

Renal Tubular Secretion of Urate in Sheep¹ (40353)LEON C. CHESLEY,² LOUIS W. HOLM, HAROLD R. PARKER,
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Renal tubular secretion of urate appears to be the rule in amphibians (1, 2), reptiles (2, 3), and birds (2, 4), where the clearance of urate exceeds the rate of glomerular filtration. In most mammals the clearance of urate is but a fraction of the filtration rate. Although the issue has been controversial, the urate of plasma probably is freely filtrable (2, 5), and there must be a net reabsorption by the tubules. In man, the urate excreted normally is only from 5 to 10% of the quantity filtered by the glomeruli (2, 6), and for some time the erroneous interpretation was that the excretion is determined simply by the balance between filtration and reabsorption. Mere comparison of the urate clearance with that of inulin does not provide any evidence for renal tubular secretion of urate in most mammals.

The data in the present paper indicate that a net renal tubular secretion of urate normally occurs in sheep, as shown by the ratio of the clearances of urate and inulin.

Material and methods. Renal clearances of inulin and urate were measured in seven ewes; two were normal nonpregnant sheep, three were in the last weeks of normal pregnancies, and two, near term, were moribund with ovine toxemia of pregnancy. The five normal ewes stood during the procedure and the two toxemic sheep lay on their sides.

Inulin was injected as a priming dose and given by constant infusion in 5% dextrose at 4 ml/min, in amounts calculated to maintain the level in plasma at about 30 mg/100 ml. An hour was allowed for equilibration and

the establishment of nearly constant rates of urinary flow before beginning the three clearance periods. Urine was obtained by Foley catheter, with two rinses of the bladder, each with 30 ml of water and about 30 ml of air. Venous blood samples were taken at the mid-point minus 5 min between collections of urine. Serum was used for the analysis. In the normal sheep the clearance periods were from 20 to 30 min; in the two oliguric sick animals the periods were from 1 to 2 hr.

In preliminary experiments, we found that the sera of blood samples from sheep (controls) had high blank readings in the method of Roe, Epstein, and Goldstein (7) for the measurement of inulin, presumably because of endogenous fructose. In each measurement of serum inulin we corrected for the blank for the particular animal, on the unproved assumption that the blank did not change significantly during the course of the observations.

We also found that urate in serum often was undetectable by Folin's (8) indirect method, although urinary concentrations were so high as to necessitate dilutions of from 50 to 100 times for analysis. We considered the possibility that some complex of urate in serum is precipitated by tungstic acid, or that there is some inhibitor of the chromogenic reaction in serum. Folin's indirect method, however, gave readable levels of color in sera that did not react in the direct method. In two experiments the indirect method gave nearly identical values in ultrafiltrates and in tungstic acid filtrates of sera. We then used both the direct and indirect methods for: (a) Diluted urines and tungstic acid filtrates of sera; (b) the same, previously treated with uricase; (c) the same, to which known amounts of urate had been added, (d) analyzed as such, and (e) analyzed after treatment with uricase. Water and reagent blanks

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were carried through all procedures. When uricase was used, each of 2 ml of water, 2 ml of diluted urine, and 5 ml of serum were placed in 50 ml volumetric flasks and 5 ml of borate buffer at pH 9.2, 50 mg of uricase, and 10 ml of distilled water were added to each. After incubation at 45° for 2 hr, 1 ml of 10% sodium tungstate was added to each and then 8 ml of N/12 sulfuric acid, slowly and with constant mixing. The preparations were diluted to volume, mixed, and filtered after standing for 10 minutes. Folin's methods were then applied to the filtrates. All readings were made with a Coleman junior spectrophotometer.

Although Folin's indirect method always gave readable color in filtrates of serum, we modified the method by precipitating the silver salt of urate from five times the usual volume of filtrate. That is, in the analysis of human serum, Folin used 5 ml of filtrate, representing 0.5 ml of serum; for measurements in ovine serum we used 25 ml of filtrate, representing 2.5 ml of serum, because of the low concentrations that ranged from 0.1 to 0.2 mg/100 ml in the normal animals and were 0.32 and 0.74 in those with toxemia.

The urate clearances that we report are based upon serum urate as measured by the modified indirect method, and urinary urate as measured by the direct method.

Results. Chromogens in urine and serum. The urinary substance(s) that developed color in both direct and indirect methods for urate was really urate, as indicated by the destruction of from 96.4 to 100% of the chromogen by uricase. Uricase destroyed virtually all of the chromogen in serum. Moreover, the indirect method is alleged to be specific for urate (8), and the clearances were calculated from indirect measurements of urate in serum.

Recovery of urate added to urine and serum.

Urinary and serum samples were mixed with equal volumes of an aqueous solution containing 0.02 mg/ml of uric acid. After standing, one aliquot was treated with uricase, as described above. The treated and untreated aliquots were then carried through the direct and indirect procedures. The recovery of urate varied from 93.5 to 108% (average, 99.6%) in the direct method and from 88 to 104% (average 96.0%) in the indirect method.

The amount of urate added to diluted urine was well within the range of the endogenous levels measured. Unfortunately, we added far too much to serum and the recoveries do not validate the estimates of endogenous levels, even though we used five times the usual volume of filtrate in the measurements.

Inulin clearances. As shown in Table I, the mean inulin clearances in the three normal pregnant ewes were 101, 98, and 115 ml/min; in the two normal nonpregnant sheep they were 73 and 70 ml/min. The clearances are well within the range that Parry and Taylor (9) observed and collected from the literature. The apparent clearances in the two sick animals varied greatly from period to period, cannot be averaged, and clearly are unreliable, perhaps because of varying delivery of urine from the ureters to the bladder in the oliguric ewes. Nevertheless, the ratios of urate/inulin clearances seem valid; we report them because they are consistent with the findings in the normal sheep, despite the profound depression in renal function.

Urate clearances. In every clearance period in every animal, pregnant or not, normal or sick, the urate clearance was greater than the simultaneous inulin clearance by from 52 to 290%, with consistent ratios from period to period in each animal. The ratio of urate clearance/inulin clearance averaged 2.46 for all observations.

Folin (8) wrote that the direct method,

TABLE I. RENAL CLEARANCES OF INULIN AND URATE IN SHEEP

Status	Weight, kg	Urinary volume, ml/min	Serum urate mg/100 ml	Clearances, ml/min		Clearance ratio Urate/inulin
				Inulin	Urate	
Pregnant	53.6	5.48	0.17	101	284	2.8
Pregnant	63.2	3.21	0.11	98	265	2.7
Pregnant	64.2	6.90	0.14	115	194	1.7
Non pregnant	59.1	7.50	0.20	73	128	1.8
Non pregnant	39.6	4.04	0.20	70	196	2.8
Toxemic	62.6	1.10-2.34	0.32	13-62	38-180	2.9-3.9
Toxemic	77.3	0.67-0.88	0.74	0.1-0.9	0.2-2.0	2.0-2.3

which we used for urine, gives urinary values that "were nearly always from 5% to over 10% higher than those obtained by the indirect method applied to diluted urines." Thus, the clearances that we report are too high by that range of percentages, but it is obvious that the clearance of urate is so much greater than that of inulin that an error of 10% is of little significance.

Thus, on the average, more than half of the excreted urate must have been secreted by the tubules. Nearly all, or all, may have been if the filtered urate had been reabsorbed, as it is in man.

Discussion. Renal tubular secretion of urate appears to be a phylogenetically ancient process that may have persisted in many, if not all, higher animals despite the later superimposition of tubular reabsorption of the substance. The current concept (2, 10) is that there are four processes involved in the excretion of urate by man and probably by other mammals. (a) Glomerular filtration of urate, (b) tubular reabsorption of nearly all of the filtered urate, (c) tubular secretion of urate, and (d) tubular reabsorption of some of the secreted urate (postsecretory reabsorption).

Praetorius and Kirk (11) described an anomalous young man with marked hypouricemia whose renal clearance of urate exceeded that of inulin; he, therefore, must have had renal tubular secretion of urate. Gutman, Yü, and Berger (12) demonstrated ratios of excreted urate/filtered urate greater than 1.0 in gouty and normal men who were loaded with a potent uricosuric agent (sulfipyrazone), thus clearly showing tubular secretion of urate in that circumstance. They suggested that in man perhaps all of the filtered urate normally is reabsorbed and whatever is excreted reaches the urine by tubular secretion.

The dalmatian coach hound is exception in that the renal clearances of urate and creatinine are virtually identical (13), or the urate clearance is the greater of the two (14).

Poulsen and Praetorius (15) observed that in the rabbit the ratio of endogenous urate to creatinine clearance averaged 0.40, with a single maximal value of 0.75. They infused urate to increase its concentrations in plasma of from 2 to 5 $\mu\text{g}/\text{ml}$ up to from 10 to 30 $\mu\text{g}/\text{ml}$, and found that the ratio of $C_{\text{Ur}}/C_{\text{Cr}}$ in-

creased to an average of 1.77 (range of 1.25–3.0 in 32 clearance periods). That is, the infusion of urate had stimulated a net tubular secretion of the substance.

Fanelli *et al.* (16) studied seven species of Old World monkeys and found that the urate clearance exceeded the inulin clearance in all but the bushbaby. In 12 species of New World monkeys, in the gibbon, and in the chimpanzee, the urate clearance was less than the inulin clearance in all but the red howler. Because of the low concentrations of urate in plasma, Fanelli *et al.* loaded the animals with urate "when indicated", and whether any animal showed a net tubular secretion of urate in the absence of loading is not specified. Net tubular secretion of urate has been observed in goats (2), calves (2), pigs (19), and guinea pigs (2, 18), but urate had been infused to raise its level in plasma. Mudge, McAlary, and Berndt (18), in their study of guinea pigs, usually infused urate but did find net tubular secretion of urate in four animals whose endogenous clearances were measured. Simmonds, Cameron, and Potter (19) recently reported that the renal clearance of endogenous urate exceeds that of inulin in pigs. Thus, the sheep is not unique in having a net tubular secretion of urate.

Summary. The simultaneous renal clearances of endogenous urate and of inulin were measured in five normal ewes, three pregnant and two not, and in two sheep moribund with ovine toxemia of pregnancy. The urate clearance exceeded the inulin clearance in every period in each sheep, with the ratio ranging from 1.7 to 3.2 and averaging 2.46.

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