Urinary Excretion of *d*-Propoxyphene Hydrochloride in Man

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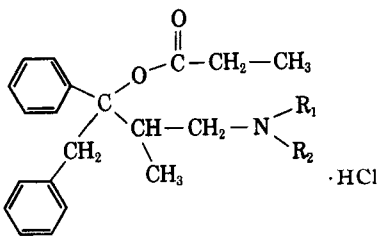
The urinary excretion pattern of *d*-propoxyphene hydrochloride in man has been determined. An assay method utilizing bromthymol blue was used to measure total excretion. Approximately 25 per cent of a 65-mg. dose was recovered in the urine within 48 hr. as a mixture of free drug and metabolite. The excretion pattern was followed with thin-layer chromatography.

THIS REPORT is concerned with the determination of the human urinary excretion pattern of *d*-propoxyphene hydrochloride[1] (*α-dextro*-4-dimethylamino - 1,2 - diphenyl - 3 - methyl - 2-propionoxybutane hydrochloride, I). This work was done preliminary to the evaluation of timed-release formulations of the drug using urinary excretion data.



	R_1	R_2
I	CH_3	CH_3
II	H	CH_3

Previous work on the metabolism of the *dl*-isomer of the drug (1) showed that one of the chief metabolic changes was removal of one of the *N*-methyl groups to form des-*N*-methylpropoxyphene (II). Only a small fraction of the dose was detectable as unchanged drug in the urine. The exact percentages of free drug and metabolite were not determined.

In this investigation an assay method was developed to measure total urinary propoxyphene excretion (free drug plus metabolite) over a 48-hr. period in subjects who received 65 mg. of the drug. Thin-layer chromatography was used to examine qualitatively the urine extracts for the presence of unchanged drug and metabolite.

EXPERIMENTAL

Drug Administration and Urine Collection.—Capsules containing 65 mg. of *d*-propoxyphene hydrochloride were administered along with 200 ml. of water at 7:30 a.m. to eight fasting healthy males on two different occasions. Food and water intake were not restricted after 1 hr. following dosage. Total urine collections were made at the following intervals: 0–2, 2–4, 4–6, 6–8, 8–10, 10–12, 12–24, 24–36, and 36–48 hr. All urine samples were refrigerated when not assayed immediately.

Assay Method.—A modification of the bromthymol blue method of Lehman and Aitken (2) was used to measure total propoxyphene excretion. A 10-ml. aliquot of the total urine volume collected at each time interval was placed in a 125-ml. separator and was made acid by the addition of 4 drops of 6 *N* hydrochloric acid. The aliquot was extracted with 50 ml. of chloroform which had been passed over an alumina (activated alumina F-20, Alcoa) column prior to use. The chloroform extract was washed with 25 ml. of 0.1 *N* sodium hydroxide and with 25 ml. of water, respectively. Twenty-five milliliters of a solution of 40 mg. of bromthymol blue (Nutritional Biochemicals Corp.) dissolved in 1 L. of pH 7.8 phosphate buffer (3) was added to the washed chloroform. After agitation for 2–3 min., the yellow chloroform layer was drained into a clean dry separator which contained 10.0 ml. of 0.1 *N* sodium hydroxide. This mixture then was shaken until the chloroform layer became colorless. The absorbance of the blue aqueous layer was determined at 620 mμ on a Beckman DU spectrophotometer. Ten-milliliter aliquots of control urine to which were added 0, 10, 25, 50, 75, and 100 mcg. amounts of *d*-propoxyphene hydrochloride were treated in a like manner. An absorbance *versus* concentration curve was prepared (Fig. 1). All measurements were calculated as "apparent" propoxyphene using this curve.

Control urines contained only a small amount of interfering substances. Examination of urine

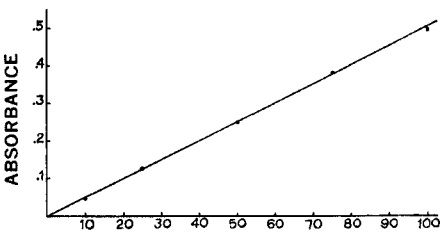


Fig. 1.—Standard curve for *d*-propoxyphene hydrochloride added to urine. The absorbance of the urine blank has been substracted.

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[1] Marketed as Darvon by Eli Lilly and Co., Indianapolis, Ind.

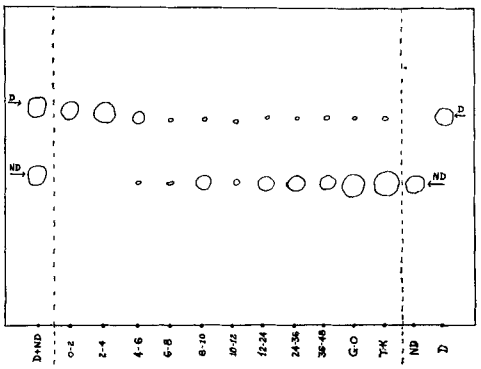

Fig. 2.—1765 MEA No. 2–60 urine–*d*-propoxyphene hydrochloride extracts. Chromatogram of urine extracts. Plate sprayed with Acid Rhodamin B, and white zones encircled. Key: D, propoxyphene; ND, des-*N*-methylpropoxyphene; 0–2, 2–4, etc., collection intervals; G.O. and T.K., pooled 48-hr. specimens.

samples obtained from the same subjects prior to drug administration showed that the blank values were consistently low and did not vary significantly with time of collection or volume of sample. An average blank absorbance of 0.050 (1 mcg./ml.) was used in this study. Making the initial chloroform extraction on acidified urine and using pH 7.8 buffer kept the absorbance of control urines at a minimum.

Experiments with *d*-propoxyphene hydrochloride added to urine showed recoveries of 89% when compared with an equal quantity of drug extracted from water. Six replicate assays of 50 mcg. of propoxyphene hydrochloride added to urine averaged 48.4 ± 1.9 mcg. when assayed by the above procedure. Equal weights of *dextro*-propoxyphene hydrochloride and des-*N*-methylpropoxyphene hydrochloride gave almost identical extinction values when assayed by this method.

Chromatography.—Extracts of the urine samples were examined chromatographically for the presence of unchanged drug and metabolite. Standard size (20 × 20 cm.) glass plates were coated with a 250 μ layer of Silica Gel G. The plates were air-dried overnight before use. The developing solvent consisted of methanol saturated with ammonium chloride–methanol (1:2). The presence of free drug and metabolite was detected by spraying the plates with aqueous Acid Rhodamin B (Allied Chemical), 0.15 mg./ml. After the sprayed plates were heated at 100–110° for 10–15 min., the reactive materials appeared as white zones against a pink background.

The urine samples were acidified and extracted with chloroform. After washing with alkali and water, the chloroform extracts were evaporated to dryness. The residues were redissolved in a small volume of chloroform and shaken with the dye-buffer solution as stated above. The yellow chloroform layer was washed with 1 vol. of 0.05 *M* phosphate buffer, pH 6. The chloroform was passed through a pledget of glass wool and evaporated to dryness. Each residue was taken up in a small specific volume of chloroform (0.2–0.5 ml.).

The samples then were applied to the plates, and the spots were dried with a stream of warm air. The plates were heated at 100–110° for 20–30 min. The spots then were overlayed with a small amount of 1:1 methanol–water, and the heating was continued for an additional 15–20 min.[2] The plates were cooled to room temperature and placed in the developing solvent. After approximately 1 hr. the plates were removed from the chamber and heated 3–4 min. at 100° to remove excess solvent. The plates were sprayed with the Acid Rhodamin B and reheated to identify the zones. A representative chromatogram is shown in Fig. 2.

RESULTS AND DISCUSSION

The small amount of unchanged propoxyphene excreted by subjects who have received the drug makes it impractical to use these values to evaluate timed-release formulations by urinary excretion data. Since considerable quantities of des-*N*-methylpropoxyphene are excreted, both unchanged drug and metabolite were measured to determine total excretion.

The acid dye method has been used widely for the determination of basic nitrogen compounds in biological fluids and tissues (4). These methods generally suffer from a lack of specificity, but this can be corrected by the use of blank determinations on specimens received prior to drug administration. The assay method given in this report will not differentiate between free drug and metabolite. No attempt was made to determine the unchanged drug/metabolite ratio in the urine samples. Therefore, all results are reported, as "apparent" or "total" propoxyphene.

Thin-layer chromatography of extracts of the separate urine collections confirmed the presence of both free and metabolized drug (Fig. 2). Some unchanged propoxyphene was found in all the samples. The greatest concentrations were found in the samples collected the first 6 hr. after drug administration. No metabolite was evident in the first 4 hr., and only traces were indicated in the 4–8-hr. period. Starting with the 8–10-hr. sample, the metabolite is present in greater concentration than the free drug. Extracts of pooled 48-hr. urine samples show the relative concentrations of the two compounds excreted, and it is evident that the des-*N*-methylpropoxyphene is present in greater concentration.

The amounts of total propoxyphene excreted in the urine within 48 hr. after the administration of 65 mg. of drug are given in Table I. An average of approximately 25% of the dose was recovered. About one-half of the total amount recovered was excreted in the first 12 hr. after dosage, as shown in Fig. 3. Figure 4 shows the excretion rate of apparent propoxyphene calculated from the mean values as shown in the table. The maximum rate of excretion occurred in the 2–4-hr. collection interval and is due

[2] Solutions of des-*N*-methylpropoxyphene maleate and of the free base produced two spots when they were chromatographed. As the solutions aged, the size of the spot associated with the pure compound decreased, while the second spot increased. Heat treatment of the plate after the solutions had been spotted served to convert the desmethyl product quantitatively to the unidentified second spot material. Chromatograms of the urine extracts also showed these same two spots when the plates were not heat treated. Since the extracts had been in chloroform solution for some time, the second spot was attributed to the instability of the metabolite. In Fig. 2, the spot designated as metabolite is the unidentified second spot material. *d*-Propoxyphene was not affected by this treatment.

TABLE I.— EXCRETION OF APPARENT *d*-PROPOXYPHENE HYDROCHLORIDE AFTER ADMINISTRATION OF A SINGLE 65-mg. DOSE

Subject	Drug Excreted per Time Period, mg. 0–2	2–4	4–6	6–8	8–10	10–12	12–24	24–36	36–48	Total	% of Dose
A	1.28	1.97	0.66	0.99	1.18	0.27	3.24	1.29	2.64	13.52	20.8
	1.07	2.83	1.22	1.75	0.90	2.07	0.04	3.24	3.37	16.49	25.4
B	1.09	1.99	1.24	0.80	0.86	0.24	3.42	3.48	2.37	15.49	23.8
	0.85	1.88	0.79	0.67	1.23	0.85	3.00	2.14	0.87	12.28	18.9
C	1.11	2.22	0.63	1.12	0.92	0.94	3.10	2.99	1.41	14.44	22.2
	0.67	1.41	1.38	1.32	0.82	0.71	3.61	4.35	1.91	16.18	24.9
D	0.85	1.49	1.85	0.76	1.16	1.58	2.34	3.27	0.67	13.97	21.5
	1.60	2.00	2.16	1.53	0.85	1.93	1.55	1.85	1.21	14.68	22.6
E	1.25	2.13	1.73	1.50	1.01	1.17	4.25	5.25	0.32	18.71	28.8
	0.35	1.59	1.95	1.40	1.11	0.52	3.11	3.11	0.29	13.43	20.7
F	0.58	1.73	1.54	0.97	1.60	2.80	4.95	2.84	1.99	19.00	29.2
	1.52	1.78	1.70	1.24	0.84	0.72	5.08	4.22	2.74	19.84	30.5
G	0.24	1.26	2.13	1.83	0.64	0.39	1.59	3.08	0.23	11.39	17.5
	1.43	3.00	0.83	0.51	0.58	1.97	2.62	1.22	4.34	16.49	25.4
H	0.51	1.18	2.16	1.95	0.53	2.94	3.03	5.34	2.29	19.93	30.7
	1.52	2.46	1.45	1.73	1.67	1.80	7.18	0.35	3.92	22.08	34.0
Mean	0.99	1.91	1.46	1.25	0.99	1.31	3.26	3.01	1.91	16.12	24.8

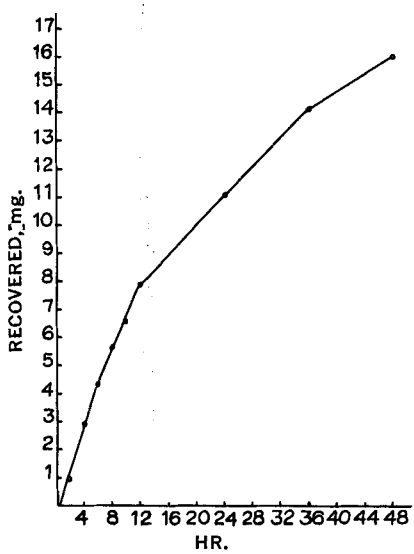

Fig. 3.—Mean accumulative urinary excretion of apparent propoxyphene hydrochloride after a 65-mg. dose.

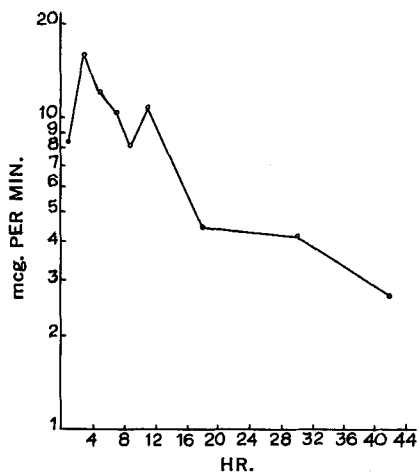

Fig. 4.—Mean urinary excretion rate of apparent propoxyphene hydrochloride.

primarily to the excretion of unchanged drug. The 10–12-hr. maximum can be attributed to the excretion of the metabolite.

SUMMARY

The urinary excretion of *d*-propoxyphene hydrochloride in humans has been followed both quantitatively and qualitatively. Approximately 25% of a 65-mg. dose was recovered as a mixture of unchanged drug and metabolite. Unchanged drug is excreted mainly in the 0–6-hr. period and metabolite in the 6–48-hr. period.

REFERENCES

(1) Lee, H. M., Scott, E. G., and Pohland, A., *J. Pharmacol. Exptl. Therap.*, **125**, 14(1959).
(2) Lehman, R. A., and Aitken, T., *J. Lab. Clin. Med.*, **28**, 787(1942).
(3) "United States Pharmacopeia," 16th rev., Mack Publishing Co., Easton, Pa., p. 929.
(4) Higuchi, T., and Brochmann-Hanssen, E., "Pharmaceutical Analysis," Interscience Publishers, Inc., New York, N. Y., 1961, p. 413.