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Excretion Patterns of Phenothiazine-S^{35} Compounds in Rats

Effect of Change in Structure on Metabolism

By THOMAS L. FLANAGAN, JACK H. NEWMAN, ALFRED R. MAASS, and EDWARD J. VAN LOON

The excretion patterns of five S^{35}-labeled phenothiazines were compared in rats following the oral administration of pharmacologically active doses. Substitution in the 2 position in the phenothiazine ring did not have any apparent effect upon the S^{35} excretion pattern. Differences in the structure of the side chain did have an effect upon the mode of S^{35} excretion. The urinary S^{35} excretion decreased and the fecal S^{35} excretion correspondingly increased as the side-chain structure was changed from 3-dimethylaminopropyl to 3-dimethylamino-2-methylpropyl to 4-methyl-1-piperazinylpropyl. At a dosage level of 9.0 mg. of free base/Kg. in rats, the urinary S^{35} excretion following the oral administration of compound II [2-chloro-10-(3-dimethylaminopropyl)phenothiazine-S^{35} hydrochloride] was twice as great as the urinary S^{35} excretion following the oral administration of compound III [10-[3-(4-methyl-1-piperazinyl)-propyl]-2-trifluoromethylphenothiazine-S^{35} dihydrochloride]. The former compound contained a chlorine atom in the 2 position of the phenothiazine nucleus, while the latter contained a trifluoromethyl grouping at this position. The increased dosage level (9.0 mg. of free base/Kg.) did not appreciably change the S^{35} excretion pattern of compound IV [10-(3-dimethylamino-2-methylpropyl)phenothiazine-S^{35} tartrate] and compound V [10-(3-dimethylamino-2-methylpropyl)-2-trifluoromethylphenothiazine-S^{35} hydrochloride].

ALTHOUGH there are reports in the literature on the urinary excretion pattern of chlorpromazine [2-chloro-10-(3-dimethylaminopropyl)-phenothiazine hydrochloride] there are few comparative studies of the excretion patterns of various phenothiazines. Christensen and Wase (1), using mice as the experimental animals, were the first to report on the urinary and fecal excretion of chlorpromazine-S^{35}. In a preliminary communication Fyodorov and Shnol (2) reported that, following the oral administration of chlorpromazine-S^{35} (aminazine-S^{35}) to rats, 16–17% of the radioactivity was excreted in the urine while approximately 76% was excreted in the feces. In other communications Fyodorov (3, 4) reported on the fate of three phenothiazine compounds in the organism: aminazine-S^{35}, promazine-S^{35}, and chlormeprazine-S^{35}. In his studies with aminazine-S^{35}, Fyodorov found a high concentration of radioactivity in the bile following oral administration. He reported (5) that a large part of the aminazine did not enter into the general blood stream but circulates in a closed circuit: intestine, portal vein, liver, bile, and back to the intestine being eliminated gradually in the feces.

In our laboratories it was shown that, following the intraduodenal administration of chlorpromazine to anesthetized dogs, the bile contained a high percentage of the drug and its metabolites (6). In similar experiments in dogs with three S^{35}-labeled phenothiazines, it was found that the phenothiazines were excreted in large but varying amounts *via* the biliary route. In light of these results it was decided to study the excretion patterns of five phenothiazine-S^{35} compounds in normal rats to see what effect changes in nucleus substitution and/or in the side chain would have upon urinary and fecal excretion following oral administration. Because of the large biliary excretion of the phenothiazines, it was recognized that the fecal pattern of each drug would not be a measure of unabsorbed drug but rather an indication of the amount of phenothiazine entering into the closed circuit system.

The structures of the phenothiazine-S^{35} compounds utilized are shown in Fig. 1.

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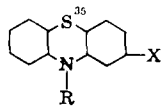

	R =	X =	Salt
Compound I:	$-CH_2-CH_2-CH_2-N(CH_3)_2$	—Cl	·HCl
Compound II:	$-CH_2-CH_2-CH_2-N\langle\rangle N-CH_3$	—Cl	·2HOOCCH=CHCOOH
Compound III:	$-CH_2-CH_2-CH_2-N\langle\rangle N-CH_3$	—CF_3	·2HCl
Compound IV:	$-CH_2-CH(CH_3)-CH_2-N(CH_3)_2$	—H	·½ $C_4H_6O_6$
Compound V:	$-CH_2-CH(CH_3)-CH_2-N(CH_3)_2$	—CF_3	·HCl

I = 2-Chloro-10-(3-dimethylaminopropyl)phenothiazine-S^{35} hydrochloride
II = 2-Chloro-10-[3-(4-methyl-1-piperazinyl)-propyl]phenothiazine-S^{35} dimaleate
III = 10-[3-(4-Methyl-1-piperazinyl)-propyl]-2-trifluoromethylphenothiazine-S^{35} dihydrochloride
IV = 10-(3-Dimethylamino-2-methylpropyl)phenothiazine-S^{35} tartrate
V = 10-(3-Dimethylamino-2-methylpropyl)-2-trifluoromethylphenothiazine-S^{35} hydrochloride

Fig. 1.—Structures and nomenclature of phenothiazine-S^{35} compounds used in the studies.

EXPERIMENTAL

Preparation of S^{35} Phenothiazines

The phenothiazine-S^{35} compounds were prepared in this laboratory by Dr. D. W. Blackburn (7).

Dosage

Study 1.—Five groups of male, albino rats, 10 rats per group, averaging between 250 to 300 Gm., were used. Each group received a single oral dose of a solution of a labeled drug by stomach tube. In the initial study the drugs were administered at dosage levels (Table I) based upon the pharmacologically active dose, *i.e.*, the dose that blocks avoidance response. The rats were fasted overnight prior to drug administration. Immediately following drug administration, each animal was placed in an individual metabolism cage. Food and water were allowed *ad libitum*. Urine and fecal collections were made at 24-hour intervals for 96 hours. Each day the animals were transferred to clean cages.

Study 2.—The purpose of the second study was to compare the excretion pattern of phenothiazine-S^{35} compounds in nonfasted rats with that of fasted rats. Three of the labeled drugs were administered by stomach tube to groups of nonfasted rats at the same dosage levels as study 1. After administration, the study was completed as described under study 1. However, only the radioactivity of the urine specimens was determined.

Study 3.—To compare the effect of drug administration at the same dosage level upon excretion, the five labeled phenothiazines were administered by stomach tube to groups of 10 rats at a dosage level of 9.0 mg. of free base content of drug per Kg. of body weight. The animals were not fasted and the experiment was completed as described in study 2.

Oxidation and Counting Procedures

The urine specimens were made to volume and a suitable aliquot was counted in a Packard Tricarb liquid scintillation spectrometer, model 314 X, using a phosphor system which contained 80 Gm. of naphthalene and 5 Gm. of 2,5-diphenyl-oxazole dissolved in a mixture of toluene, 1,4-dioxane, and absolute alcohol (1:1:0.6) in a 1 L. volume.

Each 24-hour fecal collection was placed in a 100-ml. Kjeldahl flask containing 4–5 glass beads and 50 ml. of 8 *N* nitric acid. The fecal samples were soaked overnight and then digested to remove all carbonaceous material. The samples were evaporated to 5 ml. or less and cooled to room temperature. Nine milliliters of a digestion mixture consisting of 1:1 8 *N* nitric acid and perchloric acid (60% Baker's analyzed reagent) was added to each sample and digestion continued until bumping occurred. If the digest was not clear, an additional 3 ml. of the digestion mixture was added and the digestion continued until a clear solution was obtained.

After cooling, the contents of the flasks were transferred to 150-ml. beakers and the pH of the solutions adjusted to 3.0 with alkali. The $S^{35}O_4$

TABLE I.—URINARY AND FECAL EXCRETION OF RADIOACTIVITY FOLLOWING A PHARMACOLOGICALLY ACTIVE DOSE OF DRUG

Compound	Dosage, (mg./Kg.)	Fasted or Nonfasted	Urinary Extraction of Radioactivity: % of Dose Excreted 0–24 hr. Range	0–24 hr. Av.	24–48 hr. Range	24–48 hr. Av.	48–72 hr. Range	48–72 hr. Av.	72–96 hr. Range	72–96 hr. Av.	Total Range	Total Av.
I	10	F	24.2–48.3	31.6	3.2–12.6	5.7	0.4–1.4	0.9	0.1–0.3	0.2	32.2–52.2	38.4
II	5	F	6.7–13.9	10.4	0.7–3.0	1.6	0.4–1.4	0.7	0–0.7	0.4	8.6–16.7	13.1
III	1.25	F	5.5–14.2	8.3	0.4–1.0	0.7	0.2–0.3	0.2	0.02–0.10	0.08	6.2–15.3	9.3
IV	5	F	14.7–31.5	24.3	0.6–6.4	2.3	0.3–1.2	0.5	0.02–0.20	0.10	16.9–37.8	27.2
V	2.5	F	13.0–21.3	17.9	1.1–1.7	1.4	0.4–0.8	0.6	0.2	0.2	14.7–23.4	20.1
I	10	N	28.1–39.3	34.1	3.5–5.5	4.6	1.8–3.2	2.4	1.3–2.0	1.6	37.1–49.3	42.8
III	1.25	N	7.3–11.6	8.8	1.1–2.2	1.5	0.3–0.54	0.46	0.14–0.36	0.27	9.8–14.1	11.1
V	2.5	N	13.1–23.4	18.6	1.2–2.6	2.1	0.4–0.7	0.6	0.19–0.29	0.24	14.9–26.3	21.5
			Fecal Excretion of Radioactivity									
I	10	F	20.6–43.2	28.4	13.2–32.2	21.6	2.3–6.0	3.9	0.4–1.1	0.6	50.9–59.1	54.5
II	5	F	11.0–86.4	66.8	3.1–70.2	17.8	1.3–20.1	4.8	0.6–2.0	1.1	85.1–95.9	90.5
III	1.25	F	52.6–78.6	71.8	4.5–31.9	12.9	1.7–3.5	2.3	0.7–1.2	0.9	79.5–93.4	87.9
IV	5	F	42.5–76.5	60.1	3.0–18.7	9.2	0.6–5.2	1.6	0.3–1.5	0.5	60.5–80.6	71.4
V	2.5	F	54.7–75.7	61.9	11.2–19.0	14.9	1.9–5.6	3.6	0.9–1.6	1.2	77.4–89.7	81.6

TABLE II.—URINARY EXCRETION OF RADIOACTIVITY FOLLOWING THE ADMINISTRATION OF THE SAME DOSE OF FREE BASE TO NONFASTED RATS

Compound	Dosage: As Salt	Dosage: As Free Base	% of Dose Excreted 0–24 hr. Range	0–24 hr. Av.	24–48 hr. Range	24–48 hr. Av.	48–72 hr. Range	48–72 hr. Av.	72–96 hr. Range	72–96 hr. Av.	Total Range	Total Av.
I	10	9	28.1–39.3	34.1	3.5–5.5	4.6	1.8–3.2	2.4	1.3–2.0	1.6	37.1–49.3	42.0
II	14.6	9	14.7–28.0	20.6	1.5–5.2	3.2	0.7–3.3	1.8	0.2–2.7	1.3	19.1–39.1	27.0
III	10.6	9	9.4–12.5	10.8	1.0–2.1	1.4	0.26–0.48	0.36	0.13–0.24	0.17	11.4–15.4	12.8
IV	11.2	9	25.8–37.2	30.3	1.7–3.9	2.4	0.20–0.48	0.33	0.09–0.30	0.14	27.8–41.9	33.1
V	9.9	9	19.9–22.7	21.2	2.0–5.0	3.4	0.41–0.82	0.55	0.11–0.35	0.19	23.7–27.2	25.4

was precipitated as $BaS^{35}O_4$ with 5% barium chloride solution in the usual manner. Subsequently the precipitates were filtered, dried, weighed, and counted on a gas flow counter having a micromil window (Nuclear-Chicago 192). The samples were corrected to infinite thinness. In all the studies only total radioactivity was measured. As stated previously, fasted and nonfasted rats were used in separate experiments.

DISCUSSION AND RESULTS

The urinary and fecal S^{35} excretion values obtained on the experimental animals following the administration of a pharmacologically active dose of each drug are given in Table I. The urinary S^{35} excretion in fasted and nonfasted animals was similar for each compound following the adminstration of compounds I, III, and V (Table I). However, slightly higher recoveries were obtained in the nonfasted animals over a 96-hour collection period. In the first two studies in which the animals were administered the pharmacologically active dose of drug, the average urinary recoveries of the S^{35} compounds fell into three ranges: (*a*) The highest S^{35} average recovery (38–43%) was obtained from compound I which had a dimethylaminopropyl side chain. (*b*) The lowest average urinary S^{35} recoveries, 13 and 10%, were obtained with compounds II and III, respectively, which have a 4-methyl-1-piperazinyl propyl side chain. (*c*) The urinary S^{35} excretion of the two compounds which have a dimethylamino-2-methylpropyl side chain, compounds IV and V, was intermediate, 27 and 20%, respectively.

In the comparison of compounds II with III and IV with V, the data show that the S^{35} compound which was administered at the higher dosage level (Table I) gave the higher S^{35} concentration in urine. Because of the difference in dosage levels, no attempts were made to correlate the differences in the urinary S^{35} excretion based upon substitution in the 2 position of the phenothiazine ring.

The fecal S^{35} excretion patterns also fell into three ranges as shown in Table I, and they were exactly opposite to the urinary S^{35} patterns. The fecal excretion of compounds IV and V was 82 and 71%, respectively. This 11% difference was significant at the $P = 0.01$ level ($t = 4.15$). However, no correlation was made of this difference because there was a difference in dosage level, and the respective compounds were excreted to the extent of 62 and 60% in the first 24 hours.

When the fecal S^{35} excretion data were compared from the two sets of compounds which have an identical ring structure and differ only in substitution in the side chain, compounds I with II and compounds V with III, it was seen that the two compounds which contain a 4-methyl-1-piperazinyl propyl side chain (II and III) were primarily excreted in the feces.

In the third study the five phenothiazines were administered at the same dosage level of free base (9 mg./Kg.) to nonfasted rats. When the dose of compound II was increased from 5 mg. of compound/Kg. to 14.6 mg./Kg. (equivalent to 3.1 and 9.0 mg. of free base/Kg., respectively), the urinary S^{35} excretion was increased from 13 to 27% based

upon the administered dose of drug. A threefold increase in dose caused a twofold increase in urinary S^{35} output (Table II). An eightfold increase in the dose of compound III caused only a slight increase in the urinary S^{35} excretion of this compound.

The difference in structure between compounds II and III other than the salt form is in the substitution in the 2 position in the phenothiazine nucleus; compound II contains a chlorine atom, while compound III contains the trifluoromethyl grouping. This was the only case in which substitution in the 2 position of the nucleus appeared to have an effect upon the excretion pattern.

Although the dosages of compounds IV and V were increased two- and fourfold, respectively, the urinary S^{35} excretion of these compounds was increased only slightly (Table II). The increase in urinary S^{35} excretion following the increase in dose of compound II was similar to that obtained with promazine-S^{35}, 10-(3-dimethylaminopropyl) phenothiazine hydrochloride. Fyodorov (3), following the oral administration of promazine-S^{35} to rats at dosage levels of 12–20 mg./Kg., reported that the urine contained 40% of the administered S^{35} activity. At dosage levels of 50 mg./Kg. of promazine-S^{35} to rats intragastrically by stomach tube, Walkenstein and Seifter (8) reported that approximately 65% of the S^{35} dose was excreted in the urine. Compound I (chlorpromazine-S^{35}) was administered at only one dosage level due to a limited supply of this compound.

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Relation of Cathartic Activity to Structural Modifications of Ricinoleic Acid of Castor Oil

By M. S. MASRI, L. A. GOLDBLATT, F. DeEDS, and G. O. KOHLER

Castor oil and certain of its derivatives were tested in rats to elucidate the specific structural configuration of castor oil responsible for its cathartic action. The results indicate that the hydroxyl function on carbon-12 and the double bond between carbons 9 and 10 of the ricinoleic acid moiety are essential for the cathartic action, as evidenced by the loss of activity upon either masking of the hydroxyl group or hydrogenation of the double bond. On the other hand, the naturally occurring *cis* configuration of the double bond is not essential for this action, as evidenced by the retention of the activity upon elaidinization to the *trans* configuration.

CASTOR OIL, which is obtained from the seeds of *Ricinus communis*, consists mainly of the triglycerides of an unsaturated hydroxy fatty acid, ricinoleic acid, having the formula $C_{17}H_{32}$-(OH)COOH. Castor oil acts as a cathartic for man although considerable amounts may be absorbed and utilized when fed as part of the diet of many animals. Paul and McCay (1) reported 92.1% utilization by rabbits and 99% utilization by sheep when castor oil was fed to the extent of 6%. Stewart and Sinclair (2) fed adult rats a diet containing 48.4% castor oil and found that the oil was readily metabolized with no evidence of catharsis. Perkins, *et al.* (3), recently reported that weanling rats fed 10% triricinolein "gained as much weight as those fed corn oil (10%) diets" and found (private communication) no indication of catharsis.

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Since ricinoleic acid differs from oleic acid, generally considered to be the most plentiful as well as the most widely distributed of all fatty acids in natural fats, only in having a hydroxyl group at carbon-12, it was of interest to investigate the effect of chemical modifications of the functional groups of ricinoleic acid upon the cathartic activity. The modifications planned included esterification of the hydroxyl group, hydrogenation of the double bond, and elaidinization, that is, conversion of the naturally occurring *cis* to the *trans* isomer. Materials tested included castor oil and the hydrogenated, elaidinized and acetylated oil. Castor oil, although it is predominantly the triglyceride of ricinoleic acid, is not a single chemical compound. Therefore, the investigation was extended to include highly purified methyl ricinoleate and its elaidinized, hydrogenated, and acetylated derivatives. The structural formulas for these four compounds are given in Fig. 1.

EXPERIMENTAL

Material and methods.—Castor oil grade AA,