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Localization and Pyrazinamide Inhibition of Distal Transtubular Movement of Uric Acid-2-C^{14} with a Modified Stop-Flow Technique *

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Renal tubular secretion of uric acid has been demonstrated in several mammalian species including the rabbit (1), mongrel and Dalmatian dog (2–6), and man (7). Although secretion was easily delineated in the Dalmatian coach hound (4–6), special experimental conditions were necessary for its substantiation in other species. Uric acid clearance in excess of simultaneous creatinine clearance, indicative of tubular secretion of urate, was noted by Poulsen and Praetorius in rabbits made hyperuricemic by the intravenous infusion of urate (1). In 1959, Gutman, Yü, and Berger (7) documented tubular secretion of uric acid in patients with reduced glomerular filtration, who were given infusions of uric acid and mannitol and treated with large doses of sulfinpyrazone in order to suppress tubular reabsorption. One year later tubular secretion of urate was reported in the mongrel dog under similar conditions of an osmotic diuresis and an intravenous infusion of uric acid (2, 3).

Efforts to localize the site of tubular urate secretion in the mongrel dog by stop-flow analysis have yielded conflicting results. Yü and his colleagues (3, 8) reported peak net tubular secretion in the distal segment of the nephron. However, Kessler, Hierholzer, and Gurd (6), utilizing the same experimental procedure except for the administration of probenecid, found no evidence for distal tubular secretion of urate.

Closely related to the question of tubular secretion of uric acid is the problem of the paradoxical effects of various drugs upon urinary uric acid excretion. In 1955 Yü and Gutman (9) noted that low doses of salicylates, phenylbutazone, and other uricosuric drugs caused decreased uric acid excretion and urate retention, whereas intermediate amounts had no effects upon the urate excretion rate, and large doses caused uricosuria. These findings have been explained by postulating inhibition of renal tubular secretion of urate at low doses and of tubular reabsorption with the larger amounts of drugs. At intermediate doses the effects on these two processes are approximately equivalent, and there is no net change in urate excretion. Later, administration of other drugs such as pyrazinamide and chlorothiazide was found to cause hyperuricemia by decreasing uric acid excretion without changing glomerular filtration (8, 10–13). This effect has also been attributed to inhibition of tubular secretion of uric acid.

The present study was undertaken in an attempt to define more clearly the site of renal tubular secretion of urate in the mongrel dog. The stop-flow technique was modified by the injection of uric acid-2-C^{14} into the renal artery just before the reestablishment of free flow. By this means it was possible to demonstrate transtubular movement of uric acid in the distal tubular segment and to abolish such movement by pretreatment of the dogs with pyrazinamide. Although the presence of uric acid-2-C^{14} in distal tubular urine suggests secretion, the data do not prove that there has been any net distal tubular secretion.

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Methods

Mongrel dogs weighing 15 to 20 kg were anesthetized with sodium pentobarbital. Both ureters were externalized via a flank incision and cannulated with a polyethylene tube. The left renal artery was then exposed at its origin from the aorta. With a Bowman constant infusion pump, 20% mannitol in normal saline was infused into an external jugular vein at a rate of 7.2 ml per minute. All dogs were also hydrated with 2.5% glucose in water given intravenously at a rate adjusted according to urine flow. When urine flow from the left kidney stabilized at a minimum of 7.0 ml per minute, a control sample of urine was collected for 1 minute. The catheter in the left ureter was then clamped for a period varying from 3 to 6 minutes.

Ten to 15 seconds before the release of the clamp, 5 μc of uric acid-2-C^{14} [1] (5 mc per mmole) or of inulin-C^{14} (2 μc per mg) in 0.5 ml of normal saline was injected into the left renal artery. Immediately upon release of the clamp, serial small (0.4- to 0.6-ml) urine samples were collected for 3 minutes or until a total of 20 ml of urine had been obtained. This was then followed by collection of another 1-minute control sample. Urine samples (0.1 ml) were pipetted into 3.9 ml of ethanol in a counting vial. Ten ml of toluene containing 2,5-diphenyloxazole (PPO), 0.4%, and 1,4-bis-2-(5-phenyloxazolyl) benzene

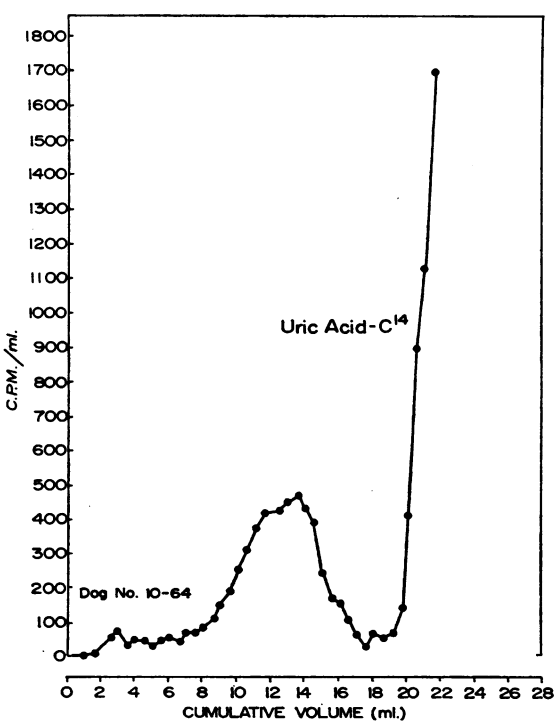

FIG. 1. DISTRIBUTION OF URIC ACID-2-C^{14} IN SERIAL URINE SAMPLES IN MONGREL DOGS.

[1] Volk Radiochemical Co., Chicago, Ill.

TABLE I

*Distribution of uric acid-2-C^{14} in serial urine samples in mongrel dogs**

	Per cent of cumulative volume																			
	0–5	5–10	10–15	15–20	20–25	25–30	30–35	35–40	40–45	45–50	50–55	55–60	60–65	65–70	70–75	75–80	80–85	85–90	90–95	95–100
	cpm/ml urine																			
Dog 1–63	0	14	10	26	32	55	57	57	100	100	116	45	29	29	18	543	1,680	4,519	7,140	9,761
Dog 2–63	3	30	0	11	49	107	111	91	62	56	35	33	297	431	1,140	3,710	5,436	6,413	7,743	9,669
Dog 3–63	0	0	9	0	0	0	83	107	74	67	59	59	58	902	17,230	19,480	23,182	26,182	29,143	33,410
Dog 4–63	20	203	281	69	77	89	131	105	163	163	168	236	243	1,116	8,133	15,967	35,946	51,060	52,372	57,788
Dog 6–63	20	7	5	28	32	134	264	21	34	37	71	114	318	516	1,088	1,927	3,425	4,145	4,975	5,007
Dog 6–64	174	184	187	216	338	618	914	1,423	1,900	2,543	3,226	0	22	41	56	79	134	166	168	4,841
Dog 10–64	0	49	49	46	59	66	152	248	378	412	743	602	424	386	456	485	2,089	2,578	8,469	12,366
Dog 22–64	0	3,025	2,897	2,488	2,854	2,404	988	0	0	0	0	0	142	504	1,356	2,320	3,413	4,313	6,091	8,680

* Sample no. 1 represents the first sample collected after the release of the ureteral clamp. The uric acid-2-C^{14} was injected into the renal artery 15 to 30 seconds before release of the clamp. The tube with the maximal radioactivity represents 100% of the cumulative volume.

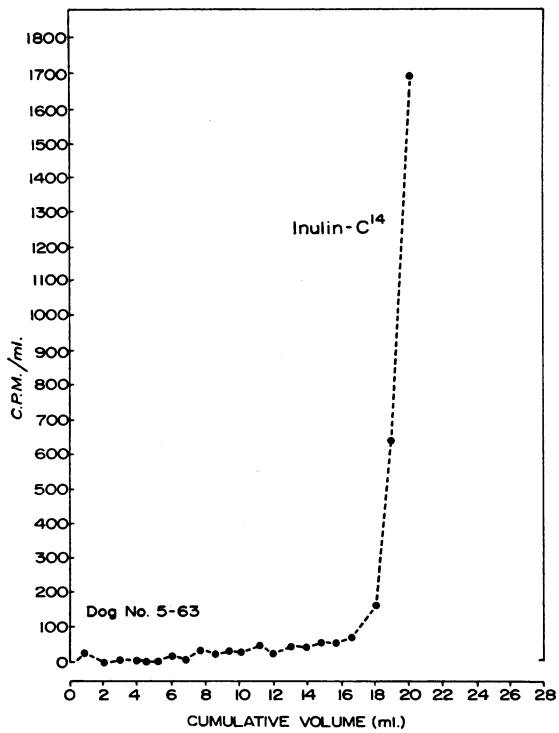

FIG. 2. DISTRIBUTION OF INULIN-C^{14} IN SERIAL URINE SAMPLES IN MONGREL DOGS.

(POPOP), 0.01%, was added and the radioactivity assayed in a Packard liquid scintillation counter. Quenching was monitored by the addition of internal standard and was found to be less than 10%. The data were corrected for quenching when present. Since the size of the serial samples was the same in any one dog but varied from dog to dog, the data in the tables are expressed as the cumulative per cent of the volume taking the sample with the highest number of counts as representing 100% of the cumulative volume. The discrepancy between the tables and the figures is due to the fact that in the latter the data are plotted as counts per minute per milliliter of the actual cumulative volume, which varied somewhat from experiment to experiment. For easier comparison, the data in the tables have been normalized on the basis of per cent of cumulative volume. Three of eight dogs that received injections of uric acid-2-C^{14} were also infused with sodium para-aminohippurate (PAH) and creatinine. This was done by adding 2.5 g of creatinine and 1.5 ml of a 20% solution of sodium para-aminohippurate per L of the mannitol solution. Creatinine was measured by the method of Bosnes and Taussky (14) and PAH by Smith and associates' method (15). Four other dogs were injected with inulin-C^{14} to serve as controls. The effect of pyrazinamide on uric acid-2-C^{14} secretion was studied as follows. Three dogs were given pyrazinamide, 0.5 g orally twice daily for 2 days. On the third day another 0.5-g oral dose was given in the morning. In the afternoon they were prepared in the manner previously described. Before the infusion of mannitol the dogs received 1.0 g of pyrazinamide iv over a 3-minute period. This was followed by a sustaining infusion of 10 mg per minute. Otherwise the experiments were performed as described above with 5 μc of uric acid-2-C^{14} being injected into the left renal artery 10 to 15 seconds before the release of the clamp.

Results

The results obtained when uric acid-2-C^{14} was injected into the renal artery 10 to 15 seconds before reestablishment of free flow of urine are shown in Figure 1 and Table I. In all cases there was an initial rise in radioactivity in the early tubes, followed by a return to base line and then a second more sustained rise indicating the appearance of new glomerular filtrate. The initial peak represents the addition of uric acid-2-C^{14} to the urine trapped within the distal segment of the nephron during a time when glomerular filtration had presumably ceased. Figure 2 represents the results obtained in one of the four dogs that were injected with inulin-C^{14}, and the data from all these experiments are shown in Table II. In contrast to the results with uric acid-2-C^{14}, there was no initial rise in radioactivity but only the second

TABLE II

*Distribution of inulin-C^{14} in serial urine samples in mongrel dogs**

	Per cent of cumulative volume																		
	0–5	5–10	10–15	15–20	20–25	25–30	30–35	35–40	40–45	45–50	50–55	55–60	60–65	65–70	70–75	75–80	80–85	85–90	90–95	95–100
	cpm/ml urine																			
Dog 5–63	0	24	0	12	0	21	5	23	24	29	56	27	46	58	55	64	164	1,204	4,563	37,396
Dog 1–64	0	0	0	15	0	0	1	23	10	9	8	19	19	20	62	466	3,167	8,154	13,935	13,957
Dog 7–64	1	1	3	0	6	8	0	10	40	521	1,463	5,974	7,006	7,232	8,101	8,421	8,632	9,107	9,483	10,950
Dog 21–64	0	0	0	41	64	61	132	149	139	304	495	1,160	1,931	2,896	3,933	5,212	6,698	7,715	8,557	9,834

* Sample no. 1 represents the first sample collected after the release of the ureteral clamp. The inulin-C^{14} was injected into the renal artery 15 to 30 seconds before the reestablishment of urine flow. The tube with the maximal radioactivity represents 100% of the cumulative volume.

TABLE III

*Effect of pretreatment with pyrazinamide on distribution of uric acid-2-C^{14} in serial urine samples in mongrel dogs**

	Per cent of cumulative volume																		
	0–5	5–10	10–15	15–20	20–25	25–30	30–35	35–40	40–45	45–50	50–55	55–60	60–65	65–70	70–75	75–80	80–85	85–90	90–95	95–100
	cpm/ml urine																			
Dog 3–64	0	0	11	0	4	19	14	0	23	46	92	98	144	129	129	124	223	332	924	4,007
Dog 8–64	0	25	52	14	22	35	29	28	63	100	103	103	148	152	226	846	2,806	5,203	18,319	15,710
Dog 9–64	13	16	4	5	0	2	5	0	11	15	10	9	15	55	30	55	88	105	235	361

* The dogs were treated with pyrazinamide for 2 days before the study. Sample no. 1 represents the first sample collected after the release of the ureteral clamp. The uric acid-2-C^{14} was injected into the renal artery 15 to 30 seconds before the reestablishment of urine flow. The tube with the maximal radioactivity represents 100% of the cumulative volume.

more sustained elevation representing the appearance of new glomerular filtrate. Inulin is known to be excreted by glomerular filtration alone, and therefore these experiments support the conclusion that the uric acid-2-C^{14} reached the urine by a transtubular route rather than by diffusion from the glomerulus.

Three dogs received creatinine and PAH in order to further aid in the tubular localization of the initial peak of uric acid-2-C^{14} radioactivity. Representative findings are shown in Figure 3. There is an initial rise in both PAH and creatinine concentrations in the early tubes due to distal reabsorption of water. This is followed by a second more sustained rise in PAH concentration alone, representing proximal tubular secretion of this substance. The initial peak of uric acid-2-C^{14} lies close to the distal creatinine and PAH peaks, thus localizing the transtubular movement of uric acid to a distal portion of the nephron. That the second or glomerular rise of uric acid radioactivity appears to coincide with the second PAH peak may be due to the greater sensitivity of the isotopic measurement compared to the chemical determination of PAH and the differing lengths of the nephron population.

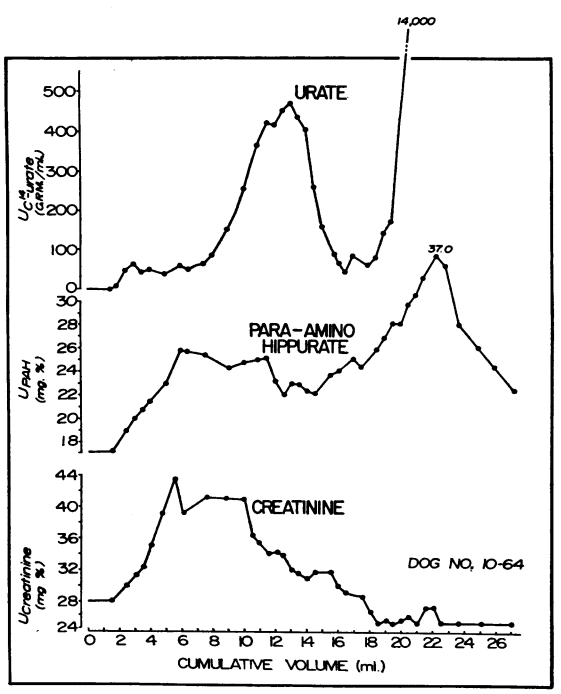

FIG. 3. LOCALIZATION OF URIC ACID-2-C^{14} TRANSTUBULAR MOVEMENT IN THE DISTAL PORTION OF THE NEPHRON. $U_{creatinine}$, U_{PAH}, and $U_{C^{14}\text{-urate}}$ = urinary creatinine, para-aminohippurate, and C^{14}-urate.

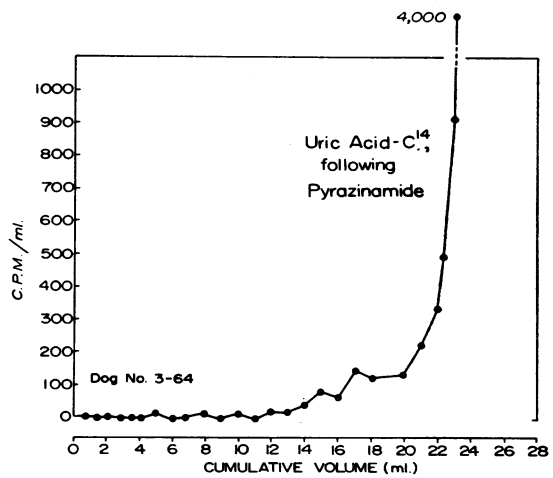

FIG. 4. EFFECT OF PRETREATMENT WITH PYRAZINAMIDE ON THE DISTRIBUTION OF URIC ACID-2-C^{14} IN SERIAL URINE SAMPLES IN MONGREL DOGS.

The results obtained in the three dogs treated with pyrazinamide are shown in Figure 4 and Table III. The initial peak of uric acid radioactivity representing tubular secretion has been eliminated, but the second or glomerular rise remains unchanged.

Discussion

Although it has usually been assumed that virtually all glomerular filtration ceases during the period of urinary stasis, the studies of Omachi and Macey indicate that this is not completely true (16). However, their data clearly demonstrate that the appearance of a glomerular filtration marker injected within 30 seconds of the restoration of free flow is restricted to the most proximal segment of the nephron. However, inulin and ferrocyanide injected 6 and 4 minutes before release of the clamp were found progressively farther down the nephron. In the present study inulin-C^{14} was absent from distal samples of the tubule when it was injected into the renal artery 15 seconds before release of the ureteral clamp. Under these circumstances the addition of radioactivity to intraluminal urine at distal sites must be attributed to transtubular movement.

Injecting uric acid-2-C^{14} 15 seconds before reestablishment of urine flow, we have demonstrated an initial peak of radioactivity in the early samples in all dogs studied. This peak represents primarily distal tubular fluid as indicated by its appearance with the creatinine peak and before the secretory peak of PAH. The argument that this distal peak represents transtubular movement of uric acid is supported by the evidence obtained with pyrazinamide-treated dogs. This drug, which is known to interfere with uric acid excretion (10, 11), almost completely abolished the initial uric acid-2-C^{14} peak that had been present in the samples representing distal tubular urine. Yü, Berger, and Gutman have presented data suggesting a similar suppression of tubular secretion of urate in the mongrel dog (8). However, in their studies with the Dalmatian coach hound, treatment with pyrazinamide led to clear-cut inhibition of both proximal and distal secretory peaks for uric acid. Although these studies demonstrate distal transtubular movement of uric acid-2-C^{14}, they do not prove that there has been any net distal tubular secretion of uric acid. Since this transtubular movement cannot be quantitated, it is not possible to determine how important a role the distal tubule plays in the over-all urinary excretion of uric acid. However, others have reported net tubular secretion of uric acid based on the finding of a ratio of uric acid clearance to creatinine clearance substantially greater than one in animals infused with large amounts of uric acid (2, 3).

Uric acid excretion in the mongrel dog thus might take place by complete glomerular filtration (17), possibly complete proximal tubular reabsorption and distal tubular secretion. With the exception of the Dalmatian coach hound, this mechanism probably is operative in other mammalian species. The action of drugs affecting urate secretion could be explained in the following manner. Agents that cause urate retention, such as pyrazinamide, would do so primarily by inhibiting distal tubular secretion. Uricosuric agents would act through a primary interference with proximal reabsorption. Some drugs, those with paradoxical effects, would act upon both reabsorption and secretion. Their net effect would be the algebraic sum of their action on each one separately. The same mechanism would explain the interactions noted among several uricosuric agents (18).

Summary

By a modification of the stop-flow technique and the renal arterial injection of uric acid-2-C^{14} just before the reestablishment of urine flow, transtubular movement of uric acid in the mongrel dog has been localized to a distal portion of the nephron. Pyrazinamide, a drug known to increase serum uric acid levels by decreasing renal excretion of urate, has been demonstrated to inhibit this distal tubular movement of uric acid.

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