Suppression of Tubular Secretion of Urate by Pyrazinamide in the Dog.* (26790)

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Pyrazinoic acid and its amide, pyrazinamide (an antituberculous agent), decrease renal excretion of urate in man, causing hyperuricemia(1-3). This is not due to less urate filtered at the glomerulus(3), hence the fall in C_{urate} must be ascribed to modification in the tubules, but whether it is the result of decreased tubular secretion or increased tubular reabsorption of urate has not been determined(3). The present study, using stop-flow and standard clearance procedures, indicates that, at least in the Dalmatian coach hound and apparently also in the non-Dalmatian dog, pyrazinamide suppresses tubular secretion of urate.

Methods. Eight experiments were carried out in female Dalmatian coach hounds, 7 in female mongrel dogs. The dogs weighed 17-21 kg. Anesthesia was not used, except for stop-flow studies (pentobarbital sodium, 30 mg/kg). Standard renal clearance technics were employed, usually under conditions of moderate mannitol or saline diuresis, with appropriate amounts of creatinine and urate added to the infusion. In 7 standard clearance experiments a priming dose of 0.5 g pyrazinamide was delivered rapidly, within 6 minutes, without concomitant mannitol diuresis; in the remaining studies a priming dose of 1.0 or 0.75 g pyrazinamide was delivered over 18 to 27 minutes, and mannitol diuresis was employed. Following the priming dose, a sustaining infusion of pyrazinamide was given at a constant rate, variously 2 to 10 mg/min. Urine and plasma specimens were collected for 56 to 87 minutes after pyrazinamide priming. Each collection period was 10 to 15 minutes in duration.

Three stop-flow studies were carried out, as previously described(4). Control stop-flow collections were first made, then pyrazinamide was infused as described. After 45 to 60 minutes, when a maximum pyrazinamide effect was anticipated, stop-flow urine samples were again collected. The analytical technics employed were as previously described(4).

Results. Dalmatian coach hound. Table I cites a representative standard clearance study, showing a fall in $C_{urate}/C_{creatinine}$ from a mean control ratio of 1.40, indicative of tubular secretion of urate, to a minimum ratio of 0.91 after infusion of pyrazinamide. Table II indicates definite declines in $C_{urate}/C_{creatinine}$ in 5 of 8 such experiments, little or none in the others in which only 0.5 g pyrazinamide was given to prime. C_{urate} fell sharply in the 3 experiments in which a 1.0 g priming dose was used. The glomerular filtration rate, as measured by $C_{creatinine}$, held relatively steady in most experiments; UV_{urate} was variably affected; P_{urate} tended to rise with constant urate infusion rates.

Fig. 1 gives the results of a stop-flow study before and after injection of pyrazinamide in a Dalmatian coach hound infused with urate at a rate of 5 mg/min. In the prestasis free flow periods of the control study, the mean $U/P_{urate}:U/P_{creatinine}$ ratio was 1.34, with urine flow 8.2 ml/min., P_{urate} 3.0 mg% and $U/P_{creatinine}$ 4.11 ml/min. Poststasis, the $U/P_{urate}:U/P_{creatinine}$ ratios also uniformly exceeded unity, with a peak value of 2.0 in the proximal segment (*i.e.*, in urine samples constituting the 80-100% aliquot of the tubular volume and the site of peak PAH secretion); and in the more distal tubular segments the ratios approximated 1.58. In the poststasis free flow periods the $U/P_{urate}:U/P_{creatinine}$ ratios returned to prestasis levels. After injection of pyrazinamide the proximal and distal secretory peaks for urate were almost completely abolished (Fig. 1), the $U/P_{urate}:U/P_{creatinine}$ ratios now approximating unity. Secretion of PAH, in contrast, was comparatively little affected by pyrazinamide.

Mongrels. In the experiments with non-Dalmatian dogs (Table II), net tubular se-

* Supported in part by a grant-in-aid from Nat. Inst. of Arthritis and Metab. Dis., N.I.H.

TABLE I. Representative Experiment Showing Effect of Pyrazinamide on Urate Excretion in Dalmatian (#2147, 16.5 kg, ♀).

Time, min.	V, ml/min.	$C_{creatinine}$, ml/min.	P_{urate}, mg %	UV_{urate}, mg/min.	C_{urate}, ml/min.	$\frac{C_{urate}}{C_{creatinine}}$
0– 5	Infuse creatinine 2.0 g					
5	S_1 started: 0.175% creatinine, 15% mannitol, 0.05% uric acid, 10 ml/min.					
45– 75	15.5	58.0	2.9	2.35	81.0	1.40
75– 96	Pyrazinamide 1.0 g intrav.					
96	S_2 replaces S_1: Pyrazinamide 1.0 g in 1000 ml S_1, 10 ml/min.					
96–105	17.7	49.3	4.0	2.20	54.8	1.11
105–135	22.7	57.8	4.9	2.73	55.6	.96
135–155	22.2	57.6	5.3	2.90	54.8	.95
155–173	19.4	51.8	5.5	2.60	47.2	.91

cretion of urate was not demonstrated ($C_{urate}/C_{creatinine} < 1$) presumably because urate loads and mannitol diuresis were moderate and adjuvant uricosuric agents were not employed (cf. #4). Nevertheless, some decline in $C_{urate}/C_{creatinine}$ occurred in the 5 experiments in which 1.0 or 0.75 g pyrazinamide was given as a priming dose. There was no consistent effect on glomerular filtration rate; changes in UV_{urate} and P_{urate} were variable. In general, the effects of pyrazinamide in the mongrels were similar to those observed in the Dalmatians but the decrease in C_{urate}/GFR was somewhat less pronounced.

The results of a stop-flow experiment before and after injection of pyrazinamide are shown in Fig. 2. In the control study, $U/P_{urate}:U/P_{creatinine}$ averaged 0.62 in both prestasis and poststasis free flow periods, with urine flow 11.8 ml/min, P_{urate} 8.4 mg%, $U/P_{creatinine}$ 2.99 ml/min. A characteristic dip in the $U/P_{urate}:U/P_{creatinine}$ curve, indicative of net tubular reabsorption of urate, is noted in the proximal tubule. As shown in Fig. 2, this proximal segment of the curve was not altered by pyrazinamide, but there was a discernible decline in $U/P_{urate}:U/P_{creatinine}$ ratios in the more distal portions of the tubule. Little suppressive effect on PAH

TABLE II. Effect of Pyrazinamide on Urate Excretion in Dogs.

Dog	PZA dose: Prime, g	PZA dose: Sustaining, mg/min.	V, ml/min. C	V, ml/min. E	$C_{creatinine}$, ml/min. C	$C_{creatinine}$, ml/min. E	P_{urate}, mg % C	P_{urate}, mg % E	UV_{urate}, mg/min. C	UV_{urate}, mg/min. E	C_{urate}, ml/min. C	C_{urate}, ml/min. E	$\frac{C_{urate}}{C_{creatinine}}$ C	$\frac{C_{urate}}{C_{creatinine}}$ E
						Dalmatian								
2147	1.0	10.0	15.5	20.8	58.0	54.7	2.9	5.4	2.35	2.75	81.0	50.9	1.40	.93
2190	1.0	10.0	11.0	20.1	51.5	69.8	3.5	5.2	2.53	3.05	72.3	43.7	1.40	.63
2190	1.0	10.0	20.0	21.6	62.6	71.1	3.1	3.9	2.92	2.37	90.6	60.8	1.45	.86
1834	.5	15.0	32.0	30.1	76.1	70.1	5.8	7.6	5.69	6.54	98.0	86.0	1.29	1.23
"Mat."	.5	5.5	3.7	4.8	81.6	80.4	1.3	1.9	.82	1.06	63.1	55.8	.77	.69
"Mar."	.5	4.0	1.0	1.7	27.8	34.1	3.0	4.0	.85	.92	28.3	23.0	1.02	.68
"Mar."	.5	2.0	.8	.8	25.4	31.0	1.4	1.4	.73	.71	52.2	50.8	2.06	1.63
"Vi."	.5	2.0	3.9	6.6	80.4	84.0	2.2	1.8	2.16	1.96	98.2	109	1.22	1.30
						Non-Dalmatian								
2334	1.0	10.0	15.3	22.4	73.4	85.4	1.9	2.0	.86	.87	45.3	43.5	.62	.51
2316	1.0	10.0	13.0	21.1	48.9	56.4	2.6	4.9	1.07	1.54	41.2	31.4	.84	.56
2508*	1.0	8.5	17.1	25.3	81.3	83.3	7.8	9.7	4.54	5.07	58.2	52.3	.72	.63
2453*	1.0	10.0	26.6	22.6	71.0	58.0	8.6	11.4	3.98	3.32	46.3	29.1	.65	.50
2334	.75	9.0	25.1	21.8	90.0	84.8	4.0	3.9	2.83	2.18	70.8	55.9	.79	.66
"Ti."	.5	4.0	1.0	5.0	63.3	76.6	2.3	2.8	.47	.78	20.4	27.9	.32	.36
"Bl."	.5	4.0	.6	2.1	66.4	67.7	4.7	4.0	1.62	1.39	35.5	34.8	.53	.51

C = mean of 3 or 4 control periods. E = mean of last four 10-15 min. experimental periods.

* Urate was infused at 32 mg/min.; in the remaining experiments rate of infusion ranged between 2-10 mg/min.

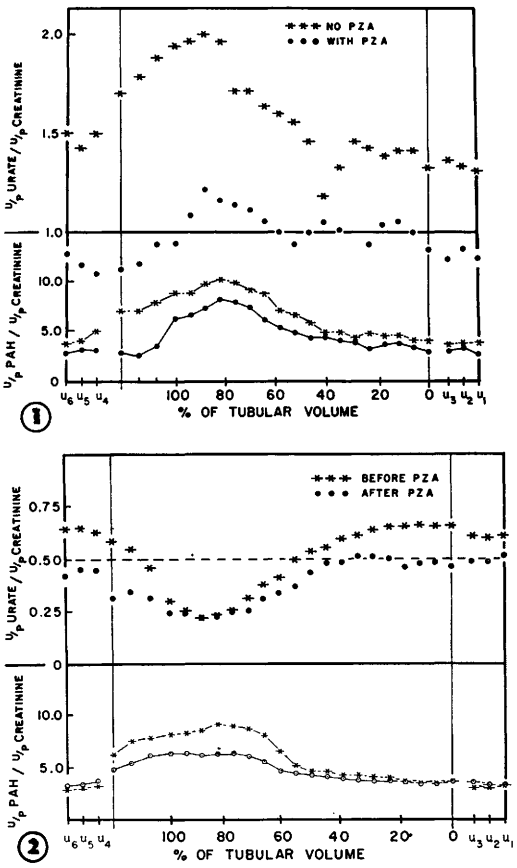

FIG. 1. Stop-flow $U/P_{urate}:U/P_{creatinine}$ ratios (above) in a Dalmatian dog before and after inj. of pyrazinamide. Proximal and more distal secretory urate peaks were almost completely abolished by pyrazinamide. There was little decrease in PAH secretion (lower curves).

FIG. 2. Stop-flow $U/P_{urate}:U/P_{creatinine}$ ratios (above) in a mongrel before and after inj. of pyrazinamide. The proximal trough, indicative of tubular reabsorption of urate, was unaffected by pyrazinamide whereas U/P ratios of the more distal peak were reduced. There was comparatively little decrease in PAH secretion (lower curves).

secretion was again noted.

Discussion. The Dalmatian coach hound is peculiarly suited to investigation of the problem at hand, that of determining whether a decline in C_{urate}/GFR is due to decreased tubular secretion or increased tubular reabsorption of urate. This distinction ordinarily cannot be made in over-all clearance studies but, in the Dalmatian, since tubular reabsorption of urate is defective whereas urate secretion is readily demonstrable(4,5), it seems permissible, because of this dissociation, to attribute lowering of C_{urate}/GFR by a drug to suppression of tubular secretion of urate. It is shown that pyrazinamide in appropriate dosage does, in fact, unequivocally depress C_{urate}/GFR in this species. The inference that this is due to suppression of tubular secretion of urate is fully borne out by the demonstration of abolition of the secretory peaks for urate in stop-flow curves.

It is shown further in these studies that pyrazinamide in appropriate dosage also depresses C_{urate}/GFR in the non-Dalmatian dog, although apparently somewhat less markedly. This effect likewise appears to be due to suppression of tubular secretion of urate. The conditions of the experiments herein cited were not such as to demonstrate net secretion of urate in mongrels (cf. 4). Nevertheless, stop-flow studies reveal no alteration by pyrazinamide in the low U/P_{urate}: $U/P_{creatinine}$ ratios in the proximal tubule, the site of net reabsorption of urate in the mongrel(4,5), but a distinct decline in the higher $U/P_{urate}:U/P_{creatinine}$ ratios in the more distal tubular segments, where net secretion of urate can be demonstrated, under appropriate experimental conditions, in the non-Dalmatian as well as Dalmatian dog (4).

Pyrazinamide causes much less reduction in C_{urate}/GFR in the dog, certainly in the mongrel, than in man(3). The qualitative effects on urate clearance and other discrete renal functions appear to be comparable, however. This applies also to the relative immunity of PAH to pyrazinamide in man (3) and dog, at least when compared to other drugs affecting tubular transport of urate, such as probenecid(6). In the chicken, on the other hand, pyrazinamide has no effect on tubular secretion of urate, except in massive dosage(7). The chicken differs markedly in its response to a variety of other drugs which affect tubular transport of urate in man and dog in a similar manner(7).

The results in the dog may well bear upon the original question(3), whether urate retention caused by pyrazinamide in man is due to decreased tubular secretion or increased tubular reabsorption of urate. At the time these alternatives were suggested,

tubular reabsorption of urate was well known to occur in man(8) whereas evidence for tubular secretion of urate, except perhaps for one abnormal instance(9), was lacking. Such evidence has since been secured, under appropriate experimental conditions, in normal man(10), as well as in the rabbit(11) and dog(4,5,12), suggesting that the mammalian kidney does indeed possess the capacity for tubular secretion of urate. There appears to be at least one precedent for inhibition of tubular secretion of urate in man, the retention of urate caused by salicylate in low dosage, presumably by competition with urate for active tubular transport(13).

Summary. Pyrazinamide depresses C_{urate}/GFR in the Dalmatian and non-Dalmatian dog. Stop-flow studies revealed unequivocal suppression of tubular secretion of urate in the Dalmatian and gave no indication of enhancement of tubular reabsorption of urate in the non-Dalmatian.

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Received June 7, 1961. P.S.E.B.M., 1961, v107.

Studies on the Sex Difference in Toxicity of a Cholinergic Phosphorothioate.* (26791)

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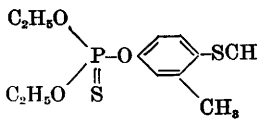
A sex difference in the acute toxicity of parathion to rats was observed in this laboratory in 1949(1). Subsequent studies have established that male rats exhibit greater resistance than females to a number of other phosphorothioates. The mechanism underlying this effect has been difficult to investigate in intact animals because the magnitude of the sex difference in susceptibility is generally relatively small. During measurements of the influence of structural changes on the toxicity of some phosphorothioates we observed a 10-fold sex difference in susceptibility of rats to 0,0-diethyl 0-(4-methylthio-m-tolyl) phosphorothioate (DMP; Bayer 29492). This compound has the following chemical structure:



The magnitude of the sex difference in susceptibility of rats to this phosphorothioate was sufficient to permit a study of factors which might be responsible for the greater resistance of male rats. The present communication describes the mammalian toxicity and anticholinesterase activity of DMP and provides evidence that the liver, under the influence of androgens, is responsible for the resistance of males toward the acute toxicity of this organic phosphate.

* Supported by a grant from U. S. Public Health Service.