Distribution, Excretion, and Metabolism of ^{14}C-Labeled Quaternary Ammonium Salt of Perphenazine in Rats

C. L. HUANG, G. M. MIR, and J. Z. YEH

Abstract □ Urinary excretion was the major route of excretion of intraperitoneally administered perphenazine dimethiodide-^{14}C in rats. Peak blood level was observed at 0.5 hr. after the administration of the compound, and brain level was above the detectable level at the same period of time. Antimicrobial activities were demonstrated. The toxicity of quaternary ammonium salt of perphenazine on mice is higher than perphenazine.

Keyphrases □ Perphenazine dimethiodide-^{14}C—synthesis □ Distribution, metabolism, excretion—perphenazine dimethiodide-^{14}C □ Antimicrobial activity—perphenazine dimethiodide-^{14}C □ UV spectrophotometry—analysis □ Paper chromatography—radio scanning

Perphenazine is a phenothiazine derivative which differs chemically from prochlorperazine only with respect to the substitution of a hydroxyethyl group for the methyl group of the latter drug. Perphenazine is approximately twice as potent dosagewise as prochlorperazine and exhibits clinical effects and side actions similar to those of chlorpromazine (1–3).

Symchowicz *et al.* (4, 5) have reported the tissue distribution of ^{35}S-perphenazine in rats after the subcutaneous injection of 0.3 mg./kg. of the compound. High concentrations were found in the lungs, adrenals, liver, kidneys, spleen, and pituitary, while the brain had only a residual activity. Blood levels were consistently low. High levels of radioactivity were found in the pituitary gland after 48 hr. and remained relatively high even after 6 days. Over 80% of the administered activity was excreted during the 24-hr. period, of which 64% was found in the feces and only 16% in the urine.

Huang and Kurland (6), who focused their attention on identification and quantitative determination of the major metabolites of perphenazine, found that unlike chlorpromazine (7, 8) the rate of excretion of perphenazine metabolites was low; approximately 44% of the administered dose was recovered in man during a 7-day period. Perphenazine glucuronides were found to be the major metabolites, representing approximately 69% of the total urinary metabolites. Perphenazine sulfoxide was found to be approximately 13% of the administered dose, and the unchanged perphenazine was usually undetectable.

It has been demonstrated that toxic properties of phenothiazine neuroleptics do not diminish by quaternization of the side-chain nitrogen but rather increase in most cases (trifluoperazine, mepazine, and promethazine). The excretion of the majority of the administered activity of quaternary derivative of mepazine, promethazine (9), and trifluoperazine (10) was consistent with predominantly fecal excretion.

Absorption of these compounds from the intraperitoneal cavity was rapid, which is reflected in a rapid increase of blood levels in rats in 0.5 hr. Brain levels were generally low and were either trace or insignificant against the background activity. All these compounds seemed to have a particular affinity for the bone, since significant activities were recorded in the bone after 5 days. No radioactivity was found in the calcium carbonate formed from the carbon dioxide collected in the expired air from the animals placed in metabolic jars, which indicated that demethylation of these quaternary compounds did not occur *in vivo*. Antimicrobial activities were also demonstrated. High intestinal activity appeared to be due to the secretory process of these drugs *via* bile. As indicated in a previous communication (10), trifluoperazine, one of the potent phenothiazine neuroleptics with a piperazinyl side chain, is excreted unchanged. It would be of interest to see how a compound of this series with a primary alcohol function on the side chain would behave *in vivo*. In this report, distribution and elimination of perphenazine dimethiodide-^{14}C in rats are described.

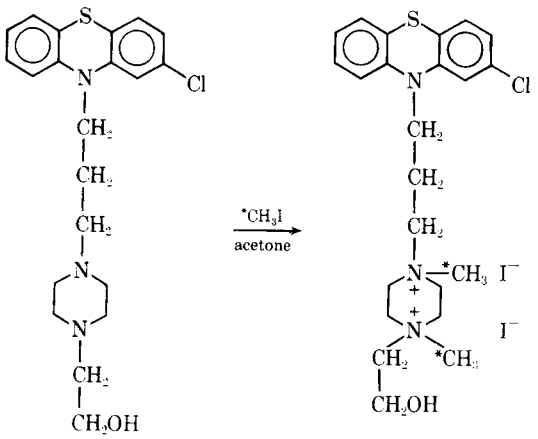
METHODS[1]

Synthesis of Perphenazine Dimethiodide (PPZ-DMEI)—Perphenazine dihydrochloride (0.53 g., 1.1 mmoles) was dissolved in water. The solution was adjusted to pH 10 and extracted several times with ether. The combined ether extracts were dried over anhydrous sodium sulfate and the solvent removed *in vacuo*. The oil (0.42 g., 0.97 mmole) remaining in the container was dissolved in 10 ml. of acetone, and methyl iodide (0.71 g., 5 mmoles) was added. The mixture was left at room temperature for 1 hr., and about 10 ml. of anhydrous ether was added to precipitate the quaternary ammonium salt. The precipitate was collected and recrystallized from methanol–ether to yield 0.36 g. (50%) of a white crystalline powder with m.p. 207–209°; $\lambda_{max.}$, 218 and 256 mμ; R_f = 0.50.

Anal.—Calcd. for $C_{23}H_{32}ClIN_3OS$: C, 40.12; H, 4.65; N, 6.10; S 4.65. Found: C, 40.38; H, 4.76; N, 6.11; S, 4.87.

Synthesis of Perphenazine Dimethiodide-^{14}C (PPZ-DMEI-^{14}C)—The free perphenazine base (121 mg., 0.3 mmole) was dissolved in 5 ml. of acetone, and 85.5 mg. (0.6 mmole) of ^{14}C-methyl iodide (11.7 μc./mg. in 5 ml. of acetone) was added. About 300 mg. of unlabeled methyl iodide was added as a carrier. The container was stoppered and left standing at room temperature for 24 hr. Then ether was added to precipitate the product. The crude material was recrystallized from ethanol–ether to yield 104 mg. (50%) of the final product with m.p. 207–209° and specific activity 3.27 μc./mg. Mixed melting point with PPZ-DMEI above did not show depression (207–209°). The authenticity and radiochemical purity of the product were established by paper chromatography coupled with a radiochromatogram scanner, Actigraph III. The physical properties of this compound were found to be identical with PPZ-DMEI. The scheme of the synthesis of this compound is shown in Scheme I.

[1] Melting points were taken in a Fisher-Johns apparatus and were corrected. UV absorption spectra were recorded on a Perkin-Elmer model 202 spectrophotometer. Elemental analysis was performed by Galbraith Laboratories. Paper chromatograms were developed in a solvent system, *n*-butanol–ethanol–water (5:2:2), and in the case of radiochromatogram, an Actigraph III (Nuclear-Chicago) was used to record the activity. Radioactivity in the tissues was recorded in a G-M counter (Tracerlab, model TGC-2), with the efficiency of 9%. All of the recordings were carried out at a constant geometry, and corrections for self-absorption were made for the thick-layer preparations.



Scheme I—Synthesis of Perphenazine Dimethiodide-^{14}C

Tissue Distribution Studies—PPZ-DMEI-^{14}C (1 mg.) was dissolved in 1 ml. of water and injected intraperitoneally into five Holtzman rats (one rat for each time period) weighing 175–250 g. The animals were sacrificed at various intervals of 0.5, 1, 2, 4, and 8 hr. after the injection. The liver, kidneys, spleen, heart, lungs, intestines, stomach, muscle, bone, and brain were isolated, rinsed with normal saline solution, and briefly dried, and their weights were recorded. The whole organ (except blood and bone) was homogenized with water 10 times its weight. An aliquot (0.5 ml.) was measured and evaporated to dryness in a planchet and the activity of the residue was recorded. The blood specimen (0.1 ml.) was measured and dried directly in a planchet, and a portion (0.1 g.) of the bone (femur, dried and ground to a powder) was placed in a planchet to record the activity.

Urinary and Fecal Excretion Studies—One milliliter of the aqueous solution of PPZ-DMEI-^{14}C (1 mg./ml.) was injected intraperitoneally into four Holtzman rats weighing 175–250 g. The animals were maintained in metabolic cages with free access to food and water. The urine and feces were collected separately every 8 hr. An aliquot (0.5 ml.) of the urine specimens was measured in a planchet and dried for recording activity. Since it was a thin-layer preparation, no correction for self-absorption was required. The feces were dried and powdered. A fraction (0.1 g.) was weighed out in a planchet to record the activity. Corrections for self-absorption were made for the preparation.

Biliary Excretion Studies—Holtzman rats weighing about 300 g. were anesthetized by a subcutaneous administration of 70 mg./kg. of pentobarbital sodium. An incision was made on the abdominal wall, and the common bile duct was cannulated with a polyethylene tube, PE50 (Clay-Adams). About 1 ml. of the test solution (1 mg./ml.) was placed directly into the intraperitoneal cavity and the incision was closed. Bile specimens were collected at the intervals of 0.5, 1, and 2 hr. All animals were sacrificed at the end of the 2-hr. period; intestines and urine in the bladder were collected and the activities were recorded.

Metabolic Studies—To study the *N*-demethylation *in vivo*, two rats were injected intraperitoneally with PPZ-DMEI-^{14}C and placed in a large metabolic jar with a constant flow of air to remove carbon dioxide produced by the animals. The air was passed through a gas washing bottle containing 40% sodium hydroxide solution. The carbon dioxide collected in the gas washing bottle was precipitated by adding 50% calcium chloride solution; the calcium carbonate thus formed was collected and dried and the activity was recorded.

Paper chromatographic technique was used to determine metabolites of PPZ-DMEI-^{14}C in urine and feces. Pooled urine (10 ml.) from three rats after the intraperitoneal administration of PPZ-DMEI-^{14}C was condensed to 1 ml. and centrifuged to remove solid precipitate. An aliquot of 0.5 ml. of the supernatant liquid was placed linearly on Whatman 3 MM paper, and the chromatogram was developed in the solvent system previously mentioned. A reference PPZ-DMEI-^{14}C was used as a control. The chromatogram was scanned in a radiochromatogram scanner, Actigraph III, and the R_f values of the corresponding spots were calculated.

Feces collected over a 3-day period after the administration of PPZ-DMEI-^{14}C were extracted with ether in a continuous extraction apparatus for 2 days to remove fatty substances. The ether extracts which did not show radioactivity nor color reaction with 50% sulfuric acid were discarded. The residue in the continuous extraction apparatus extractor was extracted with methanol for 3 days. The methanol extracts were evaporated to 1–2 ml. under reduced pressure, and an aliquot of 0.1 ml. was chromatographed on Whatman 3 MM paper and treated in the same manner described for the analysis of urinary metabolites.

Table I—Recovery of Radioactivity[a] from Urine and Feces of Four Rats after Intraperitoneal Administration of 1 mg. (1 ml.) of Aqueous Solution of Perphenazine Dimethiodide-^{14}C

Time, hr.	Urine, %	Feces, %
8	13.0 ± 4.05	0.03 ± 0.00
16	14.0 ± 3.01	0.03 ± 0.00
24	3.4 ± 0.60	0.48 ± 0.04
32	2.5 ± 0.21	9.12 ± 2.32
40	1.9 ± 0.33	0.60 ± 0.05
48	1.5 ± 0.20	0.44 ± 0.25
56	1.1 ± 0.07	0.20 ± 0.08
64	1.1 ± 0.10	0.12 ± 0.06
88	0.5 ± 0.85	2.4 ± 0.22
112	0.4 ± 0.01	0.96 ± 0.31

[a] Mean percent of the administered activity ± standard error.

Acute Toxicities—A series of dilutions of aqueous solution of PPZ-DMEI was administered intraperitoneally to a group of 10 male albino mice (Southern Animal Farms, Prattville, Ala.). Each animal was placed in an individual cage with free access to water. Acute toxicity was observed and the mortality recorded. LD_{50} was calculated according to the method of Litchfield and Wilcoxon (11).

Antimicrobial Activities—Antibacterial and antifungal activities of PPZ-DMEI were tested by using a diffusion method in which a short glass cylinder containing 0.3 ml. of the test solution was placed on a solid culture medium which was previously seeded with a test organism. After incubation at 37° for an optimum growth period for the organism, the diameter of the clear zone surrounding the glass cylinder was taken as the measure of the inhibitory effect of the drug against the organism. In this test, *Staphylococcus aureus* and *Escherichia coli* were chosen to represent a Gram-positive microorganism and a Gram-negative microorganism, respectively. Brain heart infusion agar (Baltimore Biological Laboratory, Baltimore, Md.) was used as the culture medium.

Saccharomyces carlbergensis and *Aspergillus niger* grown on Sabouraud dextrose agar medium were used in the test for antifungal activity.

RESULTS

In contrast to the excretion pattern of trifluoperazine methiodide-^{14}C, the major route of excretion of PPZ-DMEI-^{14}C was

Table II—Distribution of Radioactivity in Tissues of the Rat after Intraperitoneal Administration of Perphenazine Dimethiodide-^{14}C[a]

Organs	Time after Administration, hr. 0.5	1	2	4	8
Blood	19.84	13.84	12.54	6.64	0.64
Bone	2.42	4.21	6.06	6.20	6.70
Brain	0.08	0.08	0.14	0.11	0.15
Muscle	4.78	2.71	2.49	1.31	0.71
Lungs	0.71	0.59	0.65	0.56	0.25
Heart	0.20	0.14	0.13	0.08	0.02
Kidneys	6.13	12.18	14.53	20.93	19.93
Spleen	0.77	0.31	0.37	0.26	0.29
Stomach	1.16	0.44	0.51	0.31	0.35
Intestines	6.46	5.22	5.66	5.54	4.76
Liver	4.76	5.86	5.68	7.25	6.28
Urine	2.84	8.34	15.86	16.00	17.99
Abdominal washings	3.53	1.03	0.50	0.31	0.05
Total	53.60	54.95	65.12	65.50	58.12

[a] Expressed in terms of percent of the administered activity.

Table III—Distribution of Radioactivity in Tissues of the Rat after Intraperitoneal Administration of Perphenazine Dimethiodide-^{14}C[a]

Organs	Time after Administration, hr. 0.5	1	2	4	8
Blood	198.40	138.40	125.40	66.40	6.40
Bone	4.95	8.61	12.40	12.69	13.74
Brain	10.00	11.77	15.31	15.25	17.53
Muscle	21.72	12.40	11.31	5.96	3.24
Lungs	108.54	101.28	87.50	105.89	35.96
Heart	55.00	41.20	32.50	23.77	5.34
Kidneys	670.46	1475.90	1589.22	1978.83	1800.45
Spleen	224.58	91.22	92.50	90.13	90.38
Stomach	24.45	21.07	49.58	20.15	9.09
Intestines	94.20	69.37	83.94	115.02	72.36
Liver	117.32	138.75	132.53	173.33	167.76

[a] Expressed in terms of percent of specific activity of organ (c.p.m./g. of wet weight) against specific activity of whole body (administered activity/g. of body weight).

consistent with predominantly urinary excretion as indicated by the cumulative excretion data shown in Table I. About 55% of the administered activity was recovered during the 112-hr. period, of which 41% was found in the urine and only 14% in the feces. In all cases, urinary excretion was rapid; nearly 70% of the recovered activity appeared in the urine during the first 16-hr. period. However, fecal excretion was somewhat slow; that is, only a small amount of feces specimen was collected within the first 24-hr. period and a large amount of specimen was obtained between 24 and 32 hr. The slow fecal excretion was apparently due to the decreased intestinal motility associated with the sedative effect of the drug. The ratio of the activity between the urine and feces was 2.8:1.

The distribution pattern of radioactivity of this compound is presented in Table II. Radioactivity in the kidneys and liver prevailed over other organs 0.5 hr. after the administration. Rapid absorption of the compound was indicated by a rapid decline in the activity of the recovered abdominal washings, and it was further reflected by the high blood level with a peak at 0.5 hr. after the injection. The blood level decreased thereafter; however, the activity remained above the detectable level after 8 hr. The kidney level was almost parallel to the urinary level, which started to increase at 2 hr. and reached its peak at 4 hr. An average of 6% of the administered activity was found in the liver and 5% in the intestines during the 8-hr. period. The brain level was low but above the significant level. Activity in the brain accumulated slowly but steadily, reaching its peak at 8 hr. In other organs, the radioactivity was in the descending order of stomach, spleen, lungs, and heart during the 8-hr. period.

The overall recovery of the administered activity was between 54 and 66% in these animals. A considerable amount of the material apparently was retained in the fat and skin of the animals, because an average of 4.2 and 9.3% of the injected activity was found in the fat and skin tissues, respectively (digested with hyamine hydroxide and dried in a planchet or activity recording), of two rats (used in the excretion study) 6 days after the administration of PPZ-DMEI-^{14}C.

In contrast to chlorpromazine methiodide-^{14}C, only a low radioactivity (0.45% of the injected dose) of PPZ-DMEI-^{14}C was recovered in the bile collected from the common bile duct at 8 hr. This may be due to a weak affinity of this compound for the hepatic transport mechanism, because a compound which is not actively taken up by liver cells is not actively secreted into the bile through the liver. Activity in the intestinal content from the bile duct-cannulated animals was low (4%) but above the significant level, which suggested an active secretion of the compound to the intestinal lumen by the glands of the intestinal mucosa. Urinary excretion during the same period of time was 8% of the administered activity.

When these data were interpreted in terms of percent per unit wet weight of organs or tissues, a remarkable change in the ratio of activity between the organ was observed. The relative specific activity in the brain was higher than that in the muscles 2 hr. after the administration of the compound (Table III).

A comparative rate of biliary, intestinal, and urinary excretion of PPZ-DMEI-^{14}C and chlorpromazine methiodide-^{14}C in the rats 2 hr. after the intraperitoneal administration of these compounds is shown in Table IV.

The calcium carbonate, obtained by collecting the carbon dioxide produced by the rat after PPZ-DMEI-^{14}C administration, did not show a significant activity. This indicated that *N*-demethylation and degradation of this compound did not occur *in vivo*. However, this does not rule out the possibility that *N*-methyl exchange with neuroamines such as catecholamines might have occurred *in vivo*.

Paper chromatographic technique coupled with radiochromatogram scanner revealed that there were at least three metabolites present in the urine. One of the substances with R_f 0.50 was identified to be the unchanged PPZ-DMEI-^{14}C. A trace of the metabolite with R_f 0.66 was found to be its sulfoxide by cochromatography with an authentic perphenazine sulfoxide. Fecal metabolites are currently under investigation.

When LD_{50} (18.2 mg./kg.) was administered, the principal signs of toxicity were clonic and tonic convulsions; respiratory depression occurred between 5 and 30 min. after the intraperitoneal injection. At lower doses, respiratory depression as well as cyanosis was observed. Dosagewise, PPZ-DMEI-^{14}C (LD_{50}, 18.2 mg./kg., i.p.) appears to be more toxic than its parent compound, perphenazine (LD_{50}, 64 mg./kg., i.p.). On a molar basis, PPZ-DMEI-^{14}C has a higher toxicity than the quaternary methiodide of its analog, such as chlorpromazine and trifluoperazine.

PPZ-DMEI-^{14}C showed an inhibitory effect against the Gram-positive bacteria, *Escherichia coli*, at 10 γ/ml. No remarkable effect was observed on the yeast and fungus tested.

CONCLUSION

Intraperitoneally administered perphenazine dimethiodide-^{14}C was well absorbed by the rats. The majority (40%) of the drug was accumulated in the kidneys and was excreted in the urine; only 14% of the drug was excreted by the intestines. The ratio of the urinary to fecal excretion was 2.8:1. Peak blood level was observed 0.5 hr. after the administration of the drug. Perphenazine dimethiodide-^{14}C seemed to have a particular affinity for the bone. The activity in the bone started to rise in 0.5 hr. and reached its peak level in 1 hr. Brain level was low but above the detectable level at 0.5 hr.

Excretion of the activity of perphenazine dimethiodide-^{14}C was fairly rapid; almost 70% of the activity excreted in urine and feces was recovered in the first 32-hr. period. The quaternary ammonium salt of this compound has a toxicity higher than the parent compound, perphenazine. Antibacterial activities against Gram-positive and Gram-negative bacteria were demonstrated.

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Table IV—PPZ-DMEI-^{14}C and Chlorpromazine Methiodide-^{14}C Excretion

	Bile	Intestines	Urine
Perphenazine dimethiodide-^{14}C	0.45 ± 0.02[a]	4.10 ± 0.24	8.55 ± 1.10
Chlorpromazine methiodide-^{14}C	29.99 ± 5.88	10.09 ± 1.67	4.10 ± 0.94

[a] Percent of the administered activity ± *SE*.

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Dissolution of Slightly Soluble Powders under Sink Conditions I: Development of an Apparatus and Dissolution Studies of Salicylic Acid Powders

ISMAT ULLAH* and DONALD E. CADWALLADER†

Abstract □ A three-compartment apparatus was developed for dissolution studies of slightly soluble powders under sink conditions. The apparatus was designed to accommodate up to three phases to provide sink conditions. The apparatus could accommodate a barrier in the dissolution medium to prevent floating powders from entering and dissolving directly into the sink phase. Dissolution studies were conducted with several particle size grades of salicylic acid under nonsink as well as sink conditions. Effects of the rate of agitation and methods of introducing samples (dry or wetted suspensions) were also investigated. The data indicated that under diffusion-controlled rate of agitation, using the appropriate placement of propellers, it was possible to establish a rank order for *in vitro* dissolution times of different particle size salicylic acid powders.

Keyphrases □ Powders, slightly soluble—dissolution, sink conditions □ Particle-size effect—powder dissolution □ Diagram—powder dissolution apparatus □ UV spectrophotometry—analysis

In recent years, much attention has been focused on the problem of drug availability from solid dosage forms. The importance of *in vitro* dissolution-rate studies for solid dosage forms in determining the drug availability has been recognized (1–5), but it is now generally accepted that the *in vitro* results should be correlated to some physiologic parameter. It has been shown that unless appropriate sink conditions are maintained in certain cases, *in vitro* dissolution studies might bear little relationship to *in vivo* dissolution results (6).

Recently, several methods have been developed (3–17) to study the *in vitro* dissolution rates of drugs from solid dosage forms; however, most of these methods lack sink conditions. Only a few methods (6, 15) have been reported for dissolution studies under sink conditions, but these are not suitable for powders. Due to flotation and flocculation of slightly soluble powder, the determination of a rank order in the dissolution rates of slightly soluble powders is a problem even under nonsink conditions. Unless these floating floccules could be broken up, and the powders distributed in such a way that the relative surface areas of different particle-size powders would be available for dissolution, a rank order in the dissolution rates would not be possible. Finholt *et al.* (18, 19) encountered these problems in their attempt to study the effect of particle size on dissolution rates and while comparing the dissolution rates of powders with granules and tablets. Lin *et al.* (20) also found similar problems in the rank order in the dissolution rates of different particle-size powders.

Because certain properties of drug powders play an important part in their dissolution rates from the dosage forms, it is important that the dissolution behavior of powders be studied. An apparatus, which could give a rank order in the *in vitro* dissolution rates of powders and could also accommodate sink phases, would be of value in the development and evaluation of dosage forms where control of certain powder characteristics is important.

The objectives of this investigation were to develop an apparatus which could be used to carry out dissolution studies of slightly soluble powders under sink conditions, and to demonstrate the utility of this apparatus by obtaining an appropriate rank order in the dissolution rates of different particle-size powders of a model drug.

EXPERIMENTAL

Chemicals and Materials —The salicylic acid[1] used was USP grade. The different particle-size grades were obtained by sieving twice through Ro-Tap testing sieve shaker, using U. S. standard sieves. Isopropyl myristate[2] and polysorbate 80[3] were used. All other chemicals were reagent or certified ACS grade.

[1] Fisher Scientific Co.
[2] S. B. Penick, New York, NY 10008
[3] Atlas Chemical Industries, Inc., Wilmington, DE 19899