Hepatic Secretion and Urinary Excretion of Three S^{35}-Labeled Phenothiazines in the Dog

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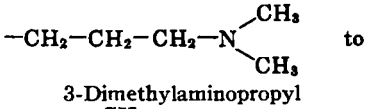
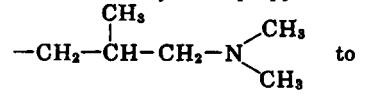
The present studies indicate that in the dog chlorpromazine, prochlorperazine, and trifluoperazine are rapidly absorbed from the intestine and that there is a rapid hepatic clearance of the phenothiazines. The biliary and urinary excretions of these compounds are greatly affected by the side chain of the phenothiazine nucleus. The "methylpiperazinylpropyl" side chain (prochlorperazine and trifluoperazine) markedly increases the rate of biliary excretion and reduces the urinary excretion of the parent compounds and/or their metabolites compared with the excretion of chlorpromazine and/or its metabolites (dimethylaminopropyl side chain). Moreover, chlorpromazine and/or its metabolites excreted in bile are reabsorbed from the intestine, resulting in an entero-hepatic recirculation.

FYODOROV REPORTED (1–3) a high degree of radioactivity in the bile of dogs and rabbits following the administration of chlorpromazine-S^{35} [1] and stated that, following oral administration (2), a large part of the chlorpromazine-S^{35} does not enter into the general blood stream but circulates in a closed circuit—intestine, portal vein, liver, bile, and back to the intestine, gradually being eliminated in the feces. Flanagan *et al.* (4) have reported on the biliary and urinary excretion patterns of chlorpromazine in the dog following intraduodenal administration. In excretion studies in rats using five S^{35}-labeled phenothiazines (5), the effect of change in structure of the side chain upon the mode of S^{35} excretion has been reported. The urinary S^{35} excretion decreased, and the fecal S^{35} excretion correspondingly increased as the side chain was changed from



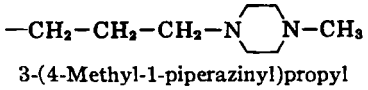
to

3-Dimethylaminopropyl



to

3-Dimethylamino-2-methylpropyl



3-(4-Methyl-1-piperazinyl)propyl

Three S^{35}-labeled phenothiazines were used in the present studies to show: (*a*) that phenothiazine drugs are readily absorbed from the intestinal tract, (*b*) that the side chain structure attached to the phenothiazine nucleus has a definite effect upon the route of excretion, and (*c*) that there is a rapid biliary recirculation of chlorpromazine and/or its metabolites.

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[1] Aminazine-S^{35}.

EXPERIMENTAL

Preparation of S^{35} Phenothiazines.—The three phenothiazine-S^{35} compounds used in the present studies were prepared by Dr. D. W. Blackburn (6). The structures of these compounds are shown in Table I.

Operative Procedure.—Mongrel female dogs, weighing 10 ± 0.2 Kg., which had been fasted for 16 hours were used in these studies. The operative procedure to insure collection of hepatic bile only was described previously (4). Urine specimens were obtained by catheterization.

Each drug was administered at a dosage level known to produce in dogs pharmacological responses, such as depression, sedation, and antiemetic activity.

In the chlorpromazine-S^{35} experiments, three dogs received only the operative intraduodenal dose—200 mg. of the labeled drug contained in 20 ml. of isotonic saline. To discover if priming had an effect upon excretion, a fourth dog was treated daily for 9 days preoperatively with 100 mg. of unlabeled chlorpromazine administered orally. On the tenth day, this dog received 200 mg. of labeled drug at the the time of operation. Bile and urine specimens were collected at 2-hour intervals for 10 hours throughout the course of each experiment.

Venous blood samples were withdrawn periodically from two unprimed dogs throughout each experiment. In the third study with an unprimed dog, periodic samples of blood were obtained from the portal, hepatic, and jugular veins in an attempt to show absorption (portal), liver clearance (hepatic), and circulation (jugular) of chlorpromazine. Cannulae were inserted into the three veins, and simultaneous blood samples were withdrawn at designated time periods. Blood specimens were not obtained from the primed dog. All bile, urine, and blood samples were analyzed for total S^{35} activity.

In another experiment, the bile collected from two of the operated dogs which had received 200 mg. of chlorpromazine-S^{35} intraduodenally was combined and made to 25 ml. volume with saline. An aliquot was assayed for S^{35} content. The remainder (20 ml.) was instilled into the duodenum of a third dog which had received no previous phenothiazine but

TABLE I.—STRUCTURES OF THE THREE COMPOUNDS

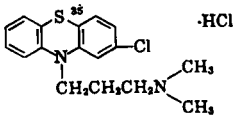
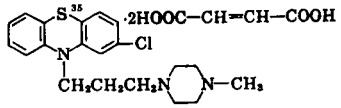
Compd.	Name	Structure
2-Chloro-10-(3-dimethylaminopropyl)phenothiazine-S^{35} hydrochloride	Chlorpromazine	
2-Chloro-10-[3-(4-methyl-1-piperazinyl)propyl]phenothiazine-S^{35} dimaleate	Prochlorperazine	
10-[3-(4-Methyl-1-piperazinyl)propyl]-2-trifluoromethylphenothiazine-S^{35} dihydrochloride	Trifluoperazine	

TABLE II.—BILIARY AND URINARY EXCRETION OF CHLORPROMAZINE-S^{35} EXPRESSED AS PER CENT OF ADMINISTERED DRUG (OPERATIVE DOSE, 200 mg.)

Collection Periods, Hr.	Dog 1[a]		Dog 2[a]		Dog 3[a]		Dog 4[b]	
	Bile	Urine	Bile	Urine	Bile	Urine	Bile	Urine
0–2	3.9	2.0	2.1	1.1	10.1	2.3	1.8	1.7
2–4	6.1	3.7	3.0	3.4	7.7	5.7	5.9	4.3
4–6	3.8	4.6	3.2	5.0	6.0	4.5	5.7	4.9
6–8	3.2	4.5	3.3	4.8	3.7	2.6	5.0	3.8
8–10	3.1	3.4	2.9	2.7	3.6	2.0	4.9	4.4
Total	20.1	18.2	14.5	17.0	31.1	17.1	23.3	19.1

[a] Single operative dose. [b] Primed for 9 days with "cold" chlorpromazine (100 mg./day) prior to receiving operative dose.

which had been prepared for quantitative collection of hepatic bile and urine. Bile and urine were then collected from this animal for a 10-hour period and assayed for S^{35} activity. Any S^{35} appearing in the collected bile or urine would necessarily arise from the intestinal reabsorption of the administered labeled chlorpromazine and/or its metabolites.

In the experiments with prochlorperazine-S^{35}, 100 mg. of the labeled drug dissolved in saline was administered intraduodenally to three dogs. Bile and urine specimens were obtained from each animal. Portal, hepatic, and venous blood specimens were obtained simultaneously from one dog. Blood specimens were not taken from the other animals.

TABLE III.—HEPATIC RECIRCULATION OF CHLORPROMAZINE-S^{35}. PER CENT OF RECOVERY OF S^{35} BILE COLLECTED FROM A CHLORPROMAZINE-S^{35}-TREATED DOG

Time, Hr.	Bile	Urine
0–2	4.1	0.9
2–4	8.8	4.1
4–6	6.6	3.6
6–8	6.5	2.9
8–10	3.4	2.7
Total	29.9	14.2

In the studies with trifluoperazine-S^{35}, 25 mg. of the labeled drug dissolved in saline was administered to a single dog. Only bile and urine specimens were collected from this animal.

Oxidation and Counting Procedures.—In the chlorpromazine-S^{35} studies, each specimen was subject to a nitric acid and a nitric-perchloric acid oxidation to convert any S^{35} material to $S^{35}O_4^-$. The $S^{35}O_4^-$ was then precipitated as $BaS^{35}O_4$, filtered, dried, weighed, and counted on a gas flow counter having a micromil window (Nuclear-Chicago 192). The samples were corrected to infinite thinness. A known amount of the original chlorpromazine-S^{35} was oxidized and counted in a similar manner tc obtain the specific activity of the administered drug.

In the prochlorperazine-S^{35} and trifluoperazine-S^{35} studies, the bile and urine specimens were made to volume; suitable aliquots were 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-diphenyloxazole dissolved in a mixture of toluene, 1,4-dioxane, and absolute alcohol (1:1:0.6).

The blood samples from the prochlorperazine-S^{35} dogs were oxidized and counted in the same manner as the specimens from the chlorpromazine-S^{35} dogs.

TABLE IV.—BILIARY AND URINARY EXCRETION OF PROCHLORPERAZINE-S^{35} AND TRIFLUOPERAZINE-S^{35} EXPRESSED AS PER CENT OF ADMINISTERED DRUG

Collection Periods, Hr.	Prochlorperazine-S^{35} Operative Dose, 100 mg.						Trifluoperazine-S^{35} Operative Dose, 25 mg.	
	Dog 5		Dog 6		Dog 7		Dog 8	
	Bile	Urine	Bile	Urine	Bile	Urine	Bile	Urine
0–2	16.7	1.4	18.7	1.4	15.6	1.9	24.5	0.4
2–4	17.8	1.9	22.3	1.8	18.3	2.5	19.4	0.8
4–6	11.8	1.6	13.6	1.6	12.2	1.9	10.4	0.5
6–8	7.8	1.1	7.6	1.1	7.8	1.3	13.3	0.5
8–10	6.1	1.0	5.7	0.9	7.3	0.8	3.9	0.1
Total	60.2	7.0	67.9	6.8	61.2	8.4	71.5	2.3

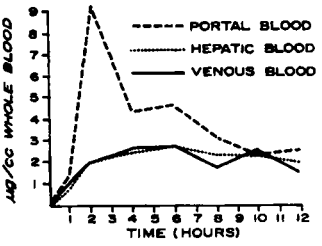


Fig. 1.—Blood levels of chlorpromazine following intraduodenal administration (200 mg.).

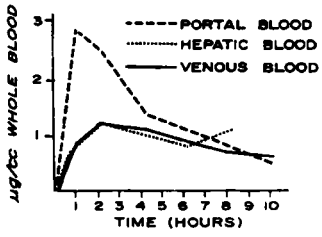


Fig. 2.—Blood levels of prochlorperazine following intraduodenal administration (100 mg.).

RESULTS AND DISCUSSION

The S^{35} concentrations of the bile and urine specimens, expressed as per cent of the administered dose for the chlorpromazine-S^{35}-treated dogs are shown in Table II.

The 10-hour recovery of S^{35} in the urine of all four animals was remarkably constant: 18.2, 17.0, 17.1, and 19.1%. Priming of a dog for 9 days with cold chlorpromazine did not appear to alter the urinary excretion of the labeled drug on the tenth day.

The 10-hour biliary excretion of chlorpromazine-S^{35} was also fairly consistent for all animals: 20.1, 14.5, 31.1, and 23.3%. The average total biliary excretion for the unprimed animals was 21.9%. The primed animal (Dog 4) excreted 23.3% in 10 hours, again suggesting that the 9 days' priming with cold chlorpromazine did not alter the rate at which the dose on the tenth day was excreted in the bile.

The collection of bile and urine from Dog 3 was continued for 24 hours. During the 22–24 hour collection period, 1.4% of the dose was recovered in the bile, while 0.7% was recovered from the urine. The total excretion during the 24-hour collection was 46.7% in bile and 24.3% in urine, amounting to a 71% total recovery of the administered dose in bile and in urine.

In the experiment where bile labeled with chlorpromazine and/or its metabolites was instilled in the duodenum, 30% of the radioactivity appeared in the bile during the 10-hour collection period, while 14% appeared in the urine (Table III). This compares with 22% for bile and 17% for urine when only chlorpromazine-S^{35} was administered. These data support the theory of the entero-hepatic circulation of chlorpromazine and/or its metabolites following its secretion into bile as postulated by Fyodorov (2).

The data obtained from bile and urine specimens following the intraduodenal administration of prochlorperazine to three dogs and trifluoperazine to one dog are shown in Table IV. The results are expressed as per cent of administered dose of drug. The biliary and urinary excretions of S^{35} in all three animals following prochlorperazine administration were consistent: 60.2, 67.9, and 61.2% for bile and 7.0, 6.8, and 8.4% for urine. The biliary excretion exceeded the urinary excretion eightfold; the average biliary excretion was 63.1%, compared with an average of 7.4% recovery from urine. The initial rate of biliary excretion of prochlorperazine-S^{35} and metabolites was high, and S^{35} activity was detected in bile within 5 minutes after intraduodenal administration of this compound.

Because of a limited supply of the radiochemical, only one animal was tested with trifluoperazine. As was observed with prochlorperazine-S^{35}, trifluoperazine-S^{35} was excreted predominantly by the biliary route. The 10-hour biliary excretion of S^{35} was 71.5% of the dose, while the urinary S^{35} excretion amounted to only 2.3%; thus, the biliary excretion was thirtyfold greater than the urinary excretion.

It would appear from these studies that prochlorperazine and trifluoperazine are excreted predominantly by the biliary route in the dog. The biliary to urinary excretion ratio was 30/1 for trifluoperazine, 8/1 for prochlorperazine, and about 1/1 for chlorpromazine. These data, together with previous excretion data in rats (5), indicate that the 3-(4-methyl-1-piperazinyl)propyl side chain affects the excretion of the phenothiazine nucleus, resulting in an increased biliary and decreased urinary excretion of the parent compound and/or its metabolites.

Systemic venous blood levels of the three chlorpromazine-S^{35}-treated dogs ranged from 3 to 7 mcg. of chlorpromazine per milliliter of whole blood, based upon S^{35} activity, with the peak concentration occurring at 4 to 6 hours post drug administration.

To determine the hepatic clearance of phenothiazines in the dog, the S^{35} content was determined on blood samples obtained simultaneously from the portal, hepatic, and jugular veins following intraduodenal administration of 200 mg. of chlorpromazine-S^{35} (Fig. 1). There was a marked difference between the concentration of S^{35} in the portal blood and that of the hepatic and jugular blood. At 2 hours, this difference was fourfold, but by 10 hours all three blood samples contained essentially the same concentration of S^{35}. This study demonstrates the rapid absorption of chlorpromazine from the intestine and its equally rapid clearance from the blood by the liver.

A similar study using 100 mg. of prochlorperazine-S^{35} administered intraduodenally again demonstrated rapid absorption, as there was a rapid increase in the portal concentration of S^{35} which reached its peak about 1 hour post drug administration. Hepatic clearance of prochlorperazine was again demonstrated by the marked difference between portal and hepatic blood concentrations of S^{35} (Fig. 2). The rapid peak of portal S^{35} concentration is in good agreement with the high level of S^{35} obtained in the bile during the first few hours after prochlorperazine-S^{35} administration.

It is fully realized that an intact entero-hepatic circulation would alter the results obtained in this study for blood levels and urinary excretion. However, it is felt that the general pattern would be the same and only the absolute values altered.

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