in 2.5 ml Krebs-Ringer bicarbonate buffer containing 10 μM of glucose and 1.38 μM of radioactive meprobamate. At the end of 2 hours, the incubation mixture was homogenized, made alkaline, and the meprobamate was extracted with ether and counted in a liquid scintillation counter. The results shown in Table IV indicate that liver metabolizes the drug to a greater extent than do the other tissues examined. Following intravenous injection of 4 mg/kg of H^3 meprobamate significant levels of radioactivity were found in all tissue examined (liver, kidney, spleen, brain, testes, muscle, fat, spinal fluid, and blood).

Summary. (1) A specific method for the determination of meprobamate in urine is described. (2) Metabolic studies indicate that about 12 to 20 percent of the unchanged drug is slowly excreted in the urine in 48 hours and that additional products are formed, including several conjugates at least one of which is a glucuronide. (3) Radioactive meprobamate has been prepared and metabolic studies have been performed. The liver is the principal site of metabolic alteration.

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Effect of Pyrazinamide and Pyrazinoic Acid on Urate Clearance and Other Discrete Renal Functions.*† (23450)

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Cullen *et al.*(1) and Gleason *et al.*(2) recently reported the occurrence of marked hyperuricemia, associated with decreased urinary urate excretion, after administration of the antituberculous agent pyrazinamide (pyrazinoic acid amide). The present report summarizes the results of clearance studies intended to determine more precisely the effect of the drug on the renal mechanisms regulating urate excretion. When it became apparent in the course of the study that the free acid (pyrazinoic acid) might be the active agent, appropriate studies with this compound also were initiated.

Methods. Fifteen male adults were available for study. Ten of these were gouty, in the intercritical phase of the disorder, and afforded opportunity to determine whether the drug could exert its effects in the face of already established hyperuricemia and, in some instances, excessive urinary urate excretion. To ascertain whether the renal effects occurred soon enough after oral administration of the drug to permit application of conventional renal clearance technics, preliminary experiments were performed in 5 subjects who received single oral doses of 3 g or 1 g. Determinations of the urate excreted in urine specimens spontaneously voided at hourly or 2-hourly intervals indicated a sufficiently prompt response, even with 1.0 g dosage. Standard renal clearance technics (3) were then employed in 10 subjects. After appropriate control periods, in which clearances of inulin, p-aminohippurate and urate were measured, the drug in 1 g or 3 g dosage was given by mouth; pyrazinamide in 4 experiments, pyrazinoic acid in 6 including 2 with 50 and 100 mg dosage. Post-medication urine collections, in 15-20 minute periods,

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TABLE I. Time of Onset and Duration of Effect of Orally Administered Pyrazinamide on Serum and Urinary Urate in 2 Gouty Subjects.

Subject	Time (hr)	Urate	
		Serum (mg %)	Urine (mg/hr)
H.G.	−2	8.0	13.6
	Pyrazinamide, 3 g oral dose		
	2	10.5	11.4
	4	9.8	3.0
	6	9.8	1.3
	10	—	1.5
	18	—	2.4
	24	10.2	—
F.C.	−2	9.6	19.1
	Pyrazinamide, 1 g oral dose		
	1	—	20.7
	2	—	9.1
	3	—	2.6
	4	—	3.7
	6	—	7.8
	12	—	9.0
	24	12.8	—

were continued for 2-3 hours. In 3 experiments the effect of the drug on Tm_{PAH} was measured. Inulin, PAH and urate were determined by the standard methods indicated elsewhere(4); many duplicate urate determinations were made also by ultraviolet spectrophotometry. Ultrafiltration of serum urate before and after medication was performed as described elsewhere(5). Pyrazinoic acid amide and pyrazinoic acid concentrations in urine were measured by the method of Allen *et al.*(6).

Results. 1. *Time of onset and duration of response to orally administered pyrazinamide.* Table I summarizes the results of 2 representative experiments in gouty subjects given single doses of 3 and 1 g pyrazinamide respectively by mouth. Reduction in urinary urate excretion was apparent within 2 hours, reached a maximum in 2-6 hours, and persisted for the duration of the observations, 12-18 hours. The serum urate level rose within 2 hours and was still elevated after 24 hours.

2. *Effect of pyrazinamide on C_{urate}, C_{inulin}, C_{PAH}, Tm_{PAH}.* In 3 experiments with 1 g dosage of pyrazinamide, UV_{urate} consistently showed some decrease in 15-40 minutes and progressively declined over the 2 hours of observation. There was no significant increase in plasma urate in this period. C_{urate} fell correspondingly, from a pre-medication mean of 6.2 ml/min to a mean minimum of 1.8 ml/min, a decline to 29% of the initial value during the period of observation. There was no decrease in C_{inulin}, consequently the ratios C_{urate}/C_{inulin} paralleled C_{urate}. C_{PAH} fell from a mean of 485 to 390 ml/min. Tm_{PAH} was not affected. Table II cites the results in representative experiments. In 3 g dosage the effects were similar but more pronounced (Table II). C_{urate} fell from 8.9 to 0.5 ml/min, or 5.5% of the initial level. There was a decline in C_{inulin} and C_{PAH}.

3. *Effect of pyrazinoic acid on C_{urate}, C_{inulin}, C_{PAH}, Tm_{PAH}.* In 3 experiments with 1 g dosage of pyrazinoic acid, UV_{urate} consistently showed a sharp decline in 15-30 minutes and reached minimum levels in 15-60 minutes (Table II), without any change in plasma urate during this period. C_{urate} fell precipitously from a pre-medication mean of 10.1 ml/min to a mean minimum of 1 ml/min. There was no change in C_{inulin} or C_{PAH}. Tm_{PAH} fell somewhat, from 73.7 to 55.5 mg/min in Case J.F. and from 65.1 to 53.1 mg/min in Case V.E. In 3 g dosage the effects of pyrazinoic acid were essentially the same (Table II). Reduction in C_{urate} could be effected by pyrazinoic acid in doses considerably less than 1 g. Thus in one experiment a single orally administered dose of 100 mg lowered C_{urate} from 14.9 to 6 ml/min (40% of the initial value) and a second dose of 100 mg given one hour later reduced C_{urate} to 3.3 ml/min (22% of the initial value). An experiment in which 50 mg was given orally resulted in a fall in C_{urate} from 8.1 to 4.7 ml/min (58% of the initial value).

4. *Rate of urinary excretion of pyrazinoic acid and pyrazinamide.* It is apparent from the preceding data that pyrazinamide and pyrazinoic acid are rapidly absorbed from the gastrointestinal tract. In the body, pyrazinamide is largely hydrolyzed, pyrazinoic acid being the principal primary product of hydrolysis(6). Urinary excretion of the drug is slow. After oral administration of 1 or 3 g of pyrazinamide, less than 1% of the administered dose appeared unchanged in the urine in 2 hours, 2-3% as the free acid. Pyrazinoic acid is more rapidly excreted by the kidneys, 25-30% appearing in the urine in 2 hours

TABLE II. Results of Representative Experiments Employing Clearance Technics (Values Corrected to Standard Surface Area, 1.73 M^2).

Subject	Period (min.)	UV_{urate} (γ/min.)	C_{urate} (ml/min.)	C_{inulin} (ml/min.)	C_{urate}/C_{in} (%)	C_{PAH} (ml/min.)	Tm_{PAH} (mg/min.)
L.W.*	-45 to 0	470	4.5	86.4	5.2	332	
		Pyrazinamide, 1 g given orally					
	0- 15	464	4.4	87.5	5.0	351	
	15- 30	325	3.1	81.5	3.8	308	
	30- 75	150	1.4	87.9	1.6	329	
	75-120	138	1.3	87.5	1.5	292	
M.E.*	-135 to -90	575	5.3	103	5.2	567	
	- 30 to 0	599	5.5	94.0	5.9		67.1
		Pyrazinamide, 1 g given orally					
	0- 40	525	4.8	94.4	5.1		68.1
	40- 80	366	3.4	90.7	3.8		63.8
	80-100	327	3.0	95.0	3.2		74.5
	100-120	313	2.8	100	2.8		72.8
S.Z.*	45 to 0	994	8.9	125	7.1	505	
		Pyrazinamide, 3 g given orally					
	0- 32	623	5.5	90.6	6.1	392	
	32- 60	210	1.9	92.5	2.1	410	
	60- 90	125	1.1	92.5	1.2	365	
	90-120	64	.6	84.0	.7	408	
	120-180	55	.5	80.0	.6	386	
D.C.	-45 to 0	486	7.3	111	6.6	544	
		Pyrazinoic acid, 1 g given orally					
	0- 15	415	6.2	106	5.9	550	
	15- 30	55	.9	101	.9	515	
	30- 60	60	.9	105	.9	520	
	60- 89	65	1.0	100	1.0	520	
	89-120	66	1.0	100	1.0	528	
J.F.*	-135 to -90	733	7.9	124	6.4	479	
	- 30 to 0	914	9.8	120	8.1		73.7
		Pyrazinoic acid, 1 g given orally					
	0- 19	827	8.9	122	7.3		73.7
	19- 38	195	2.1	140	1.5		70.5
	38- 58	126	1.3	132	1.0		59.8
	58- 98	119	1.3	139	.9		58.7
	98-158	128	1.3	123	1.0		55.5
J.A.	-45 to 0	554	6.8	118	5.8	443	
		Pyrazinoic acid, 3 g given orally					
	0- 15	540	6.4	115	5.6	439	
	15- 30	92	1.1	121	.9	467	
	30- 45	58	.7	121	.6	429	
	45- 75	59	.7	120	.6	433	
	75-120	64	.8	123	.6	428	

* Gouty subjects.

when administered orally in 1-3 g dosage.

5. *Side reactions.* Like nicotinic acid, which has a similar structure, pyrazinoic acid and pyrazinamide may elicit transitory peripheral vasodilatation and dizziness, particularly in 3 g dosage. This reaction was marked in Case S.Z. and may account in part for the fall in C_{inulin} and C_{PAH}. No other side reactions were noted. Of interest is the failure to precipitate acute gouty arthritis in any of our gouty subjects. This has been noted by others, but only after several weeks of therapy, and presumably only in patients with gouty diathesis.

Discussion. It has been pointed out previously(7) that a variety of uricosuric drugs, when given in low dosage, may cause a transitory fall in C_{urate}; occasionally to as low as 50% of the pre-medication level in the case of salicylate. The reduction in C_{urate} was

much more pronounced with pyrazinamide and pyrazinoic acid, however, consistently reaching levels of 5-10% of the control value when appropriate dosages were employed. No uricosuric effect was noted under the conditions of these experiments.

This striking reduction in C_{urate} cannot be ascribed to a decrease in filtered urate. It occurred when C_{inulin} was not affected, and ultrafiltration of the serum urate in 2 cases, before and again 4 and 8 hours after medication when the serum urate was distinctly elevated, showed no change in the virtually complete filtrability of the serum urate. It would seem to follow that the reduction in C_{urate} was due to enhanced tubular reabsorption of urate, calculated to exceed 99% of the filtered load in most cases. Another possibility, previously considered(7), is that tubular secretion of urate may occur in man, and that the drugs in question inhibit this process. In this event, the marked (in large dosage almost complete) suppression of UV_{urate} implies that the filtered load of urate presented to the tubules is virtually completely reabsorbed, and that the urate appearing in the urine derives largely from tubular secretion of urate. This process, then, would be analogous to that postulated for potassium.

In this connection, the relative independence of tubular secretory mechanisms for PAH might be pointed out. Previous studies with probenecid(4) and phenylbutazone(8), in doses which markedly increase C_{urate} by inhibition of tubular reabsorption of urate, showed more marked lowering of Tm_{PAH} in equivalent dosage. Beyer(9) has commented in some detail on this aspect of the problem of tubular transport mechanisms.

The data indicate that pyrazinoic acid, a product of hydrolysis of pyrazinamide in the body, produces more rapid and more marked reduction in C_{urate} than does pyrazinamide in equivalent dosage. This suggests that the free acid may be largely or wholly responsible for the effect on urate transport.

Summary. 1. The effect of pyrazinamide and pyrazinoic acid on the renal clearance of urate, inulin and p-aminohippurate, in some instances also Tm_{PAH}, was studied in 10 human subjects. 2. A rapid and marked reduction in urate clearance was noted, without decrease in the filtered urate load. The implications as to the renal mechanisms regulating urate excretion are discussed. 3. Data indicating that the effect of pyrazinamide is ascribable largely to the action of pyrazinoic acid are presented.

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