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Urinary Excretion of Three Oral Cholecystographic Agents in Man. (30367)

EVAN W. MCCHESNEY AND WILLIAM F. BANKS, JR.
Sterling-Winthrop Research Institute, Rensselaer, N. Y.

Data on the absorption and excretion of 2 orally administered cholecystographic agents have been reported from this laboratory(1,2). It was found that of a 3 g dose of iopanoic acid* to man, 36% was excreted in the urine within 132 hours, while 62% was excreted in the feces. Of the urinary excretory product about ⅞ was not extractable by ether at pH 6-7, therefore was presumed to be present as the glucuronide conjugate(1,3,4). Butyryl-iopanoic acid (tyropanoic acid†) was found to be 94% excreted within 108 hours following a 4.5 g dose of the sodium salt, but its partition between urine and feces was almost exactly 1:1. Although it was also shown that tyropanoate is excreted in the bile as a glucuronide, its partition between free and conjugated forms in the urine was not determined(2). It has been stated(5,6‡) that 70% of an oral dose of bunamiodyl§ is excreted in the urine. A more recent report, however, indicates that the urinary excretion in man is only 25% of the dose in the first 30 hours post-medication(7).

In view of several recent reports of renal damage in subjects receiving bunamiodyl(8-13), and the suggestion that cholecystographic agents might cause oliguria under certain circumstances by physical blockage(14), it has been of interest to determine the extent to which the comparatively insoluble contrast media are excreted in the urine in the non-conjugated form.

Materials and methods. Three inmates of the Oklahoma State Prison served as subjects for this experiment.‖ Each received orally, in experimental cross-over design, 3 g iopanoic acid, 4.5 g sodium tyropanoate, or 4.5 g of sodium bunamiodyl. There was a lapse of 2 weeks between the first and second medications, and a lapse of 4 weeks between the second and third medications. The radiopaques were given at 9 P.M. on the first day

* Available from Winthrop Laboratories, New York, under the trade name of Telepaque®.

† Winthrop Laboratories registered name for tyropanoic acid is Bilopaque.

‡ Although ref. 5 makes it clear that this statement applies specifically to the dog the idea has become fairly widespread that it also applies to man (see ref. 9).

‖ These samples were collected under the supervision of Dr. C. K. Wisdom of Stough-Wisdom Research, Inc.

§ Bunamiodyl is a product of E. Fougera Co., Hicksville, N. Y. (trade name, Orabilex).

TABLE I. Urinary Excretion of 3 Radiopaque Compounds in Man Following Ingestion of Standard Diagnostic Dosages* (Data Given as % of Dose).

Radiopaque	Subject	Time after administration, hr 0–12	12–36	36–60	60–84	84–108	0–108
Iopanoic acid	A	3.1 (.17)†	16.5 (2.16)	5.5 (.51)	1.9 (.09)	.4 (.02)‡	27.4 (2.95)
	B	6.7 (.15)	15.0 (.39)	7.3 (.20)	7.0 (.13)	4.0 (.09)	40.0 (.96)
	C	13.5 (.38)	10.9 (.53)	9.2 (.30)	5.2 (.23)	4.9 (.32)	43.7 (1.76)
	Mean	7.8 (.23)	14.1 (1.03)	7.3 (.34)	4.7 (.15)	3.1 (.14)	37.0 (1.89)
Tyropanoate	A	17.9 (1.91)	16.2 (1.33)	7.0 (.71)	2.1 (.23)	.8 (.04)	44.0 (4.22)
	B	23.0 (2.70)	18.8 (1.58)	12.8 (.92)	6.5 (.35)	2.0 (.16)‡	63.1 (5.71)
	C	16.1 (1.02)	17.6 (.81)	3.4 (.25)	2.4 (.07)	2.2 (.08)	42.7 (2.23)
	Mean	19.0 (1.88)	17.5 (1.24)	7.7 (.63)	3.7 (.07)	1.7 (.09)	49.9 (4.04)
Bunamiodyl	A	17.4 (1.27)	9.8 (.58)	5.3 (.45)	1.6 (.07)	.5 (0)	34.4 (2.37)
	B	14.8 (.87)	18.1 (1.57)	6.8 (.57)	3.3 (.23)	2.4 (.11)	45.4 (3.35)
	C	26.8 (.91)	16.5 (.38)	5.4 (.22)	2.9 (.15)	1.3 (.04)‡	52.9 (1.70)
	Mean	19.7 (1.02)	14.8 (.84)	5.8 (.41)	2.6 (.15)	1.4 (.05)	44.2 (2.41)

* 3 g iopanoic acid, 4.5 g of tyropanoate and bunamiodyl.
† Values in parentheses represent extractable (presumably unchanged) radiopaque.
‡ Samples too small to permit this determination; values estimated.

of the experiment; urine was collected quantitatively for the first 12 hours, and then for four 24-hour periods thereafter. Aliquots of these samples were shipped (frozen) to this laboratory, where they were analyzed for total organic iodine by chloric acid digestion (15). Aliquots buffered at pH 4.5 were also extracted with 3 vols of chloroform/hexane = 1/1. This procedure removes the unchanged drugs quantitatively, but would not be expected to remove more than traces of glucuronide conjugates. Organic iodine was determined in the extracts after evaporation of the solvents, to establish the partition of the excretory products between unchanged drugs and conjugates. The results are presented in Table I.

Solubility of radiopaques as affected by pH. It proved to be very difficult to determine the solubility of the radiopaques in urine, since on acidification they precipitate out in a form so finely divided that filtration is virtually impossible. Accordingly, a simulated urine was prepared by dissolving 30 g urea and 11 g disodium phosphate dodecahydrate in a liter of water. To about 200 ml of this solution the radiopaque was added, as the sodium salt, in sufficient amount so that on adjustment of the pH to 7.5 a slight but definite precipitation would occur. With the aid of filter cel and repeated filtration this suspension was clarified, and an aliquot was removed for analysis. The pH was then adjusted successively to 7.0, 6.5, 6.0, 5.5, and 5.0, each time removing a clarified aliquot sufficient for analysis. The results of this experiment are presented in Table II. They show little change in the aqueous solubility of bunamiodyl and tyropanoate over the pH range 6.5-7.5, but a decrease in solubility of about 87% for each pH unit below 6.5. This would be expected from their pKa values of approximately 5.5.¶ Iopanoic acid, however, begins to precipitate immediately and in quantity as the pH falls below 7.5 and its solubility decreases by about 87%, for each pH unit below 7.5. (Since these data were obtained at room temperature, it may be anticipated that the solubilities at 37°C would be somewhat higher.) At the normal pH of

TABLE II. Solubilities of 3 Radiopaque Compounds in 3% Aqueous Urea, 0.03 M Phosphate, at Various pH Values. (Solubilities given as mg %, calculated as the acid.)

pH	Iopanoate	Tyropanoate	Bunamiodyl
7.5	418	520	373
7.0	83	520	373
6.5	23	512	373
6.0	7.3	224	192
5.5	2.5	73	59
5.0	1.4	29	22

¶ The pKa values of iopanoate, tyropanoate, and bunamiodyl titrated in 50% methanol have been determined by Dr. H. E. Jorgensen of this Institute as 5.83, 5.68, and 5.48, respectively. However, when iopanoate is titrated in water, an apparent pKa of 6.2 is obtained.

urine, therefore, bunamiodyl and tyropanoate are about 25 times as soluble as iopanoate.

Discussion. The mean total excretion of iodine in the urine (37% in 108 hours) found herein following administration of iopanoic acid agrees well with that previously reported (1), especially considering the fact that the present report deals with only 3 cases. The earlier findings on tyropanoate have also been essentially confirmed (50% of the dose excreted in 108 hours, as compared to 47% (2)). The excretion of bunamiodyl in the urine of man found herein is much less than the 70% observed in the dog, but the 44% excreted by man in 108 hours does correlate quite well with the 25% reported for a 30-hour period(7). In fact, subject A excreted almost exactly 25% of the bunamiodyl dose in the first 30 hours.

The mean fraction of the radiopaques excreted in an extractable (and, presumably, unchanged) form is the highest for tyropanoate and the lowest for iopanoic acid, but averages less than 10% of the total urinary iodine for all 3 compounds. When account is taken of the aqueous solubilities as shown in Table II, it does not appear that precipitation in the renal tubules would represent a serious hazard for any of these compounds. For example, taking the extreme cases from Table I the following relationships hold:

1) For iopanoic acid, subject A excreted in the 12-36-hour period 2.16% of the dose (65 mg) in unchanged form. Urine volume was 1250 ml; therefore, this concentration (5.2 mg%) would exceed the solubility of the compound at pH 5.5, but not at pH 6. The same mg% concentration was found in the 0-12-hour sample from this subject, which was of very small volume (100 ml). However, the concentration of unchanged iopanoate did not exceed its solubility at pH 5 in any other sample from this, or the other 2 subjects.

2) For tyropanoate, subject B in the 0-12-hour period excreted 2.70% of the dose (120 mg) in a volume of 600 ml. This concentration (20 mg%) is considerably less than the solubility of the compound at pH 5.

3) For bunamiodyl, subject A in the 0-12-hour period excreted 1.27% of the dose (57 mg) in a volume of 445 ml. This concentration (13 mg%) also does not exceed the solubility of the compound at pH 5.

The experiments reported herein were performed on subjects apparently quite normal with respect to their state of hydration, urine flow rates, and urinary pH. One cannot assume without qualification or reservation, therefore, that the same absence of hazard of crystalluria characterizes patients who are not normal with respect to these parameters. It is also obvious that a different situation may exist when, as a result of poor gallbladder visualization following a standard dose, the patient is after a very brief interval given 2 or 3 times the recommended amout of the cholecystographic agent. However, recent clinical observations(12) suggest that solubility relationships *per se* probably have no bearing on the problem of renal shutdown in cholecystographic procedures.** As has been shown above, the standard dose of bunamiodyl in normal subjects does not produce urinary concentrations of unchanged drug even approaching its limiting solubility at pH 5. Yet this compound has caused numerous cases of renal shutdown(16,17). However, Wennberg *et al*(12) have demonstrated that bunamiodyl causes a significant decrease in creatinine clearance in man, and this suggests that the renal shutdown may be caused by a specific biochemical mechanism. They also found that the less soluble iopanoate causes only a very moderate decrease in creatinine clearance. At least one of the cases of renal shutdown following iopanoate administration has been complicated in interpretation by the known prior administration of another cholecystographic agent(12).

Summary. The excretion of 3 oral cholecystographic agents in the urine has been studied in 3 normal subjects in experimental cross-over design, following administration of standard diagnostic dosages. For the 0-108-hour period the total mean excretion, based on organic iodine determination was: for iopanoate, 1.1 g (37%), for tyropanoate 2.2 g (49.9%), and for bunamiodyl, 2.0 g

** It is true that "microcrystals" were observed in the renal tubules of one of the cases of fatal oliguria following bunamiodyl(9), but these were not specifically demonstrated to be crystals of the radiopaque.

(44.2%). For all 3 compounds less than 8% of the urinary iodine was in a solvent-extractable (presumably unchanged) form. In view of the relatively small amounts of the radiopaques excreted in the urine in an unchanged form, and their solubilities at physiological pH values, it does not appear that renal damage due to actual physical blockage should represent a significant hazard. Nevertheless, the suggestion of Nelson(14) that these compounds should be accompanied by an adequate fluid intake seems in order.

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Biologic Relationship of Endotoxin and Other Toxic Proteins. V. Alterations of Blood-Brain Barrier and Susceptibility to Snake Venom.* (30368)

EDWARD V. STAAB,† ROBERT A. GOOD,‡ AND RICHARD M. CONDIE§
Pediatric Research Laboratories, Variety Club Heart Hospital, University of Minnesota, Minneapolis

Hypersusceptibility to water moccasin venom and a variety of other snake venoms has been established in rabbits by parenteral administration of Gram-negative endotoxin(1). The physiologic locus of the endotoxin-induced hypersusceptibility state (EIHS) is unknown. The role of the central neurotoxic component of venom has been suggested by various observations in the course of these studies. First, the early death of the endotoxin-treated animals challenged with moccasin venom is preceded by labored respirations, convulsions, paresis, and a variety of autonomic symptoms, indicating profound effects on the nervous system.

Second, of the 3 major families of venomous snakes, Elapidae, Viperidae, and the Crotalidae, only venoms from the latter 2 have been associated with central neurotoxic components. The Elapidae venoms, with the exception of tiger snake venom, did not elicit the typical EIHS; 2 of 3 Crotalidae venoms and the single Viperidae venom used did(2-6).

The third factor suggesting the possible importance of central neurotoxins was the earlier demonstration(7) that parenteral administration of *E. coli* endotoxin alters the

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† Medical Fellow, Dept. of Radiology, Univ. of Minnesota.
‡ American Legion Memorial Heart Research Professor of Pediatrics and Microbiology.
§ Research Fellow, Cardiovascular Clinical Research Center, Grant 6314.