3%), accounting for about 74% of the total radioactivity in the bile. About 7% of the radioactivity in the urine could be attributed to *p*-chlorobenzophenone, *p*-chlorobenzhydrol, *p*-chloro-*p'*-hydroxybenzophenone, about 15% to the glucuronides of the latter two compounds, and about 5% to piperazine and 2-[2-(1-piperazinyl)ethoxy]ethanol.

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