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STUDIES ON RENAL URATE SECRETION IN THE DOG[1, 2]

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ABSTRACT

NOLAN, R. P. AND E. C. FOULKES: Studies on renal urate secretion in the dog. J. Pharmacol. Exp. Ther. **179**: 429-437, 1971. An understanding of the mechanism of drug action on urate excretion requires selective study of unidirectional fluxes. We have utilized two techniques in an attempt to characterize the urate secretory flux in the dog. Results of double indicator dilution studies with ^{14}C-urate and ^{3}H-inulin indicate that less than 10% of the postglomerular urate is extracted from the inulin space. Since the size of this urate extraction imposes an upper limit on the magnitude of urate secretion, it follows that the secretory flux is relatively small. A rapid flux of ^{14}C-urate from postglomerular blood into the tubular lumen was also demonstrated with a modified stop-flow technique. This flux could be inhibited by a number of agents, including *p*-aminohippurate, pyrazinoate, probenecid, chlorothiazide, furosemide, ethacrynate and lactate, but not by chlormerodrin. These data are further evidence that a number of similarities exist between drug effects on urate excretion in man and the dog.

A three component system for urate excretion, including filtration at the glomerulus, reabsorption from the tubular lumen and secretion into the tubular lumen, is believed to occur in many mammalian species, including man (Praetorius and Kirk, 1950; Gutman *et al.*, 1959) and the mongrel dog (Lathem *et al.*, 1960; Yu *et al.*, 1960). This latter species has been used by numerous investigators to study the mechanism of action of drugs which alter urate clearance in man. Unfortunately, however, some drugs apparently affect urate clearance differently in man than in the mongrel dog; for example, salicylate is reported to have a biphasic effect on urate clearance in man (Yu and Gutman, 1959) but to be slightly uricosuric in the dog (Mudge *et al.*, 1968). These apparent species variations may simply represent quantitative differences. Alternatively it is possible, of course, that the drugs act differently in the two species or that urate is handled by differing mechanisms.

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Our earlier attempts to study secretory fluxes of urate under free-flow conditions with the precession technique were unsuccessful: urate does not precede simultaneously injected inulin into urine under our conditions (Banks and Foulkes, 1971).

Direct demonstration of urate secretion has recently been achieved with a modified stop-flow technique (Zins and Weiner, 1968). The present paper is an extension of the study of urate secretion in the dog, both under stop-flow and free-flow conditions. Our findings indicate that only relatively small amounts of urate undergo secretion, but that this flux is very rapid and can be inhibited by a variety of drugs. These data further reveal many similarities between man and the mongrel dog in terms of drug action on urate excretion.

METHODS. Mongrel dogs weighing 6 to 20 kg were anesthetized with 30 mg/kg of sodium pentobarbital and placed on a heating pad for maintenance of body temperature. The trachea was cannulated and the lungs ventilated with a positive pressure respirator (Harvard Apparatus Co., Inc., Millis, Mass.). A polyvinyl catheter [3 mm inside diameter (I.D.)] was positioned in the left femoral artery for blood pressure recordings (Harvard Apparatus pressure transducer) and blood sampling.

Double indicator dilution technique. Silverman *et al.* (1970) have shown that by analysis of renal

venous recovery patterns for glomerular markers and certain solutes after injection into the renal artery it is possible to determine the extent of loss of these solutes from the postglomerular extracellular space. Since urate secretion must include movement of urate out of the postglomerular extracellular space, we used the double indicator dilution technique to characterize urate uptake on the peritubular side of tubular epithelial cells.

Surgical preparation of the animal involved exposure of the left renal artery and vein by a midline abdominal approach. The ovarian (spermatic) vein emptying into the renal vein was ligated to prevent contamination of renal venous blood. The renal vein was cannulated with a polyvinyl catheter (3 mm I.D.) fitted with a Teflon tip. Renal ischemia during the cannulation procedure usually lasted less than one minute. The renal venous catheter was connected to an extracorporeal flow probe and flowmeter system (model 300 P1 probe and model 321 electromagnetic flow meter, Carolina Medical Electronics, Inc., King, N.C.) for continuous monitoring of renal blood flow. A polyvinyl tube (3 mm I.D.) led from the flow probe into a 250-ml Teflon collecting reservoir. Blood was returned with a Harvard Apparatus pulsatile pump from the reservoir through a heat exchanger into a jugular vein. After tracer injections, the renal venous blood, instead of entering the reservoir, was collected as two- to four-second samples for a period of one minute. The complete collection of the venous effluent over this interval prevented any significant recirculation of injected tracer. Collected blood was replaced by an equal volume of a 6% dextran saline solution (clinical grade C, Mann Research Laboratories Inc., New York, N.Y.) added to the venous reservoir. The renal vein-jugular vein extracorporeal shunt was primed initially with a 6% dextran saline solution containing 500 U/kg b.wt. of sodium heparin. Total volume of the shunt was maintained at approximately 50 ml with level controllers (Cole-Parmer Instrument Co., Chicago, Ill.) attached to the venous reservoir. Intra-arterial injections were administered through a curved 25-gauge needle attached to a 10-inch polyethylene catheter (PE 20, Clay-Adams, Parsippany, N.J.) and inserted into the renal artery against the direction of blood flow. A similar approach was used for determination of renal venous recovery patterns in the rabbit; details are described elsewhere (Foulkes, 1971).

Modified stop-flow technique. The procedure used in these experiments resembled that previously described by Zins and Weiner (1968). In essence, it involves the infusion of ^{14}C-urate and ^{3}H-inulin into the thoracic aorta after occlusion of ureteral cannulas. Under these stop-flow conditions ^{14}C-urate filtration is limited, thereby permitting the unmasking of a ^{14}C-urate secretory flux. One significant difference between the modified stop-flow technique in the present paper and that used by Zins and Weiner was that by using tracer levels of urate we were able to study urate secretion under steady-state conditions. In contrast Zins and Weiner infused a high concentration of urate, thus altering the concentration gradient between capillaries and tubular lumen. In spite of this difference the qualitative results of the two studies appear similar.

A PE-60 catheter was inserted into the right femoral artery and passed up into the thoracic aorta; the catheter was kept patent by a slow saline infusion. Both ureters were exposed by midline abdominal incision and cannulated with PE-60 catheters. Both femoral veins were cannulated for i.v. infusions. One intravenous solution containing 500 mg/100 ml of uric acid dissolved in lithium carbonate was infused at 1.0 ml/min. The other intravenous solution, 20% mannitol, 27 mM $NaHCO_3$ and 3 mM KCl in saline, was infused at 0.5 ml/min/kg. Stop-flow studies were performed 40 to 60 minutes after start of i.v. infusions. At the time of the stop-flow studies plasma urate levels were generally between 40 and 70 μg/ml (mean, 53 μg/ml).

Materials and analytical methods. Chemical urate analyses were performed by a modification of the method of Liddle *et al.* (1959). Radioactivity was determined on a Packard Tri-Carb scintillation counter (Packard Instrument Company, Inc., Downer's Grove, Ill.) with automatic external standard. Samples were counted in Bray's solution (Bray, 1960), with silicon dioxide (Cab-O-Sil, Packard) added to plasma samples. Determination of the pH of arterial blood samples was made on a Radiometer pH meter 27 with a microelectrode unit (type E 5021).

Uric acid-2-^{14}C was purchased from International Chemical and Nuclear Corporation (Irvine, Calif.) and ^{3}H-methoxy inulin from New England Nuclear Corporation (Boston, Mass.). ^{14}C-allantoin was formed by the uricase oxidation of urate-2-^{14}C in Tris buffer, pH 9.5, at room temperature. Completion of the urate oxidation was verified by spectrophotometric analysis. Purified uricase was obtained from Worthington Biochemical Corporation (Freehold, N.J.).

RESULTS. *Double indicator dilution technique.* Preliminary experiments were performed on a series of rabbits for evaluation of the usefulness of this technique in the study of urate secretion. The rabbit was chosen for these studies because

net secretion can be readily demonstrated in this species with conventional techniques (Beechwood *et al.*, 1964). Because Beechwood *et al.* (1964) have shown that creatinine, the extracellular marker used by Silverman *et al.* (1970), inhibits urate transport in the rabbit, we performed studies to evaluate the use of inulin as a marker. Our results in the rabbit confirmed the earlier observation of Chinard (1955) in the dog that creatinine and inulin have identical recovery patterns in renal venous blood. Thus, in spite of the large molecular size of inulin its distribution throughout the postglomerular extracellular space is not diffusion limited.

Figure 1A shows results from one of five rabbit control studies. Serial renal venous samples were obtained immediately after instantaneous intrarenal arterial injection of a small volume of saline (0.2 ml) containing ^{14}C-urate, ^{3}H-inulin and 60 μg of unlabeled carrier urate. Fractional recoveries of tracer urate and inulin in renal venous blood are shown as a function of time. Percent recoveries of both urate and inulin are calculated on the basis of nonfiltered solute only. In most venous samples shown in figure 1A, ^{3}H recovery exceeded that of ^{14}C, indicating a larger postglomerular ^{14}C space than ^{3}H space. Figure 1B shows a study in the same animal five minutes after i.v. administration of 200 mg of *p*-aminohippurate (PAH), an agent believed to inhibit urate secretion in the rabbit (Moller, 1967a,b). Under these conditions, the difference between the tracer urate and inulin spaces was markedly decreased. Similar results were also obtained with one other rabbit treated with PAH. Clearly, the double indicator dilution technique permits visualization of urate extraction from the postglomerular inulin space and the inhibition of this extraction by certain drugs. Control *in vitro* experiments with both rabbit and dog blood had shown that red cells are essentially impermeable to ^{14}C-urate over incubation periods as long as 10 minutes. We shall therefore assume that the postglomerular extraction may be equated with cellular uptake at the peritubular membrane (PTM).

Figure 2 depicts a typical result from one of eight double indicator dilution studies in six mongrel dogs. In this study, 0.4 ml of saline containing ^{14}C-urate and ^{3}H-inulin was injected into the renal artery. Fractional recoveries of tracer urate and inulin are again shown on the ordinate and time on the abscissa. ^{14}C-urate recovery was less than that of ^{3}H-inulin in early samples. The small net gain of ^{14}C-urate seen in later venous samples represents net addition of ^{14}C into peritubular capillary blood and presumably reflects at least in part reabsorption of filtered urate.

The results of all the dog studies carried out under a variety of conditions are summarized in table 1. In all cases a loss of ^{14}C-urate from the postglomerular ^{3}H-inulin space was observed in early samples. The point in time at which ^{14}C-urate recovery changed from loss to net gain, referred to as the crossover time, is denoted by the symbol t_x. The mean artery-vein transit times for tracer urate and inulin are denoted as $\bar{t}_{ur}$ and $\bar{t}_{in}$, respectively. The percent loss of ^{14}C-urate from the postglomerular ^{3}H-inulin space equals the difference between the total percent recoveries of tracer inulin and urate at time t_x. This observed loss represents a minimum loss for two reasons: 1) there might have been some

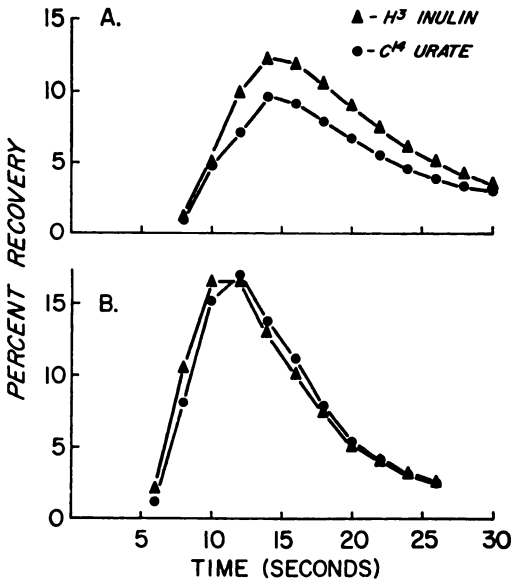

FIG. 1. Renal venous recovery patterns for ^{14}C-urate and ^{3}H-inulin in a New Zealand White rabbit. Data from 2.7-kg male. At time $t = 0$ a solution of 0.2 ml of saline containing 3 μc of ^{14}C-urate, 6 μc of ^{3}H-inulin and 60 μg of unlabeled urate was injected into the renal circulation. ▲——▲, ^{3}H recovery; ●——●, ^{14}C recovery. A. Control study. Renal blood flow (direct measurement) = 14.2 ml/min. B. Effect of PAH on recovery patterns. PAH, 200 mg, (1.0 ml of 20% PAH) was administered i.v. five minutes prior to study. Renal blood flow = 16.4 ml/min.

addition of ^{14}C from cells into peritubular capillary blood prior to time t_s, making the observed loss a net rather than actual loss and 2) ^{14}C-urate extraction from postglomerular inulin space likely was occurring also after time t_s, but was masked by the net addition of tracer from cells to capillary blood. For the reasons indicated above, the mean percent extraction may well have been larger than the value of 7% shown in table 1.

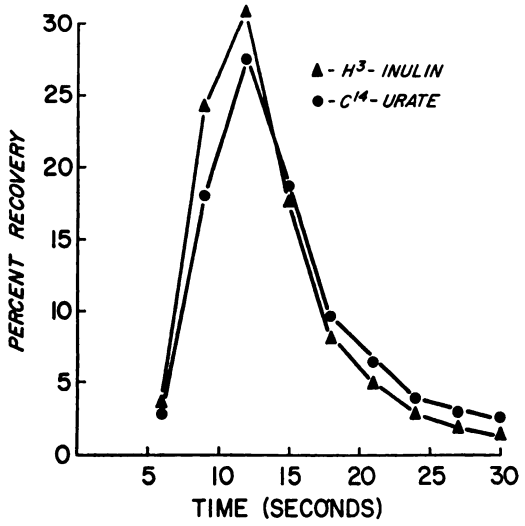

FIG. 2. Renal venous recovery patterns for ^{14}C-urate and ^{3}H-inulin in the mongrel dog. Data from dog 178, 17.9-kg male. Intrarenal arterial injection of 4 μc of ^{14}C-urate and 10 μc of ^{3}H-inulin in 0.4 ml of saline occurred at time $t = 0$, ▲——▲, ^{3}H recovery; ●——●, ^{14}C recovery. Renal blood flow (direct measurement) = 8.0 ml/min/kg b.wt. Mean blood pressure = 130 mm Hg.

Modified stop-flow technique. Figure 3 shows a typical result from a control modified stop-flow study. Four minutes after occlusion of ureteral cannulas, ^{14}C-urate and ^{3}H-inulin were infused into the thoracic aorta. After one minute of tracer infusion the ureteral clamps were released and serial 0.3-ml urine fractions obtained. The ratio of tracer urate/inulin in urine fractions (U ur*/in*) divided by that ratio in the infusate (I ur*/in*) is shown on the ordinate and cumulative volume of collected urine along the abscissa. Under conditions of ^{14}C-urate secretion we would expect higher relative levels of ^{14}C in urine than in the infusate; thus (U ur*/in*)/(I ur*/in*) would be greater than one.

In the experiment depicted in figure 3 no tracer activity was observed in more distal urine fractions; only in later (more proximal) samples were significant amounts of tracer observed. For a significant number of the more proximal urine fractions shown in figure 3 the expression on the ordinate clearly assumed values greater than 1, implying the existence of a ^{14}C secretory flux.

TABLE 1

Results from double indicator dilution studies

Dog	Weight	Sex	Mean Blood Pressure	Renal Blood Flow[a]	t_s[b]	$\bar{t}_{ur}$[c]	$\bar{t}_{in}$[c]	Postglomerular Extraction of ^{14}C-Urate	Conditions
	kg		*mm Hg*	*ml/min/kg b.wt.*	*sec*	*sec*	*sec*	%	
178A	17.9	M	130	8.0	14	15	13	10.2	Antidiuresis
178B	17.9	M	130	8.7	11	13	12	11.0	Antidiuresis[d]
182	10.8	M	140	8.2	12	14	13	2.5	Antidiuresis[d]
183	13.8	M	130	5.3	11	20	16	1.3	Antidiuresis[e]
184A	12.8	M	140	10.5	7	8	7	7.9	Antidiuresis
184B	12.8	M	140	11.7	6	7	7	5.4	Antidiuresis
188	17.2	F	130	12.0	8	9	8	9.0	Diuresis[f]
189	18.5	M	130	9.6	8	16	9	6.4	Diuresis[f]
Mean	15.2		134	9.3	10	13	11	7.0	

[a] Obtained by direct measurement of renal venous outflow.

[b] t_s, time at which ^{14}C-urate recovery changed from loss to gain (see text).

[c] $\bar{t}_{ur}$ and $\bar{t}_{in}$, mean renal artery-vein transit times for ^{14}C-urate and ^{3}H-inulin.

[d] Carrier urate, 1 mg, included in tracer injection.

[e] Carrier urate, 0.4 mg, included in tracer injection.

[f] A solution of 10% mannitol in saline was infused i.v. at 0.5 ml/min/kg.

Contrasted to these studies with ^{14}C-urate were experiments in two mongrel dogs in which ^{14}C-allantoin was infused with ^{3}H-inulin into the thoracic aorta during a modified stop-flow study and urine samples were obtained one minute after the start of infusion. A representative result from one kidney is shown in figure 4. The ratio of tracer allantoin/inulin in urine (U al*/in*) divided by that ratio in the infusate (I al*/in*) is plotted against cumulative volume of collected urine as above. It can be seen that allantoin was not secreted under the present conditions. Since allantoin is the only known breakdown product of urate in the dog, it seems likely that the ^{14}C secretion observed in the urate studies represented ^{14}C-urate and not a metabolite.

Secretory delay for ^{14}C-urate. In order to select suitable control conditions for drug studies, further experiments with tracer urate and inulin were performed in which the time interval between start of tracer infusion and release of ureteral clamps was varied. This interval includes both the transit time of tracers from thoracic aorta to site of postglomerular uptake at the PTM and the time required for secretion across

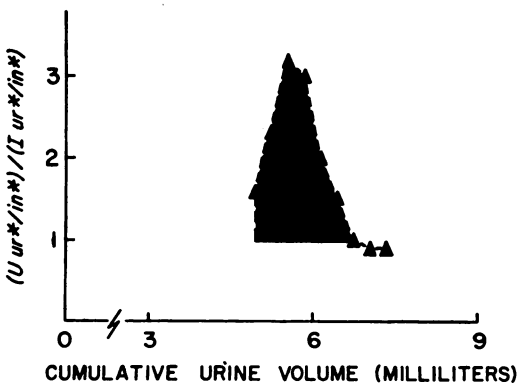

FIG. 3. Demonstration of ^{14}C-urate secretion. Data from dog 149, 9.0-kg male mongrel dog. Control urine flow = 6.6 ml/min. Plasma urate = 43 μg/ml. Blood pressure 200/130 mm Hg. Intravenous infusions as indicated in METHODS. After a four-minute occlusion of ureteral cannulas 5 μc of ^{14}C-urate and 60 μc of ^{3}H-inulin in saline were infused into the thoracic aorta over a one-minute interval. Ureteral clamps were release at the end of the tracer infusion. Shaded area can be quantitated with the expression

$$\sum_{n=1}^{n=x} (R_n u/i - 1).$$

See text for explanation.

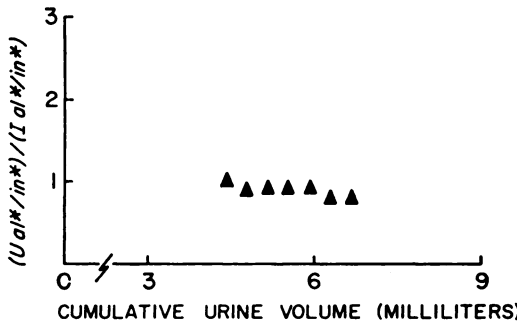

FIG. 4. Inability of mongrel dog to secrete ^{14}C-allantoin. Data from 11.8-kg female. Control urine flow = 4.9 ml/min. Mean blood pressure = 135 mm Hg. Intravenous infusions same as listed in METHODS for urate modified stop-flow studies. After a four-minute occlusion of ureteral cannulas 4 μc of ^{14}C-allantoin and 50 μc of ^{3}H-inulin were infused into the thoracic aorta over a one-minute interval. Ureteral clamps were released at the end of the tracer infusion.

the cell. For simplicity this time interval will be referred to as the secretory interval. The results from all studies with varied secretory intervals are summarized in table 2. The number of kidneys for which secretion of ^{14}C-urate could be demonstrated is shown for each secretory interval tested. In these studies secretion was consistently demonstrable at secretory intervals $\geq$ 30 seconds. Even with intervals as short as 14 to 20 seconds secretion was seen in most studies, whereas with intervals $\leq$ 12 seconds clear-cut ^{14}C-urate secretion was observed in only two of seven kidneys.

Effect of drugs on ^{14}C-urate secretion. In order to study the effects of different drugs on urate secretion it was necessary to choose control conditions under which secretion was consistently demonstrable. Therefore a secretory interval of one minute was used for all drug studies. To facilitate comparisons between control and drug-treated animals it was desirable to obtain some rough quantitation of urate secretion. This was obtained by graphic integration of the shaded area under the curve depicted in figure 3 as follows:

$$\text{Area} = \sum_{n=1}^{n=x} (R_n u/i - 1)$$

where $R\ u/i = (U\ ur^*/in^*)/(I\ ur^*/in^*)$, n = a urine fraction containing significant amounts of tracer urate and inulin and $R\ u/i > 1$ only between the limits $n = 1$ and $n = x$. This integration yielded a mean value of 7.3 ± 0.8

TABLE 2

Demonstration of ^{14}C-urate secretion as a function of time

Tracer infusion was terminated simultaneously with, or immediately after release of ureteral clamps.

Time Interval	Fractional Number of Kidneys Showing Secretion
sec	
9	0/2
12	2/5
14	5/6
20	3/4
30	5/5
60	15/15
70	2/2

TABLE 3

Drug Effects on ^{14}C-urate secretion

All drugs, except lactate, were administered by i.v. injection over a 1 to 10-minute interval and the modified stop-flow study was performed within 15 minutes after completion of drug administration. In the lactate studies an aqueous i.v. solution containing 0.67 M lactate, 27 mM $NaHCO_3$ and 0.9% NaCl was infused at 0.5 ml/min/kg b.wt. immediately prior to the study. Statistical comparison of control and drug-treated groups was based on the Student's *t* test.

Drug	Dose	N[a]	$\sum_{n=1}^{n=s} (R_n u/i - 1)$	P value
	mg/kg			
Control		5	7.3 ± 0.8[b]	
PAH	200	4	0.0	<.001
Chlorothiazide	50	4	0.0	<.001
Probenecid	30	4	0.3 ± 0	<.001
Pyrazinoate	40	7	2.4 ± 1.3	<.025
Lactate	850–1700	7	1.2 ± 0.3	<.001
Chlormerodrin	7	6	5.3 ± 2.3	N.S.
Ethacrynate	2	4	0.7 ± 0.7	<.001
Ethacrynate	12	4	2.9 ± 1.0	<.025
Ethacrynate	80	2	0.5	
Furosemide	2	4	6.1 ± 1.2	N.S.
Furosemide	12	6	7.6 ± 3.0	N.S.
Furosemide	40	8	0.5 ± 0	<.001

[a] *N*, number of kidneys.

[b] Values given are mean ± S.E.

(S.E.) for a group of five control kidneys with a one-minute secretory interval; this mean value served as a control for the drug studies.

Zins and Weiner (1968) have previously shown that PAH and chlorothiazide inhibit urate secretion under modified stop-flow conditions. We could readily confirm these observations. The effect of administration of 200 mg/kg of PAH (all drugs were administered as the sodium salt) and 50 mg/kg of chlorothiazide is shown in table 3. Both of these drugs caused a decrease of our integrative estimate of secretion to zero, suggesting complete secretory blockade. Similarly, ^{14}C-urate secretion was depressed after administration of 30 mg/kg of probenecid, 40 mg/kg of pyrazinoate and 0.85 to 1.7 g/kg of *dl*-lactate. Blood pH was frequently monitored during the lactate studies, and adjustments in the respiratory rate were made when necessary to correct an acidosis.

A dose of 7 mg/kg of chlormerodrin did not significantly affect urate secretion under conditions of respiratory acidosis (blood pH ranges 7.28–7.35) induced by slight hypoventilation.

Ethacrynate, in doses of 2, 12 and 80 mg/kg, caused a depression of urate secretion. Furosemide had no apparent effect at 2 mg/kg. At 12 mg/kg furosemide did depress urate secretion in two out of six kidneys; the group mean at this dosage was not significantly different from that of the control group, however. At 40 mg/kg furosemide consistently blocked secretion.

DISCUSSION. Evidence that the mongrel dog is capable of secreting urate into the renal tubular lumen was first presented in 1960 by Lathem *et al.* and Yu *et al.* Both groups showed that under appropriate experimental conditions the clearance ratio of urate to a glomerular marker [C_{urate}/glomular filtration rate (GFR)] can assume values greater than 1. Unfortunately this approach is of limited usefulness for characterization of urate secretion because the net secretion may conceal a reabsorptive flux which would affect the interpretation of studies on the secretory flux. Recently Zins and Weiner (1968) demonstrated that a modified stop-flow technique could be used to study certain aspects of the secretory flux. We have used a similar technique to extend the study of urate secretion. In addition, we have used the double indicator dilution technique to demonstrate the first step of the secretory process, cellular uptake of urate at the PTM.

Because urate is readily metabolized to allantoin by hepatic uricase it is necessary to have some degree of confidence that the ^{14}C transport observed in the present studies represented urate transport. There seems little likelihood that the ^{14}C extracted from the postglomerular inulin space in the double indicator dilution studies represented anything other than urate.

There is no evidence supporting the existence of a plasma uricase capable of oxidizing ^{14}C-urate in the short passage from renal artery to site of extraction. In the modified stop-flow studies, on the other hand, since some recirculation of tracers could occur it is possible that some ^{14}C-allantoin formed in the liver is presented to the kidneys. Our stop-flow studies with ^{14}C-allantoin indicate that this compound does not cross the tubular epithelial cells into the lumen. We have not experimentally ruled out the possibility that ^{14}C-urate, after crossing the PTM, is converted to ^{14}C-allantoin, which then enters the tubular lumen. This would require the presence of uricase activity in renal tissue, however, and Platts and Mudge (1961) were unable to demonstrate uricase activity in kidney slices. Thus it seems likely that oxidation of ^{14}C-urate was not a significant factor in the present study.

It is of interest to compare the cellular uptake of urate by renal cells *in vivo* and *in vitro*. Rabbit kidney cortex slices are reported to accumulate significant amounts of urate (Platts and Mudge, 1961; Berndt and Beechwood, 1965) whereas urate accumulation by mongrel dog kidney cortex slices is reported to be negligible (Platts and Mudge, 1961). These observations parallel our own observations of more significant postglomerular uptake of ^{14}C-urate in the rabbit than mongrel dog.

The ^{14}C-urate recovery pattern in the double indicator dilution studies can be interpreted in terms of the bidirectional nature of urate transport across tubular epithelial cells. As noted above, the loss of ^{14}C-urate observed in early venous samples is an essential component of the secretory flux. Since a net urate reabsorption normally occurs in the mongrel dog we might have expected a net addition of ^{14}C-urate from the tubular lumen into postglomerular blood. Indeed, total ^{14}C recovery slightly exceeded that of tritium. This "excess" ^{14}C must represent reabsorbed material. That we observed a loss of ^{14}C-urate in early renal venous fractions suggests that appearance of reabsorbed ^{14}C-urate in postglomerular blood was delayed. This delay must include both the transit time from renal artery to renal vein and the transcellular transit time from tubular luminal membrane to peritubular capillary lumen. If we assume that all the ^{14}C added from cells to postglomerular capillary blood represents reabsorbed ^{14}C, *i.e.*, that there is no back-diffusion of ^{14}C which is taken up at PTM, then the value for t_a in table 1 approximates the minimum time required for reabsorbed urate to reach the renal vein. The mean value for t_a in table 1 was 10 seconds, leading to an estimated reabsorptive transcellular transit time of less than 10 seconds. In similar studies Chinard *et al.* (1957) reported that the transcellular transit time for reabsorbed glucose was less than 10 seconds.

Our results also indicate that the urate secretory flux in the dog is very rapid. Indeed, it was shown with the modified stop-flow technique that tracer urate secretion often occurred within 14 seconds after intra-aortic tracer injection. It seems reasonable to assume that ^{14}C-urate would first appear in peritubular capillary blood within two seconds after intra-aortic injection. Thus the transcellular transit time from PTM to tubular lumen was in several studies as short as 12 seconds. In similar studies we have found that the transcellular delay for PAH is of a comparable magnitude (Nolan, 1970). We have no explanation for the discrepancy between our results with PAH and those previously obtained by Chinard (1956) under different experimental conditions.

Evidence suggesting that the urate secretory flux is rather small can be obtained by analysis of clearance data reported in the literature. After administration of high doses of pyrazinamide (Yu *et al.*, 1961) and PAH (Zins and Weiner, 1968), C_{urate}/GFR was depressed by about 18%. Assuming that: 1) in the dog these drugs act only by inhibiting urate secretion (*i.e.*, they do not enhance urate reabsorption), 2) secretory inhibition was essentially complete in these studies and 3) prior to drug administration 20% of the urate entering the kidney was being excreted (*i.e.*, filtration fraction = 0.25 and C_{urate}/GFR = 0.80), it follows that 18% of 20%, or only about 4%, of the total amount of urate entering the kidney was being secreted under control (drug-free) conditions. This value would correspond to 5% of the postglomerular urate and is of the same general order of magnitude as that found for the percent loss of ^{14}C from the postglomerular inulin space during the double indicator dilution studies (7%). In any event, it is clear from the earlier work with pyrazinamide (Yu *et al.*, 1961) and PAH (Zins and Weiner, 1968) that, in spite of the relatively

small magnitude of the secretory flux, secretory inhibition can be reflected in significant depressions of urate clearance.

The drug studies reported in the present paper rely on a measure of secretion provided by the stop-flow procedure. Clearly this measure can at best provide only a semiquantitative value for secretion. Nonetheless, the approach appears to be useful in detecting changes in urate secretion after drug administration.

Zins and Weiner (1968) have reported that the urate secretory flux in the mongrel dog can be inhibited by several drugs, namely PAH, chlorothiazide and salicylate. We have confirmed their results with PAH and chlorothiazide and added several additional drugs to this list of inhibitors. In addition to chlorothiazide, we evaluated three other diuretic agents. Under our conditions chlormerodrin did not significantly alter urate secretion. Similarly, this drug has been reported to have only slight effects on PAH secretion in the dog (Kessler *et al.*, 1958). Both ethacrynate and furosemide were shown to block secretion, although the former drug was more potent than the latter. Whether or not this secretory inhibition is the explanation for the hyperuricemia observed in the dog (Zins *et al.*, 1970) and commonly observed clinically after chronic administration of these diuretics cannot be concluded from the present study. Indeed, other investigators have reported evidence suggesting that increased urate reabsorption secondary to volume depletion may play a role in diuretic induced hyperuricemia in man (Hull *et al.*, 1967) and the mongrel dog (Zins *et al.*, 1970). Either mechanism, secretory inhibition or enhanced reabsorption, or a combination of the two could account for the observed urate retention.

The finding that urate secretion is inhibited by lactate contrasts with the lactate stimulation of PAH secretion in the mongrel dog (Mudge and Taggart, 1950; Cohen and Randall, 1964). Both PAH and urate are generally assumed to be transported by the organic acid secretory mechanism (Weiner and Mudge, 1964). However, some aspect of the secretory process must differ for PAH and urate since lactate has opposite effects on their secretion. Interestingly, these *in vivo* effects of lactate are somewhat comparable to observations by Platts and Mudge (1961) that lactate stimulates PAH uptake by rabbit kidney cortex slices but has little effect on urate uptake.

The information now available suggests a number of similarities between dog and man. Among the drugs believed to inhibit urate secretion in man at appropriate doses are probenecid (Yu and Gutman, 1955), salicylate (Yu and Gutman, 1959; also see, however, Podevin *et al.*, 1968) and pyrazinoate (Yu *et al.*, 1957; Podevin *et al.*, 1968; Gutman *et al.*, 1969). These three drugs have also been shown to inhibit urate secretion in the dog (see present paper; also Zins and Weiner, 1968).

A further possible similarity between man and the dog is illustrated by the action of lactate. Lactate is known to cause a marked depression of urate clearance in man (Nichols *et al.*, 1951; Yu *et al.*, 1957). Although Reem and Vanamee (1964) have reported that under certain conditions lactate also depresses urate clearance in the mongrel dog, we have been unable to confirm these results (unpublished observations). The present demonstration that lactate inhibits urate secretion may explain the depression of urate clearance in man. Thus it is quite possible that lactate has qualitatively similar effects on urate transport in man and the dog even though this similarity is not readily reflected in quantitative changes in urate excretion in the dog.

Similarly although probenecid and salicylate are apparently less potent uricosuric agents in the dog than in man (Sirota *et al.*, 1952; Yu *et al.*, 1960; Lathem *et al.*, 1960; Mudge *et al.*, 1968; Yu and Gutman, 1959), these differences are likely quantitative rather than qualitative. Thus it is clear that, although some quantitative differences do exist, there are many similarities in the mechanism of drug action on urate transport in man and the dog.

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