Comparative Distribution, Excretion, and Metabolism of ^{14}C-Labeled Quaternary Ammonium Compounds of Promazine, Chlorpromazine, and Triflupromazine

C. L. HUANG, J. Z. YEH, and I. A. MUNI

Abstract □ The tissue distribution and biotransformation patterns of the quaternary methiodides of promazine, chlorpromazine, and triflupromazine in rats were investigated. After parenteral administration, high concentrations of these compounds were found in the liver, kidneys, and intestines. The majority of these compounds was metabolized by the liver and excreted in the intestines *via* the bile duct. In contrast to the corresponding tertiary amine, the majority of these compounds was excreted unchanged; however, a trace of chlorpromazine sulfoxide methiodide was detected in the urine.

Keyphrases □ Promazine, chlorpromazine, triflupromazine ^{14}C-methiodides—synthesis □ Triflupromazine, chlorpromazine, promazine ^{14}C-methiodides—synthesis □ Biotransformation, distribution—quaternary phenothiazine compounds □ IR spectrophotometry—identity □ UV spectrophotometry—identity □ Scintillometry—analysis □ Radiochromatography—analysis

Molecular modification of phenothiazines with an aim to isolate sedative effect from antihistaminic activity has been contemplated by quaternization of the tertiary amino nitrogen. Albanus *et al.* (1) prepared promethazine hydroxyethyl ammonium chloride[1] and demonstrated that this compound had an antihistaminic activity similar to that of the parent compound but lacked sedative effect. Hanngren (2) reported that this compound caused a decrease in the volume and acidity of the gastric juice in human subjects by inhibiting the gastric basal secretion.

Levine *et al.* (3–5) studied the rate of absorption of monoquaternary ammonium compound of benzomethamine by using *in vivo* intestinal loops in rats. The data indicated that the quaternary ammonium salt was poorly absorbed by the gastrointestinal tract due to its high degree of ionization and the positive charge which promotes the formation of a nonabsorbable complex with the intestinal mucin. Levine stated that more than a passive diffusion was involved in the kinetics of intestinal absorption of benzomethamine and other onium compounds.

The rate of excretion of onium compounds is dependent upon the route of administration and the degree of ionization of the compound. By subcutaneous administration, some 7–37% of radioactivity was recovered in the urine during the same period of time. In a comparative study made by Hansson and Schmitterlöw (6) and Allgen *et al.* (7) on *in vivo* behavior of tertiary and quaternary promethazine, markedly different excretion patterns were found between these compounds. Both compounds were excreted mainly *via* the kidneys, and only 25% was found in the feces after subcutaneous administration. However, with oral administration, tertiary promethazine was excreted mainly in the urine, whereas the majority of the quaternary compound appeared in the feces.

Recently, distribution and excretion patterns of quaternary ammonium salts of mepazine, promethazine, trifluoperazine (8), and perphenazine (9) with one or two moieties of ^{14}C-methyl groups attached to the terminal nitrogen have been studied. The rate of absorption of the i.p. administered ^{14}C-methiodide of these compounds was rapid, as evidenced by the quick disappearance of radioactivity from the injected site and further reflected in fairly high blood levels with peaks, in most cases, at 0.5 hr. after the administration of the compounds. Contrary to the previous speculations of a high blood-brain barrier for these compounds, brain levels of the compounds were low but above the significant level. This finding may provide direct evidence as to why these compounds are active on the CNS.

It appears that the distribution and biotransformation pattern of quaternary phenothiazine compounds are quite different from those of the corresponding tertiary amino derivatives. The majority of the administered quaternary ammonium compounds is found in organs such as liver, intestines, kidneys, gastric mucosa, and pancreas; the majority of the tertiary amine derivatives is found in the lungs and brain. Tertiary phenothiazine derivatives are known to be metabolized *in vivo* largely through four pathways: sulfoxidation, hydroxylation, glucuronidation, and *N*-demethylation; however, quaternary derivatives undergo these metabolic processes to a much lesser extent.

Apparently, two types of major excretion patterns exist, the urinary and fecal types, for these methiodide derivatives. Perphenazine methiodide exhibited the former type of excretion, while methiodides of mepazine, promethazine, and trifluoperazine belonged to the latter category. The difference in the metabolic behavior between these compounds was apparently due to the difference in the affinity of these compounds for the liver cells; perphenazine methiodide could not enter into the active metabolic process of the liver, whereas other compounds actively participated in the excretory mechanism of the liver and thus were excreted in the intestines *via* the bile duct.

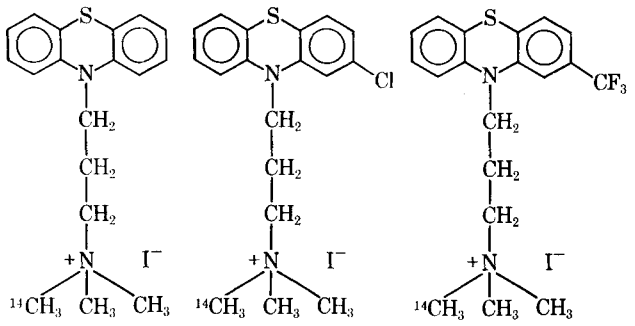
The toxicity of these phenothiazine derivatives did not diminish by quaternization, as evidenced by the fact that the LD_{50} of these compounds administered by the i.p. route was higher than that of the corresponding tertiary amino derivatives. Antimicrobial activities were also retained by these quaternary ammonium salts in that growth of *Staphylococcus aureus* and *Escherichia*

[1] Aprobit, Recit Co., Stockholm, Sweden.

coli was inhibited at the concentrations of 1 and 10 mcg./ml., respectively.

Review of the literature indicated that only a few quaternary ammonium compounds of phenothiazine derivatives were studied extensively in regard to their distribution, excretion, and metabolism in animals. It is the purpose of this study to compare the *in vivo* behavior of three quaternary compounds of promazine, chlorpromazine, and trifluprom azine which are similar in structure except for the functional group attached to the Position 2.

In this report, comparative studies on the tissue distribution and elimination of the methiodide of three structurally related phenothiazine derivatives—promazine, chlorpromazine, and trifluprom azine—are presented. These three compounds differ only by the presence of substituent groups at the Position 2 on the aromatic ring system (Structures I, II, and III).



promazine methiodide-^{14}C I

chlorpromazine methiodide-^{14}C II

trifluprom azine methiodide-^{14}C III

METHODS[2]

Synthesis of Promazine Methiodide (PRZ-MEI) and Promazine Methiodide-^{14}C (PRZ-MEI-^{14}C)—Promazine hydrochloride (0.5 g.) was dissolved in 5 ml. of water, and the solution was adjusted to pH 10 (pHydrion paper) and extracted several times with benzene. The benzene extracts were combined and the solvent removed under reduced pressure to leave an oil (0.42 g.). The oil was dissolved in 5 ml. of acetone, and 0.2 g. of methyl iodide was added. Crystallization commenced within 15 min. at room temperature. The crystals were collected and recrystallized from acetone to yield 0.57 g. (90%) of PRZ-MEI with m.p. 255–257° and R_f 0.71; $\lambda_{max.}$, 210 and 354 mμ.

Anal.—Calcd. for $C_{18}H_{23}IN_2S$: C, 50.70; H, 5.40; N, 6.57. Found: C, 50.80; H, 5.50; N, 6.70.

The free base of promazine (265 mg., 0.93 mmole) obtained was dissolved in 1.5 ml. of acetone and pipeted into the capillary guard of a breakseal tube which contained 85.5 mg. (0.60 mmole) of ^{14}C-methyl iodide (11.7 μc./mg.). The capillary seal was broken to allow 1 ml. (163 mg., 0.60 mmole) of the solution to enter the tube under negative pressure. The tube was stoppered immediately and allowed to stand overnight at room temperature. About 100 mg. of unlabeled methyl iodide was added to ensure a complete quaternization of the promazine base. Then ether was added to the mixture until turbidity occurred. Crystallization commenced within 15 min. The crystals were collected and washed with ether to yield 345 mg. (87%) of PMZ-MEI-^{14}C with m.p. 255–257° and specific activity 3.19 μc./mg. Radiochromatograms showed only one spot with an identical R_f (0.71) with PMZ-MEI, and mixed melting point with PMZ-MEI did not show depression (255–257°).

Synthesis of Chlorpromazine Methiodide (CPZ-MEI) and Chlorpromazine Methiodide-^{14}C (CPZ-MEI-^{14}C)—Chlorpromazine hydrochloride (0.5 g.) in 5 ml. of water was adjusted to pH 10 and extracted several times with benzene. The combined benzene extracts were dehydrated with anhydrous sodium sulfate and evaporated *in vacuo* to leave 0.43 g. of an oil. To the acetone solution (5 ml.) of the oil, 0.2 g. of methyl iodide was added. The mixture was shaken occasionally and left at room temperature overnight. Then ether was added to the mixture to precipitate the product. The precipitate was collected and recrystallized from acetone–ether to yield 0.49 g. (81%) of CPZ-MEI with m.p. 145–147°; $\lambda_{max.}$, 216 and 256 mμ.

Anal.—Calcd. for $C_{18}H_{22}ClIN_2S$: C, 46.91; H, 4.78; N, 6.08. Found: C, 47.23; H, 5.11; N, 5.96.

The oily free base of chlorpromazine (190 mg., 0.6 mmole) in 2 ml. of acetone was reacted with 85.5 mg. (0.6 mmole) of ^{14}C-methyl iodide (11.7 μc./mg.) as described for promazine. The reaction was allowed to proceed for 12 hr. at room temperature; then a suitable amount of ether was added to initiate crystallization. The crystals were collected and recrystallized from acetone–ether to yield 218 mg. (80%) of CPZ-MEI-^{14}C with m.p. 145–147° and specific activity 2.89 μc./mg.

The radiochemical purity and authenticity of this compound were checked by paper chromatography (R_f, 0.79) coupled with a radiochromatogram scanner. The mixed melting point of this compound with CPZ-MEI did not show depression (145–147°).

Synthesis of Trifluprom azine Methiodide (TFP-MEI) and Trifluprom azine Methiodide-^{14}C (TFP-MEI-^{14}C)—Trifluprom azine hydrochloride (0.5 g.) was dissolved in water, and the solution was adjusted to pH 10 by adding sodium hydroxide solution. The oily precipitate appearing in the mixture was extracted several times with ether. The combined ether extracts were dehydrated with anhydrous sodium sulfate and the solvent removed *in vacuo*. The free base of trifluprom azine (0.45 g.) was dissolved in 5 ml. of acetone, and methyl iodide (0.2 g.) was added. The mixture was allowed to stand at room temperature overnight; then a suitable amount of ether was added to initiate crystallization. The crystals were collected and recrystallized from acetone–ether to give 0.38 g. (60%) of TFP-MEI with m.p. 169–170° and R_f 0.81; $\lambda_{max.}$, 216 and 258 mμ.

Anal.—Calcd. for $C_{19}H_{22}F_3IN_2S$: C, 46.16; H, 4.49; N, 5.67. Found: C, 46.33; H, 4.48; N, 5.71.

To the free base of trifluprom azine (224 mg., 0.6 mmole) in 2 ml. of acetone was added ^{14}C-methyl iodide (11.7 μc./mg., 85.5 mg., 0.6 mmole). The reaction was allowed to proceed for 12 hr. at room temperature; then ether was added until crystallization commenced. The crystals were filtered off and recrystallized from acetone–ether to yield 188 mg. (60%) of TFP-MEI-^{14}C with m.p. 169–170° and specific activity 3.11 μc./mg. Radiochemical purity and authenticity of the product were checked by chromatography coupled with radiochromatogram scanner, mixed melting point (169–170°), and cochromatography (R_f 0.81) with an authentic sample of TFP-MEI.

Excretion Studies—In the excretion studies, 1 mg. each of the quaternary ammonium compounds of promazine (3.19 μc./mg.), chlorpromazine (2.89 μc./mg.), and trifluprom azine (3.11 μc./mg.) was injected i.p. to six albino rats weighing 250–280 g. The animals were maintained in metabolic cages with free access to food and water. The urine and feces specimens were collected separately every 8 hr. for a period of 5 days. Urine specimens were diluted five times with water; an aliquot of 0.5 ml. was measured in a planchet, dried, and the activity recorded. It was a thin-layer preparation and no correction for the self-absorption was required. Feces specimens were dried and powdered. An aliquot of 0.1 g. was placed in a planchet and the activity measured. Corrections were made for the self-absorption.

Tissue Distribution Studies—Three groups of albino rats (five rats in each group) weighing 250–280 g. were administered through the intraperitoneal route with 1 mg. each of PRZ-MEI-^{14}C, CPZ-MEI-^{14}C, and TFZ-MEI-^{14}C, respectively. The animals were housed in individual cages and were sacrificed at the following intervals: 0.5, 1, 2, 4, and 8 hr. The following organs and tissues were isolated: liver, lungs, kidneys, spleen, heart, stomach, intestines, blood, brain, muscle (femoral), and bone (femur). The surface of the intestines

[2] Melting points were taken on a Fisher-Johns apparatus and were corrected. UV and IR absorption spectra were recorded on a Perkin-Elmer model 202 and model 137 infracord spectrophotometer, respectively. Paper chromatograms were developed in a solvent system; *n*-butanol–ethanol–water (5:2:2). Albino rats were purchased from Southern Animal Farms, Prattville, Ala. Radioactivity in the tissues was recorded in a G-M counter (Tracerlab, model TGC-2), and radiochromatograms were scanned in a radiochromatogram scanner, Actigraph III (Nuclear-Chicago).

Table I—Urinary and Fecal Excretion of Quaternary ^{14}C-Methiodides of Promazine (PRZ), Chlorpromazine (CPZ), and Triflupromazine (TFZ)[a]

Time Intervals, hr.	Urinary Excretion			Fecal Excretion		
	PRZ	CPZ	TFP	PRZ	CPZ	TFZ
8	12.28	7.23	5.67	0.41	0.40	0.13
16	4.82	1.22	0.97	29.12	19.48	16.96
24	3.98	0.53	0.37	5.20	14.71	13.44
32	2.62	0.44	0.25	3.96	10.28	4.39
40	1.16	0.30	0.17	4.88	5.42	3.00
48	0.85	0.28	0.13	2.61	1.12	0.47
56	0.90	0.22	0.14	1.84	0.52	0.34
64	0.82	0.15	0.07	1.72	1.25	0.87
72	0.86	0.17	0.10	1.10	0.21	0.26
96	0.80	0.42	0.28	1.82	0.82	1.39
120	0.74	0.40	0.28	0.96	0.34	0.52

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

was rinsed with saline to remove mechanical contamination of the administered material, and the peritoneal cavity was washed several times with detergent to recover the unabsorbed portion of the activity. The whole organs, except for the blood and bone, were homogenized in a homogenizer and diluted with water to an extent that 16 ml. represented 1 g. of the organ. An aliquot of 150 mcg. of the dilution was measured in a planchet, dried, and the activity recorded.

Metabolic Studies—Since the ^{14}C-methyl group is attached to the nitrogen of the 10-substituted side chain of the phenothiazine ring system, it was essential to study its fate *in vivo*. In this study, three rats were injected i.p. with ^{14}C-methiodide (1 mg.) of these compounds. The animals were maintained in a large metabolic jar, and the expired air was passed through a washing bottle containing 40% sodium hydroxide solution for 24 hr. to collect carbon dioxide. The carbon dioxide trapped as sodium carbonate was treated with a calcium chloride solution; the precipitate of the calcium carbonate thus formed was collected and the activity determined.

A portion (usually 0.5 ml.) of the urine collected 24 hr. after the intraperitoneal administration of these compounds was placed linearly on Whatman No. 3 chromatographic paper along with radioactive standards. The chromatogram was developed by an ascending technique in the usual solvent system (10). The chromatogram was dried and scanned in a radiochromatogram scanner to record the activity, and the R_f value of the spot was calculated. Feces collected over a 3-day period were placed in a continuous extraction apparatus and extracted successively with ether for 2 days and then with methanol for 3 days. The methanol extracts were reduced to about 1 ml. *in vacuo*, and an aliquot of 0.1 ml. was placed linearly on Whatman No. 3 chromatographic paper along with the radioactive standard. The chromatogram was developed by an ascending technique in the solvent system described for the urine specimens. After the chromatogram was dried, the activity was recorded in a radiochromatogram scanner and the R_f value was calculated.

Synthesis of Methiodide of Chlorpromazine Sulfoxide—About 0.5 g. of chlorpromazine sulfoxide was dissolved in 5 ml. of acetone and reacted with 0.5 ml. of methyl iodide. The crystal appeared spontaneously. The compound was recrystallized from the acetone–ether mixture to a crystalline powder with m.p. 242–243°. The R_f value was 0.65. This material was used as a reference compound for the identification of metabolites of chlorpromazine methiodide.

Biliary Excretion Studies—Male Holtzman rats weighing about 300 g. were anesthetized by subcutaneous administration of 70 mg./kg. of pentobarbital sodium. Through an abdominal incision, the bile duct was cannulated with a polyethylene tube (PE50, Clay-Adams Inc.). About 1 ml. of the drug solution (1 mg./ml.) was dropped directly into the abdominal cavity before the incision was closed. Bile was collected at the intervals of 0.5, 1, and 2 hr. All animals were sacrificed at the end of 2 hr., and the entire intestines were removed and the radioactivity was recorded as described previously (*Tissue Distribution Studies*). The radioactivity in the urine and bile was determined according to the procedure described under the urinary excretion study.

RESULTS

Excretion of PRZ-MEI, CPZ-MEI, and TFP-MEI—Table I summarizes the urinary and fecal excretion of radioactivity by rats after a single intraperitoneal administration of PRZ-MEI-^{14}C, CPZ-MEI-^{14}C, and TFP-MEI-^{14}C. Total radioactivity recovered in the urine in 5 days was: PRZ-MEI, 29.83%; CPZ-MEI, 11.36%; and TFP-MEI, 8.43% of the administered radioactivity. The majority of the ^{14}C in the urine was recovered within the first 8 hr., and the first 24-hr. excretion accounted for 70–90% of the total urinary radioactivity recovered during the 5-day period. However, fecal excretion was the major route of excretion, which is approximately 2–6 times the urinary excretion for these compounds. The radioactivity in the feces was low in the first 8 hr., but the activity reached its peak in 16 hr. and declined slowly thereafter. Occasional fluctuation of radioactivity was observed which was due to the uneven fecal excretion by these animals. Total radioactivity recovered during the 5-day period was: PRZ-MEI, 53.62%; CPZ-MEI, 54.55%; and TFP-MEI, 51.37% of the administered activity. The ratio between the urinary and fecal excretion for PRZ-MEI, CPZ-MEI, and TFP-MEI was 1:1.8, 1:5, and 1:7.3, respectively. The combined radioactivity of PRZ-MEI recovered in the urine and feces was the highest (83.45% of the administered activity), followed by CPZ-MEI (65.91%) and TFP-MEI (59.80%). The difference in the rate of excretion in these compounds is apparently due to the different substituent groups attached to the Position 2.

Tissue Distribution of PRZ-MEI, CPZ-MEI, and TFP-MEI (Tables II and III)—The blood levels of these ^{14}C-compounds were generally low with peaks at 0.5 hr. The concentrations declined slowly after 1 hr. but remained significant until after 8 hr. Among the three compounds tested, PRZ-MEI showed the highest blood level. The brain level of PRZ-MEI showed an interesting trend of increasing radioactivity in contrast to the decreasing trend of the blood level during the 8-hr. period. However, the brain levels of CPZ-MEI and TFP-MEI were almost parallel to the blood levels. The brain levels declined after 0.5 hr. and were insignificant after 2 hr. In all cases, the intestines showed the highest radioactivity followed by the muscle, liver, and kidneys. Apparently, the liver was

Table II—Distribution of Radioactivity in the Tissues of Rats after Intraperitoneal Administration of Quaternary ^{14}C-Methiodides of Promazine (A), Chlorpromazine (B), and Triflupromazine (C)

Organs	0.5 hr.			1 hr.			2 hr.			4 hr.			8 hr.		
	A	B	C	A	B	C	A	B	C	A	B	C	A	B	C
Blood	1.36	0.36	0.33	1.04	0.32	0.26	0.65	0.29	0.20	0.51	0.16	0.09	0.37	0.03	0.07
Brain	0.04	0.01	0.01	0.06	0.01	0.01	0.09	<0.01	<0.01	0.16	<0.01	0.01	0.20	<0.01	<0.01
Heart	0.08	0.05	0.03	0.08	0.05	0.05	0.06	0.03	0.04	0.03	0.02	0.03	0.03	0.01	0.02
Intestines	9.63	18.28	15.76	16.15	25.97	24.58	21.37	26.61	28.66	30.26	33.54	46.27	39.69	50.91	44.02
Kidneys	1.76	1.37	0.95	1.98	0.60	0.69	1.33	0.40	0.87	0.97	0.38	0.35	0.65	0.13	0.28
Liver	6.24	8.38	9.50	10.36	6.43	7.08	7.92	3.20	6.03	6.10	2.50	2.63	4.21	1.12	1.73
Lungs	0.11	0.12	0.04	0.08	0.11	0.14	0.12	0.13	0.07	0.16	0.15	0.02	0.12	0.09	0.02
Muscle	8.38	0.67	3.83	3.47	3.07	1.85	2.78	2.08	1.50	2.40	1.37	1.00	1.89	0.40	0.32
Spleen	0.12	0.66	0.79	0.16	0.38	0.55	0.18	0.13	0.24	0.27	0.27	0.10	0.23	0.08	0.05
Stomach	0.59	0.68	1.71	1.12	0.74	1.13	1.98	1.06	1.37	1.14	0.43	0.88	1.61	0.32	0.68
Urine	0.41	2.13	2.50	0.78	3.07	4.35	1.72	5.67	5.07	4.63	6.98	5.76	11.63	12.04	6.46
Abdominal washings	9.32	4.67	6.54	6.68	1.51	1.96	3.12	0.40	0.60	2.86	0.16	0.10	1.38	0.06	0.05

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

Table III—Relative Activity[a] of the Intraperitoneally Administered Promazine Methiodide-^{14}C (A), Chlorpromazine Methiodide-^{14}C (B), and Triflupromazine Methiodide-^{14}C (C) in the Tissues of Rats

Organs	0.5 hr.			1 hr.			2 hr.			4 hr.			8 hr.		
	A	B	C	A	B	C	A	B	C	A	B	C	A	B	C
Blood	13.6	3.6	3.3	10.2	3.2	2.6	6.5	2.9	2.0	6.0	1.6	0.9	3.7	0.3	0.7
Brain	2.5	1.5	1.6	7.7	1.5	1.0	11.6	0.8	0.7	20.7	0.3	0.2	25.9	0.2	0.1
Heart	21.6	16.3	10.0	21.6	15.2	18.0	16.2	10.0	13.6	8.1	6.9	11.0	8.1	3.2	6.4
Intestines	99.3	282	220	166	389	420	220	436	433	312	446	723	409	699	676
Kidneys	152	176	143	172	86.0	96.9	116	48.0	118	83.8	50.0	43.1	56.6	16.0	38.4
Liver	116	229	276	194	178	219	148	84.0	176	113	58.1	68.7	78.4	30.1	43.1
Lungs	16.2	22.5	6.4	11.9	23.6	24.3	17.7	21.2	5.7	32.6	14.8	5.7	17.7	12.8	4.0
Muscle	38.2	3.0	8.5	15.7	13.9	4.1	12.7	9.5	2.5	10.9	6.3	1.4	8.5	—	1.0
Spleen	43.6	268	326	58.1	163	231	65.3	47.6	75.3	98.1	75.3	33.3	83.6	24.8	19.2
Stomach	73.3	48.8	92.7	140	30.8	107	247	58.6	140	142	22.1	84.9	201	41.1	65.3

[a] A value of 100 represents nonspecific tissue distribution. Relative activity = μc./g. tissue/(total activity/body weight) × 100.

the major organ that metabolized these compounds; it showed a peak level between 0.5 and 1 hr. and maintained a fairly high level throughout the 8-hr. period. The radioactivity accumulated in the intestines reflected the fact that heptobiliary excretion was the major route of elimination for these compounds. The overall radioactivity in the kidneys was lower than that in the liver; however, the specific activity of the kidneys was higher than that of the liver when the radioactivity was expressed in terms of percent specific activity (Table III). The kidney level had its peak at 0.5 to 1 hr. and then steadily declined. The increase of radioactivity in the urine recovered from the urinary bladder by autopsy coincided with the decrease in the kidney level. Abdominal washings were obtained to estimate the rate of absorption of these compounds after the intraperitoneal administration. The recovered radioactivity indicated that the rate of absorption of these compounds by rats was quite rapid; 4.67–9.32% of the administered activity remained in the abdominal cavity at 0.5 hr. but only 0.05–1.38% was recovered after 8 hr.

When the tissue distribution data were expressed in terms of relative activity in respect to the specific activity of the whole body, the distribution pattern of the activity in each organ remained the same. However, the relative specific activity of these organs changed (Table III). The specific activity of PRZ-MEI-^{14}C in the kidneys became the highest, followed by that in the liver at 0.5 hr. The specific activity of TFP-MEI-^{14}C in the spleen was higher than that in the liver and kidneys. A considerably high specific activity was found in the stomach, which had a higher level than that of the heart and lungs. The specific activity of these compounds in the intestines was 1–3 times higher than that of the whole body at 0.5 hr. and eventually it became 4–7 times at 8 hr.

Metabolic Studies—N-*Demethylation* in vivo—No radioactivity was detected in the carbon dioxide collected from animals administered with the ^{14}C-methiodide of promazine, chlorpromazine, and triflupromazine. This finding indicated that *N*-demethylation of the quaternary ammonium compounds did not occur *in vivo*.

Urinary Metabolites—A paper chromatogram of the urine specimens collected from animals given PRZ-MEI-^{14}C and TFP-MEI-^{14}C showed only one spot as indicated on the recording of a radiochromatogram scanner. The R_f values matched those of the corresponding original compounds, indicating that both compounds were excreted in the urine unchanged. The chromatogram of the urine specimens from the CPZ-MEI group of animals showed a trace of the second metabolite with an R_f value of 0.66. It was identified to be its sulfoxide by comparing the R_f value (0.66) and UV absorption spectrum (240 and 310 mμ) with those of the authentic specimen of chlorpromazine sulfoxide methiodide.

Fecal Metabolites—The ether extracts of the feces specimens from animals given the compounds appeared greasy and showed no radioactivity. Most of the radioactivity in the feces was found in methanol extracts; however, about 10–15% of the radioactivity was left in the residue after the methanol extraction. A paper chromatogram of the methanol extract showed only one spot, as indicated on the recording of a radiochromatogram scanner. The R_f values of the spot matched each of the original starting materials (PRZ-MEI, 0.73; CPZ-MEI, 0.78; and TFP-MEI, 0.81).

A study was conducted to establish the rate of fecal excretion of CPZ-MEI-^{14}C after various routes of administration. During the 5-day period, 55% of the administered activity was recovered in the feces after an intraperitoneal administration and about 57% by a subcutaneous route; after an oral route of administration, nearly 98% of the material was recovered within 48 hr. Thus, it was established that fecal excretion was the main route of elimination of CPZ-MEI irrespective of the route of administration. The ratio between the urinary and fecal excretion was as follows: intraperitoneal route, 1 : 5, subcutaneous route, 1 : 2.2; and oral route, 1 : 320. By parenteral routes of administration, usually a slight inflammation was observed at the site of injection.

DISCUSSION

Fecal excretion was the major route of elimination of the *N*-methiodide of promazine, chlorpromazine, and triflupromazine. A substituent group of either Cl or CF_3 at Position 2 on the promazine molecule caused a remarkable change in the rate of elimination of these compounds. The rate of absorption of these compounds after the intraperitoneal route of administration was rapid, as indicated by a rapid disappearance of the compounds from the site of injection. Urinary excretion represented about 8–30% of the administered activity, and 51–55% of the activity was found in the feces. The total excretion in the urine and feces combined was 60–83% of the administered dose in a 5-day period, and a slow excretion continued after 5 days. Apparently, a substituent group of Cl and CF_3 at Position 2 on the promazine molecule remarkably changed the rate of elimination of these compounds, as evidenced by the fact that the extent of excretion for PRZ-MEI (83%) was the highest, followed by CPZ-MEI (66%), with TFP-MEI (60%) the lowest.

By parenteral routes of intraperitoneal and subcutaneous administration of CPZ-MEI, the radioactivity recovered in the feces was 55% and 57%, respectively. However, because of the ionic nature of this compound, it was poorly absorbed by the stomach. Therefore, with oral administration, 98% of the compound was recovered in the feces, and urinary excretion represented only a trace of the compound (9).

Blood level of PRZ-MEI was the highest among the three compounds with a proportionally high brain level. It appeared that the blood-brain barrier is low for PRZ-MEI, which is contrary to the general concept that quaternary ammonium compounds have a high blood-brain barrier. However, the brain level of CPZ-MEI and TFP-MEI was very low and was insignificant against the background after 2 hr.

The majority of the i.p. administered compounds was metabolized by the liver and excreted in the feces. This metabolic route was confirmed by cannulating the common bile duct of animals previously treated with CPZ-MEI. The bile specimen collected in this manner was subjected to paper chromatographic analysis as described previously for urine specimens. A single spot with R_f 0.78 was identified to be the unchanged CPZ-MEI.

Apparently the bile excretion is the major source of radioactivity in the intestines. This is evidenced by the fact that when the common bile dust was ligatured, only a trace of radioactivity was detected in the intestines.

Except for the intestines, the liver and the kidneys are the major organs representing most of the activity. However, the specific activity in the spleen was considerably higher than in other organs.

The methyl groups attached to the terminal nitrogen are quite stable, as evidenced by the negative finding of the radioactivity in the expired air of the animals pretreated with the quaternary compounds. Chromatographic analyses indicated that the majority of the urinary and fecal metabolites was the unchanged drug, except for a metabolite of CPZ-MEI which was identified to be the sulfoxide of CPZ-MEI.

SUMMARY

1. The i.p. administered ^{14}C-methiodides of the three structurally related phenothiazine derivatives—promazine, chlorpromazine, and triflupromazine—were well absorbed by the rats.

2. Promazine methiodide showed higher blood and brain levels than the 2-chloro (chlorpromazine) and 2-trifluoromethyl (triflupromazine) substituted analogs.

3. Liver was the major organ which metabolized the drug and eliminated it to the intestines through biliary excretion.

4. The majority (51–55%) of the administered radioactivity was recovered in the feces, and urinary excretion represented 8–30% of the administered activity.

5. The methyl groups attached to the terminal nitrogen were stable and not demethylated in the metabolic process. No radioactivity was detected in the carbon dioxide collected from the expired air of the animals.

6. Paper chromatography revealed that chlorpromazine methiodide was metabolized to its sulfoxide, while promazine methiodide and triflupromazine methiodide were excreted unchanged.

REFERENCES

(1) L. Albanus, E. Hansson, and C. G. Schmitterlöw, *Acta Pharmacol. Toxicol.*, **18**, 105(1961).

(2) A. Hanngren, *ibid.*, **21**, 116(1964).

(3) R. M. Levine, M. R. Blair, and B. B. Clark, *J. Pharmacol. Exp. Ther.*, **114**, 78(1955).

(4) R. M. Levine and E. M. Pelikan, *ibid.*, **131**, 319(1961).

(5) R. M. Levine, *Pharmacologist*, **3**, 67(1961).

(6) E. Hansson and C. G. Schmitterlöw, *Arch. Int. Pharmacodyn. Ther.*, **131**, 309(1961).

(7) L. G. Allgen, L. Ekman, L. Reio, and S. Ullberg, *ibid.*, **126**, 1(1960).

(8) C. L. Huang and J. Z. Yeh, to be published.

(9) C. L. Huang, G. M. Mir, and J. Z. Yeh, reported at 115th Annual Meeting of American Pharmaceutical Association, Miami meeting, May 1968.

(10) C. L. Huang, *Int. J. Neuropharmacol.*, **6**, 1(1967).

ACKNOWLEDGMENTS AND ADDRESSES

Received December 18, 1969, from the *Department of Pharmacology, School of Pharmacy, University of Mississippi, University, MS 38677*

Accepted for publication February 26, 1970.

This work was supported in part by the Public Health Research Grant MH-11927 from the National Institute of Mental Health, Chevy Chase, Md.

Conformations of *erythro*- and *threo*-Dimethylacetylcholine Iodides in the Solid State

ELI SHEFTER*, PHYLLIS SACKMAN*, WILLIAM F. STEPHEN, Jr.†, and EDWARD E. SMISSMAN†

Abstract □ The structures of *erythro*- and *threo*-α,β-dimethylacetylcholine iodides were determined by X-ray crystallographic procedures. The two molecules have substantially different conformations. The conformation of the *threo*-compound appeared to be dominated by coulombic attraction between the carbonyl oxygen and the quaternary nitrogen group, while in the *erythro*-analog the acyloxy oxygen atom was involved in a similar intramolecular interaction.

Keyphrases □ *erythro*-α,β-Dimethylacetylcholine iodides—structure determinations □ *threo*-α,β-Dimethylacetylcholine iodides—structure determination □ Conformation, structural—*erythro*- and *threo*-α,β-dimethylacetylcholine □ X-ray crystallography—structure determination

Substitution of methyl groups on the α- and/or β-carbons of the acetylcholine molecule (ACh) has dramatic effects on both the muscarinic activity of the analog and the hydrolysis rate of the molecule in the presence of acetylcholinesterase (AChE) (1, 2). Pharmacological studies (2) on the *erythro*(±)- and *threo*(±)-α,β-dimethylacetylcholine compounds indicated that the racemic *erythro*-material is over 300 times more potent as a muscarinic agent than the racemic *threo*-compound. However, *erythro*(±)-dimethylacetylcholine has approximately one-tenth the activity of ACh. When relative rates of hydrolysis by AChE of the two molecules are compared, a reverse situation is found, *i.e.*, the *threo*(±)-material is hydrolyzed at approximately one-tenth the rate of ACh and the *erythro*(±)-analog is negligibly hydrolyzed and possibly acts as an antagonist. In consideration of these results, it was deemed worthwhile to carry out structural studies on these molecules to learn to what extent their electronic and steric features might account for their observed properties. The crystal structures of the *erythro*- and *threo*-compounds are reported in this article.

EXPERIMENTAL

The iodide salts of the racemic mixtures of the two compounds crystallized as prisms from ethanol–ether (for *erythro*-material) and ethanol–benzene (for *threo*-compound) solutions. The individual *threo*-crystals were found to be optically active, and the crystal chosen for the X-ray study was found to contain the α(R)β(R) enantiomer. The crystal data for these compounds are:

erythro-		*threo*-
7.166 (2) Å	*a*	7.592 (3) Å
14.715 (5) Å	*b*	13.229 (4) Å
11.802 (3) Å	*c*	13.322 (3) Å
99.38 (3)°	β	
1.61 g./cm.^{3}	Density meas.	1.54 g./cm.^{3}
1.617 g./cm.^{3}	Density calcd.	1.495 g./cm.^{3}
$P\,2_1/c$	Space group	$P\,2_12_12_1$