(160) Riegelman, S., and Fischer, E. Z., *ibid.*, **51**, 206 (1962).
(161) Rollefson, G. K., Bartlett, P. D., Hammett, L. P., and Long, F. A., *J. Am. Chem. Soc.*, **77**, 2666a(1959).
(162) Schmir, G. L., and Bruice, T. C., *ibid.*, **80**, 1173 (1958).
(163) Schroeter, L. C., THIS JOURNAL, **50**, 891(1961).
(164) Schroeter, L. C., *ibid.*, **51**, 258(1962).
(165) Schroeter, L. C., and Higuchi, T., *ibid.*, **47**, 426 (1958).
(166) Schroeter, L. C., Higuchi, T., and Schuler, E. E., *ibid.*, **47**, 723(1958).
(167) Scott, M. W., and Lachman, L., *ibid.*, **51**, 125(1962).
(168) Shah, N. P., and Amis, E. S., *Anal. Chim. Acta*, **11**, 401(1954).
(169) Shou, S. A., *Pharm. Acta Helv.*, **34**, 398(1959).
(170) Siegel, S., Lachman, L., and Malspeis, L., THIS JOURNAL, **48**, 431(1959).
(171) Stern, M. J., King, L. D., and Marcus, A. D., *ibid.*, **48**, 641(1959).
(172) Swintosky, J. V., Rosen, E., Robinson, T. J., Chamberlain, R. E., and Guarini, J. R., *ibid.*, **45**, 37(1956).
(173) "Tables of Chemical Kinetics: Homogenous Reactions," National Bur. Standards, Circular 510, U. S. Dept. of Commerce, Washington, D. C., 1951, and Suppl. 1, 1956.
(174) Taft, R. W., Jr., in Newman, M. S., "Steric Effects in Organic Chemistry," John Wiley & Sons, New York, N. Y., Chap. 13, 1956.
(175) Taft, R. W., Jr., *J. Am. Chem. Soc.*, **74**, 589, 599, 2729, 3120(1952).
(176) Taft, R. W., Jr., *ibid.*, **75**, 4321(1953).
(177) Tanaka, N., *J. Pharm. Soc. Japan*, **81**, 604(1961).
(178) Tanaka, N., Harada, K., and Sugawara, S., *ibid.*, **81**, 903(1961).
(179) Tanaka, N., and Nakagaki, M., *ibid.*, **81**, 591, 597, 600(1961).
(180) Tingstad, J. E., Macdonald, L. H., and Meister, P. D., Preprint D-I symposia papers, Scientific Section A.PH.A., 1962 meeting.
(181) Tishler, F., Sinsheimer, J. E., and Goyan, J. E., THIS JOURNAL, **51**, 214(1962).
(182) Tommila, E., and Hietala, S., *Acta Chem. Scand.*, **8**, 257(1954).
(183) Tommila, E., Koivisto, A., Lyrra, J. P., Antell, K., and Heimo, S., *Ann. Acad. Sci. Fennicae Ser.* A II, **47**, 3(1952).
(184) Tommila, E., and Maltamo, S., *Suomen Kemistilehti B*, **28**, 73, 118(1955).
(185) Udani, J. H., and Autian, J., THIS JOURNAL, **49**, 376(1960).
(186) Walker, G. C., *Bull. Ontario Coll. Pharm.*, **10**, 95 (1961).
(187) Walker, G. C., *ibid.*, **11**, 3(1962).
(188) Wallach, D. P., private communication.
(189) Webb, N. E., Jr., Sperandio, G. J., and Martin, A. N., THIS JOURNAL, **47**, 101(1958).
(190) "Webster's New Collegiate Dictionary," 2nd ed., G. and C. Merriam Co., Springfield, Mass., 1953.
(191) Willi, A. V., *Trans. Faraday Soc.*, **55**, 433(1959).
(192) Whittet, T. D., "Decomposition of Medicaments. . ." presented at Symposium on Drug Stability at 19th International Conference of Pharmaceutical Sciences, Zurich, Switzerland, September 1959.
(193) Windheuser, J. J., and Higuchi, T., THIS JOURNAL, **51**, 354(1962).
(194) Yamana, T., Koike, H., and Tamura, Y., *J. Pharm. Soc. Japan*, **81**, 1528(1961).
(195) Yamana, T., and Mizukami, Y., *ibid.*, **81**, 1381(1961).
(196) Yunker, M. H., and Higuchi, T., THIS JOURNAL, **47**, 621(1958).
(197) Yunker, M. H., Szulczewski, D., and Higuchi, T., *ibid.*, **47**, 613(1958).
(198) Zvirblis, P., Socholitsky, I., and Kondritzer, A. A., *ibid.*, **45**, 450(1956).

Research Articles

Biliary and Urinary Excretion Patterns of Chlorpromazine in the Dog

By T. L. FLANAGAN, L. W. REYNOLDS†, W. J. NOVICK, T. H. LIN, I. M. RONDISH, and E. J. VAN LOON

Chlorpromazine instilled intraduodenally in dogs was excreted in bile and urine as "free" and "bound" chlorpromazine and chlorpromazine sulfoxide. Based upon chromatographic studies and ultraviolet analysis, chlorpromazine and its sulfoxide undergo two types of binding. Strong alkaline treatment hydrolyzed one type while treatment with β-glucuronidase hydrolyzed both types. In urine, the concentration of the "bound" phenothiazines liberated by alkaline hydrolysis was 2–3 times as great as the "free" material. In bile, the concentration of this type of "bound" chlorpromazine and chlorpromazine sulfoxide was 10 to 15 times greater than the "free" forms of these materials.

VARIOUS investigators have reported on the urinary excretion of chlorpromazine and/or its metabolic products in man and experimental animals (1–9). Ross, Young, and Maass (10), Walkenstein and Seifter (11), and Fishman and Goldenberg (12) reported that the side chain which is attached to the phenothiazine nucleus is demethylated. Nadeau and Sobolewski (9) reported that β-glucuronidase treatment increases the amount of phenothiazine-like extractable material from human urine, and Lin, Reynolds, Rondish, and Van Loon (13) reported on the isolation and characterization of glucuronic acid conjugates of chlorpromazine in human urine. Fyodorov (14) administered chlorpromazine-S^{35} to dogs and found that the bile was distinguished by a high degree of radioactivity, especially 3–6 hours after administration.

The purposes of the present studies were (*a*) to obtain an insight into the distribution and

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metabolism of chlorpromazine by following its biliary and urinary excretion in the anesthetized dog and (*b*) by the use of chlorpromazine-S^{35}, to study further the metabolites present in urine and to elucidate the type of binding in urine.

EXPERIMENTAL

In the first experiments, mongrel female dogs weighing approximately 10 Kg. were anesthetized by intraperitoneal pentobarbital and operated so as to expose the duodenum and the common bile duct. A polyethylene cannula was inserted into the common bile duct about 1 in. above the major duodenal papilla and held in place by means of sutures. The open end of the cannula was brought to the outside of the animal and terminated in the collection tube. The cystic duct was clamped off to insure collection of only hepatic bile throughout the experiment. At the same time a catheter was inserted into the urinary bladder and all residual urine was drained and discarded.

One-half hour later, 200 mg. of chlorpromazine hydrochloride, dissolved in 10 ml. of saline, was administered to the animal intraduodenally. Bile samples were obtained at hourly intervals and urine samples at 2-hr. intervals for a period of 8 or 10 hours following drug administration. Each sample was analyzed individually for "free" and "bound" chlorpromazine and chlorpromazine sulfoxide by the method of Flanagan, *et al.* (15). The samples were not analyzed for glucuronic acid conjugates.

"Bound" chlorpromazine and "bound" chlorpromazine sulfoxide are the terms given to the chlorpromazine and chlorpromazine sulfoxide which can be extracted from the aqueous residue, following the extraction of the free components, after the aqueous residue is subjected to an alkaline hydrolytic treatment. The analytical procedure determined the ether-extractable chlorpromazine metabolites as either chlorpromazine or its sulfoxide. If demethylation of the side chain occurred, the demethylated components would be determined as chlorpromazine and/or chlorpromazine sulfoxide, since their absorption spectra are similar to the parent compounds.

In the next series of experiments the dogs received a daily oral dose of chlorpromazine hydrochloride for varying periods of time (Table I) prior to the operation. The operative dose was 200 mg. of chlorpromazine hydrochloride in 10 ml. of isotonic saline administered intraduodenally. Before the administration of the operative dose, control samples of bile and urine were obtained to determine any excretion due to the previous chlorpromazine priming.

In the experiments with chlorpromazine-S^{35}, each dog was anesthetized with pentobarbital and the bladder was emptied by inserting a catheter which was allowed to remain *in situ* for the subsequent collection of urine samples. Chlorpromazine hydrochloride, 25 mg./Kg., containing about 60 μc. of S^{35}, were injected into a surgically-exposed loop of the duodenum. Urine samples were collected every 2 hours.

Each sample was reduced *in vacuo* to about one-third of the original volume and 50–100 μl. spotted on $^3/_4$ inch strips of Whatman 3MM paper. The

TABLE I.—BILIARY AND URINARY EXCRETION IN PRIMED DOGS[a]

Dog	Collection Period, hr.	Daily Priming Dose of Chlorpromazine, mg.	No. of Days of Priming	% of Dose Recovered and Calculated as Chlorpromazine Hydrochloride — Free Chlorpromazine Bile	Free Chlorpromazine Urine	Free Chlorpromazine Sulfoxide Bile	Free Chlorpromazine Sulfoxide Urine	Total Recovery of Free Components, Bile and Urine	Bound Chlorpromazine Bile	Bound Chlorpromazine Urine	Bound Chlorpromazine Sulfoxide Bile	Bound Chlorpromazine Sulfoxide Urine	Total Recovery of Bound Components, Bile and Urine	Free *vs.* Bound Components
5	8	200	14	0.9	2.3	0.6	3.6	7.4	3.7	2.7	10.3	4.5	21.2	1:2.9
6	8	200	2	0.5	1.7	0.3	2.6	5.1	2.6	2.1	4.1	1.4	10.2	1:2.0
7	8	100	7	0.1	0.3	0.1	1.0	1.5	0.8	0.9	1.2	0.7	3.6	1:2.4
8	8	100	6	0.3	1.5	0.1	1.3	3.2	1.1	2.9	1.7	3.0	8.7	1:2.7

[a] Operative dose, 200 mg. of chlorpromazine hydrochloride intraduodenally.

TABLE II.—BILIARY AND URINARY EXCRETION IN DOGS FOLLOWING A SINGLE 200-MG. DOSE OF CHLORPROMAZINE HYDROCHLORIDE INTRADUODENALLY

Dog	Collection Period, hr.	% of Dose Recovered and Calculated as Chlorpromazine Hydrochloride — Free Chlorpromazine Bile	Free Chlorpromazine Urine	Free Chlorpromazine Sulfoxide Bile	Free Chlorpromazine Sulfoxide Urine	Total Recovery of Free Components, Bile and Urine	Bound Chlorpromazine Bile	Bound Chlorpromazine Urine	Bound Chlorpromazine Sulfoxide Bile	Bound Chlorpromazine Sulfoxide Urine	Total Recovery of the Bound Components, Bile and Urine	Free *vs.* Bound Components
1	8	0.3	1.1	<0.1	0.6	2.0	2.9	3.9	2.8	1.4	11.0	1:5.5
2	10	0.2	1.1	<0.1	1.4	2.7	2.4	2.3	1.9	1.8	8.4	1:3.1
3	10	0.3	1.1	0.1	1.6	3.1	3.6	3.7	5.4	3.2	15.9	1:5.1
4	10	0.1	0.9	<0.1	1.3	1.3	1.3	4.6	1.2	1.0	8.1	1:6.2

strips were developed in a descending direction with a solvent system consisting of 100 parts of isoamyl alcohol, 100 parts of water, 15 parts of ethanol, and 10 parts of formic acid.

A portion of the remaining urine was adjusted to pH 13 and extracted with ether to remove the "free" chlorpromazine and "free" chlorpromazine sulfoxide. The aqueous residue was divided into two fractions. One fraction was hydrolyzed with strong alkali, ether extracted, and the ether extract and the aqueous residue were chromatographed. The pH of the second fraction was readjusted to 5.0 and hydrolyzed with mammalian β-glucuronidase. After an ether extraction, the extract and the hydrolyzed urine were chromatographed in the same manner as the original urine.

The metabolites were detected by two independent procedures: (*a*) the chromatograms were sprayed with nitrite reagent as described by Flanagan, *et al.* (15), and (*b*) the developed strips were fed into a scanner (Nuclear-Chicago, model No. 1620-A) and the radioactive areas were detected in this manner.

In all the experiments the animals were maintained by a slow 5% dextrose-saline intravenous infusion and additional pentobarbital as required.

RESULTS AND DISCUSSION

In Table II are listed the per cent recoveries of chlorpromazine and chlorpromazine sulfoxide in bile and urine of four unprimed dogs which received an operative dose of 200 mg. of chlorpromazine hydrochloride intraduodenally. In bile, the concentrations of "bound" chlorpromazine and "bound" chlorpromazine sulfoxide were much greater than the concentrations of "free" chlorpromazine and "free" chlorpromazine sulfoxide. "Free" chlorpromazine sulfoxide was not obtained from the bile specimens of these dogs until 5–6 hours after administration of chlorpromazine; "bound" chlorpromazine sulfoxide was obtained in the first hour following administration of the drug. The ratio of "bound" chlorpromazine to "bound" chlorpromazine sulfoxide was approximately 1:1 in the test period for three of the dogs. The bile recovery of the bound components was 10–20 times greater than the recovery of the free components.

In urine the amount of "free" chlorpromazine and "free" chlorpromazine sulfoxide was much greater than that obtained in the bile. The recovery of "bound" chlorpromazine was from 2–5 times greater than the recovery of "free" chlorpromazine, while the "bound" chlorpromazine sulfoxide was 1.3 to 3 times greater than the "free" sulfoxide.

In the next experiment, four dogs received priming doses of chlorpromazine hydrochloride to see what effect priming would have upon the excretion pattern of the drug. In these animals the control bile samples contained measurable concentrations of "free" and "bound" chlorpromazine and chlorpromazine sulfoxide, although the last oral dose had been administered 18 hours prior to the operation. In the dog that was primed with 200 mg. of chlorpromazine hydrochloride for 14 days, there was an increased recovery of "free" and "bound" chlorpromazine and chlorpromazine sulfoxide. In these primed dogs the ratio of "bound" chlorpromazine to "bound" chlorpromazine sulfoxide in bile varied from 1:1.5 to 1:3, while in three of the unprimed dogs this ratio was 1:1.

The urinary values obtained for "bound" chlorpromazine on the primed animals were lower than the values obtained on the single-dose animals.

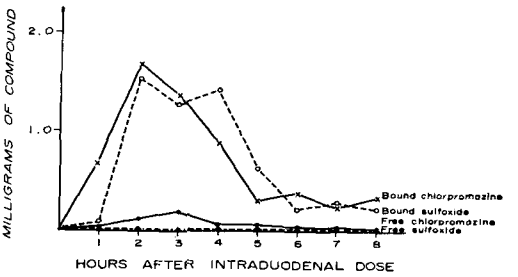

Fig. 1.—Dog No. 1, nonprimed biliary excretion.

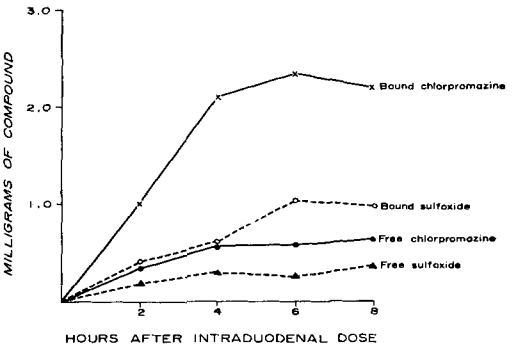

Fig. 2.—Dog No. 1, nonprimed urinary excretion.

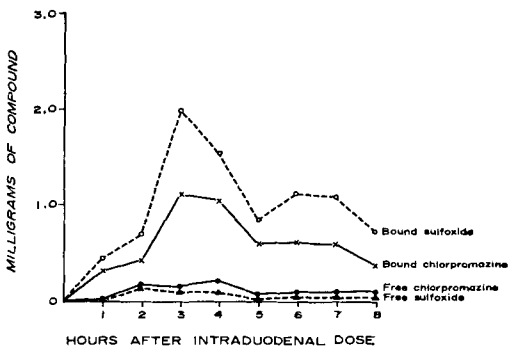

Fig. 3.—Dog No. 6, primed biliary excretion.

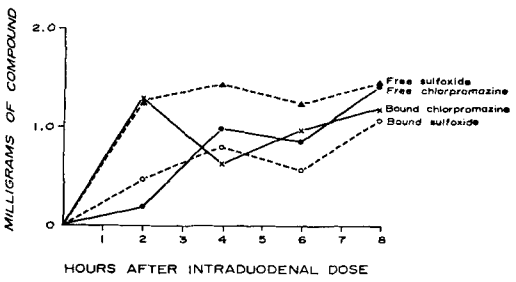

Fig. 4.—Dog No. 6, primed urinary excretion.

However, the ratio of "free" *vs.* "bound" components was increased in the primed animals due to the increased excretion of "free" components by these animals.

Figures 1 and 2 show the biliary and urinary time excretion curves of the four components in a nonprimed dog, while Figs. 3 and 4 illustrate the same curves for a primed dog. The patterns obtained on these two dogs are typical of those obtained on the other test animals.

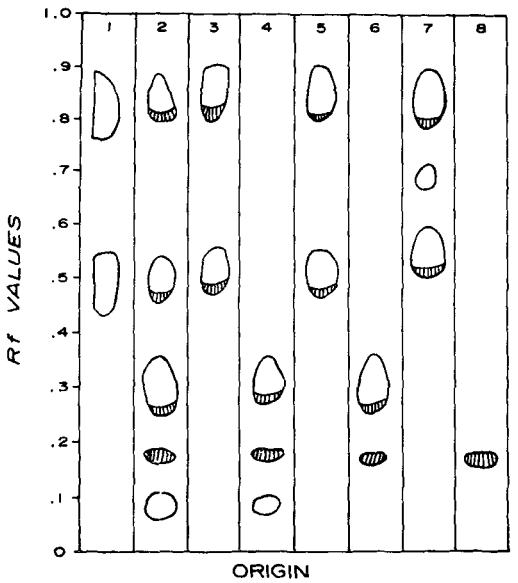

Fig. 5.—Col. 1, Chlorpromazine and chlorpromazine sulfoxide standards; Col. 2, whole urine; Col. 3, ether extract of whole urine (alkaline side); Col. 4, aqueous residue; Col. 5, fraction 1, ether extract after alkaline hydrolysis; Col. 6, aqueous residue; Col. 7, fraction 2, ether extract after β-glucuronidase hydrolysis; Col. 8, aqueous residue.

In the chromatographic studies with chlorpromazine-S^{35} on noncannulated, primed and unprimed dogs, four radioactive peaks were observed. The two peaks following the solvent front corresponded to chlorpromazine and chlorpromazine sulfoxide. The two remaining peaks were attributed to bound metabolites of these compounds. No attempt was made to separate chlorpromazine and chlorpromazine sulfoxide from their demethylated components as did Fishman and Goldenberg (12). Typical chromatographic patterns obtained on urine from dogs administered chlorpromazine-S^{35} by spraying the strips with nitrite reagent are shown in Fig. 5.

In all the experiments, the R_f values obtained for the metabolites by the use of the color developing spray corresponded to the R_f values obtained radiochemically.

The chromatographic patterns of chlorpromazine and chlorpromazine sulfoxide are presented in Col. 1 (Fig. 5). Column 2 shows the chromatographic pattern obtained for whole urine; the spots between the two "bound" metabolic spots represent an endogenous urinary constituent which was obtained with this spray for blank urine specimens. The dark areas behind the chlorpromazine and chlorpromazine sulfoxide were always present and they were purple in color as compared to a light pink shade of the pure compounds. The darker areas did not show increased radioactivity over that obtained for the pink shaded areas. Column 3 shows the pattern for the ether extract of whole urine made alkaline. The "bound" material remained in the aqueous phase (Col. 4).

In regard to Col. 4, it was believed initially that the two "bound" metabolites corresponded to "bound" chlorpromazine and "bound" chlorpromazine sulfoxide. However, following alkaline hydrolysis and subsequent ether extraction, the spot having an R_f of approximately 0.1 disappeared from the aqueous residue, while the R_f 0.3 spot remained (Col. 6). The chromatographic pattern obtained for the ether extract (Col. 5), as well as ultraviolet analysis of this extract, showed both chlorpromazine and chlorpromazine sulfoxide to be present. The R_f 0.1 spot was a mixture of "bound" chlorpromazine and "bound" chlorpromazine sulfoxide, and the type of binding is such that it can be broken by strong alkaline hydrolytic treatment. The "bound" components in this area did not resolve themselves into separate and distinct entities. The separation was not as sharp as that obtained by Lin, *et al.* (13), with human urine.

The chromatogram obtained for the aqueous residue after hydrolysis with β-glucuronidase and subsequent extraction with ether is presented in Col. 8. Only the endogenous spot remained; β-glucuronidase hydrolyzed both "bound" areas. In the ether extract (Col. 7) three spots appeared; two of the spots corresponded to chlorpromazine and chlorpromazine sulfoxide, the small spot in between may be one of the metabolites resulting from partial or total demethylation of the side chain.

REFERENCES

(1) Dubost, P., and Pascal, S., *Ann. pharm. franç.*, **11**, 615(1953).
(2) Kok, K., *Acta Physiol. et Pharmacol. Neerl.*, **4**, 388 (1955).
(3) Salzman, N. P., Moran, N. C., and Brodie, B. B. *Nature*, **176**, 1122(1955).
(4) Berti, T., and Cima, L., *Farmaco Pavia Ed. Sci.*, **11**, 451(1956).
(5) Salzman, N. P., and Brodie, B. B., *J. Pharmacol. Exptl. Therap.*, **118**, 46(1956).
(6) Berti, T., and Cima, L., *Farmaco Pavia Ed. Sci.*, **12**, 159(1957).
(7) Henriksen, U., Huus, I., and Kopf, R., *Arch. intern. pharmacodynamie*, **109**, 39(1957).
(8) Nadeau, G., and Sobolewski, G., *Can. Med. Assoc. J.*, **80**, 826(1959).
(9) Nadeau, G., and Sobolewski, G., *ibid.*, **81**, 658(1959).
(10) Ross, J. J., Jr., Young, R. L., and Maass, A. R., *Science*, **128**, 1279(1958).
(11) Walkenstein, S. S., and Seifter, J., *J. Pharmacol. Exptl. Therap.*, **125**, 283(1959).
(12) Fishman, V., and Goldenberg, H., *Proc. Soc. Exptl. Biol. Med.*, **104**, 99(1960).
(13) Lin, T. H., Reynolds, L. W., Rondish, I. M., and Van Loon, E. J., *ibid.*, **102**, 602(1959).
(14) Fyodorov, N. A., *Z. Nevropat psiknat.*, **57**, 761(1957).
(15) Flanagan, T. L., Lin, T. H., Novick, W. J., Rondish, I. M., Bocher, C. A., and Van Loon, E. J., *J. Med. Pharm. Chem.*, **1**, 263(1959).