Human Urinary Excretion of Piperazine Citrate from Syrup Formulations

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Abstract □ A modified colorimetric method using Folin's amino acid reagent was used for the quantitative determination of piperazine in human urine. Comparative urinary excretion trials were carried out on five subjects using two different piperazine citrate syrup formulas. Within each individual, the percentage and pattern of excretion of piperazine citrate after oral administration of a newly developed syrup formula, equivalent to 3.5 g. piperazine hexahydrate, compared favorably with a commercial syrup sample taken in this investigation as a standard.

Keyphrases □ Piperazine citrate (syrup formulations)—determination in human urine, excretion profiles □ Excretion profiles—piperazine citrate syrup formulations, comparison of formulations, colorimetric method developed □ Colorimetry—analysis of piperazine citrate syrup formulations, human urine

Piperazine is the agent of choice in ascariasis, and its preparations are always given orally. Piperazine is readily absorbed from the GI tract (1). A portion of the absorbed drug is degraded in the body, and the remainder is excreted in the urine (2). Quantitative determination of piperazine in urine has not been widely studied. Titration of the monoperiodate piperazine derivative has been employed (3); however, this was not sufficiently accurate for the present study. A potentiometric method was described (4), which required long preliminary treatment of the urine. A lengthy quantitative color reaction of piperazine with acidified benzoquinone also was published (5).

In this paper, a modified colorimetric method of Rogers (6) was used to achieve higher sensitivity and more accurate results. In Rogers' method, readings were taken at 480 nm. after standing for 10 min. The present work has shown that this length of time would allow neither for the optimum stability and intensity of the color derivative nor for the reading at its maximum wavelength.

The aim of this study was to compare the amount of piperazine excreted in the urine over 24 hr. after oral administration of a newly developed syrup formula, equivalent to 3.5 g. piperazine hexahydrate, against a commercial syrup sample. This dose was chosen according to the normal adult dose range for the treatment of ascariasis.

Table I—Reagents Used in Tests

Reagent	Reagent Blank, ml.	Test Sample, ml.	Standard, ml.
Urine, 1:25 dilution	—	2	—
Water	4	2	2
Piperazine citrate (20 mcg./ml.)	—	—	2
2% borax in water	4	4	4
95% alcohol	10	10	10
0.5% Folin's amino acid reagent	2	2	2

Table II—Recovery of Piperazine Citrate after *In Vitro* Addition to Human Urine

Piperazine Citrate Added to Urine, mg./ml.	Mean Recovery[a], mg./ml.	Standard Deviation
5	5.0264	±0.065
4	4.0896	±0.120
3	3.0362	±0.070
2	2.0554	±0.104
1	1.0278	±0.061

[a] Average of five determinations using the modified method.

EXPERIMENTAL

Determination of Piperazine in Urine—Piperazine was determined quantitatively in urine by its color reaction with Folin's amino acid reagent[1]. The color intensity increased rapidly from 0 to 18 min. and was stable for 10 min. (Fig. 1). The maximum absorbance of the color was determined by scanning the developed color under visible light at a range of from 800 to 320 nm. and was found to be at 490 nm. The color obeyed Beer's law in concentrations between 0 and 200 mcg./ml. Each urine sample was measured for the exact volume, and a dilution of 1:25 with water was made for each sample including the control urine. This dilution was used for testing according to Table I using the various reagents in the tabulating order. After standing for 20 min., readings[2] were carried out within 5 min. at 490 nm. The value of the unknown sample was then compared with the standard sample reading.

The validity of this method was verified by adding various known amounts of piperazine citrate to the urine. This urine was tested and assayed for the amount of piperazine present according to the described method. Each experiment was repeated five times, and the standard deviation was found to be ±0.42 (Table II).

Urinary Excretion of Piperazine—Five Caucasian subjects, three men and two women ranging from 23 to 38 years of age, in normal state of health as determined by prior physical and laboratory medical examination, were used in this trial. Each was administered an oral dosage of 35 ml. of piperazine citrate syrup equivalent to 3.5 g. piperazine hexahydrate. At least 48 hr. elapsed between the administration of the new formula and the commercial sample[3]. Prior to the oral administration of either syrup sample, a urine sample was collected from each individual to be used as an internal control. The amino acids and other naturally occurring chromogens

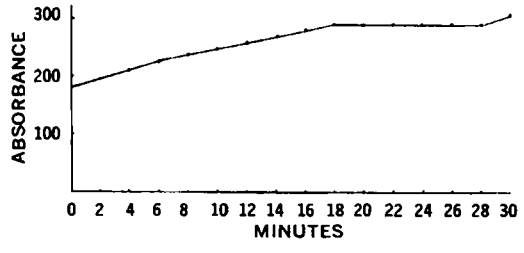

Figure 1—*Stability of the colored derivative of piperazine with Folin's amino acid reagent (piperazine citrate concentration, 100 mcg./ml.).*

[1] Sodium 1,2-naphthoquinone-4-sulfate.
[2] Beckman DB-G grating spectrophotometer.
[3] Antepar, Lot Y813, Burroughs Wellcome Co.

Table III—Total Urinary Excretion of Piperazine (in Milligrams) Calculated as Hexahydrate after Oral Administration

Subject[a]	Dose[b]	1 hr.	2.5 hr.	4.5 hr.	6.5 hr.	9 hr.	13 hr.	20 hr.	24 hr.	Total Percent Excretion	t^c Value
A	I	50	633	761	528	429	127	—[d]	—[d]	72	
27, 110	II	76	682	927	458	412	109	—[d]	—[d]	75	
B	I	156	479	604	305	216	356	347	—[d]	70	
33, 73	II	196	476	530	340	275	391	353	77	75	
C	I	153	562	647	372	261	177	50	18	64	0.0982
38, 79	II	66	596	588	318	306	220	70	27	63	
D	I	103	502	688	293	223	158	65	41	59	
23, 57	II	46	334	582	413	486	104	73	61	60	
E	I	54	263	160	30	—[d]	—[d]	—[d]	—[d]	15	
29, 56	II	69	127	219	106	—[d]	—[d]	—[d]	—[d]	15	

[a] The first number is for age (years) and the second number is for body weight (kilograms). [b] I = new syrup formula, 3.5 g. piperazine hexahydrate. II = commercial syrup sample, 3.5 g. piperazine hexahydrate. [c] The t test of significance, where $t = \bar{x}_1 - \bar{x}_2/s\sqrt{n_1n_2/n_1 + n_2}$, $p = 0.05$, and $df = 8$ (7). The t value was calculated with the pooled variance of the two syrup samples. [d] Zero excretion.

present in normal urine had been found to give colored derivatives with the reagent used. Urine samples were collected, whenever possible, at 1, 2.5, 4.5, 6.5, 9, 13, 20, and 24 hr. after oral administration. The values obtained for the samples were corrected by subtracting the value of the normal urine reading for each individual. Piperazine concentration in the diluted urine sample was then calculated by comparing these corrected values with the standard reading carried out simultaneously. The total piperazine concentration in the urine sample was calculated by multiplying its dilution factor and urine volume. The total amount of piperazine excreted and the percentage of excretion of each individual was then ascertained as piperazine hexahydrate.

RESULTS AND DISCUSSION

The results of the total urinary excretion of piperazine calculated as hexahydrate after the oral administration of an amount equivalent to 3.5 g. of piperazine hexahydrate are shown in Table III and Fig. 2.

The percentage and pattern of urinary excretion of piperazine calculated as hexahydrate varied from individual to individual, as seen in the five subjects. The total urinary recovery varied from 15 to 75%. However, the results and pattern of urinary excretion for both syrup formulas compared favorably within each individual.

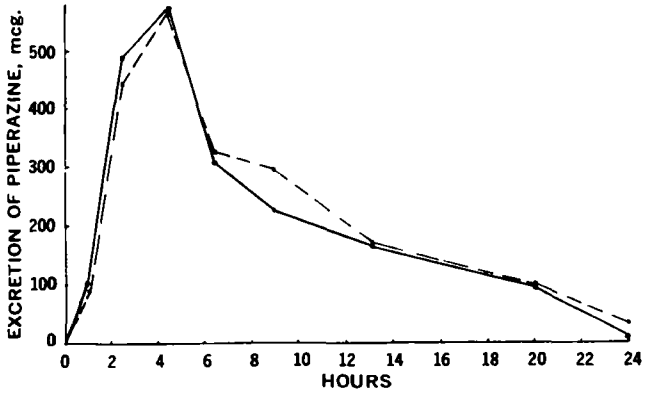

Figure 2—*Urinary excretion of piperazine after oral administration of the syrup formulas (average data for five individuals). Key:* —○—, *piperazine syrup, new formula; and* --●--, *piperazine syrup, commercial sample.*

Urinary excretion began 1 hr. after the oral administration, and the rate was maximal between 2 and 6 hr. Excretion was nearly completed in 24 hr.

CONCLUSION

1. The modified colorimetric method of analysis of piperazine in urine is accurate and simple to perform.
2. The percentage and pattern of urinary excretion of piperazine varied from individual to individual.
3. The percentage and pattern of urinary excretion after oral administration of the newly developed syrup formula, equivalent to 3.5 g. piperazine hexahydrate, compared favorably with that of the commercial syrup sample with each individual.
4. The maximum urinary excretion occurred 2–6 hr. after oral administration and was nearly completed at 24 hr. for both syrup formulas under investigation.
5. The t test of significance, where the t-value was calculated with the pooled variance of the two syrup samples, showed no significant difference.

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