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Ionic Composition of Urate-Containing Spheres in the Urine of Domestic Fowl

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ABSTRACT. Birds excrete urate in their urine in the form of small spherical concretions. In addition to urate, these concretions contain protein and inorganic ions. Energy dispersive x-ray microanalysis was used to determine the inorganic ion composition of the urate-containing spheres to gain a better understanding of how the spheres are formed. Ureteral urine was collected from six female white leghorn domestic fowl, *Gallus gallus*. The urine was filtered to separate the spheres into size categories to determine if the ionic composition varied with the size of the spheres. The spheres were placed on scanning electron microscope stubs, dried and coated with carbon. All samples were examined at an accelerated voltage (kV) of 20. The results showed that in all birds, the spheres contained the ions calcium and potassium (approximately 70% and 30%, respectively, of the total inorganic ions present). However, in one bird, the percent of calcium and potassium was reversed (i.e., calcium 30% and potassium 70%). In all birds, chloride and magnesium were also detected, but in comparatively small amounts (less than 1%). There were no significant differences in the ion content of spheres of different sizes. These data suggest that calcium and potassium may play an important role in the formation of the spheres and are not random inclusions as the spheres are formed. We suggest that the spheres form to facilitate the excretion of urate without blocking or damaging the renal tubules. COMP BIOCHEM PHYSIOL 118A;3:585–588, 1997. © 1997 Elsevier Science Inc.

KEY WORDS. Domestic fowl, inorganic ion, nitrogen, sphere, urate, uric acid, x-ray microanalysis

INTRODUCTION

In terrestrial vertebrates, nitrogen is liberated as ammonia during gluconeogenesis. This ammonia is combined with carbon atoms to form compounds that must be excreted to prevent toxic levels from accumulating in the blood. In avian urine, the nitrogen compound present in the highest concentration is uric acid (and/or the salts of uric acid). Although from an osmoregulatory perspective, uric acid is considered to be an efficient form to excrete nitrogen because of its low solubility, this characteristic can also be a liability due to the tendency of uric acid to precipitate from solutions. Indeed, the normal concentration of uric acid in avian plasma is very near the aqueous solubility of this compound. However, at the normal pH of avian plasma (ca. 7.52) (11), 99.1% of the uric acid will be in the form of urate salts because of the low pKa (5.75) of the proton at position nine of the uric acid molecule. Salts of urate have a greater solubility than the acid form of the molecule and are less likely to precipitate in plasma.

Evidence suggests that there is little protein binding of uric acid (urate) in avian plasma (9,20). This suggests that because of their size, the urate salts should pass freely through the filtration barrier of the renal corpuscle. The pH of the filtrate in the early proximal tubule is not different from plasma (ca. 7.52) (7); thus, the majority (99.1%) of the uric acid should be in the form of urate salts, most probably as sodium or potassium salt. However, as the filtrate moves along the proximal tubule, the concentration of the urate salts in the tubule fluid is markedly increased compared with that of the plasma. This is because in addition to being freely filtered, urate is avidly secreted by the cells of the proximal tubule and water is reabsorbed through the tubular epithelium (4). Moreover, urate is not reabsorbed by the proximal renal tubule of birds, although 60–70% of the water filtered is reabsorbed by solute-linked flow (8). These three processes (filtration, secretion, water reabsorption) increase the concentration of urate salts in the proximal tubule to levels that exceed the solubility limits of uric acid and that of the most soluble salts of uric acid (8).

Crystalline precipitates of urate do not form because urates are chemically "bound" together with protein in small spheres (0.5–15 μm in diameter). These form a colloidal suspension that moves along the renal tubules without blocking and/or damaging the cells of the tubules. Our previous work has shown that these spheres are about 65% uric

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acid and about 5% protein on a mass basis (2). However, our analysis leaves several questions. First, we have accounted for only about 70% of the mass of the spheres. Second, because of the pH of the tubule fluid, the majority of the uric acid is in its anionic form. Moreover, proteins tend to have a net negative charge. Therefore, it is unresolved how the urate and the protein (two negatively charged species) combine to form the small spheres. Our present study was directed toward determining the inorganic ion content of the spheres. In particular, we wish to determine whether positively charged divalent ions are present within the spheres to serve as ligands between the urate and protein.

MATERIALS AND METHODS

Urine Preparation

Ureteral urine was obtained from six female white leghorn domestic fowl, *Gallus gallus*. The animals were approximately 6 months old and egg layers. The animals were housed in outdoor enclosures with free access to food (Hickman's layer feed, Arizona; 4% calcium, 0.2% salt, 9% added mineral) and water. To collect urine, the birds were brought to the laboratory and placed in a sling designed to minimize their movement. Ureteral urine was obtained by inserting a closed-end cannula into the cloaca as far as the coprodeum. The cannula had openings that were placed over the ureters. Ureteral urine (uncontaminated by cloacal contents) that entered the cannula was transferred to tubes that were maintained at 41°C in a water bath. During the urine collection, the birds showed no visible signs of stress or an unusually high rate of urine flow. Half a milliliter of urine was collected and diluted with 4.5 ml of distilled water and mixed on a vortex stirrer. The diluted urine was filtered through polycarbonate filters (Poretics, Livermore, CA) to separate the spheres into size categories. The filters had pore sizes of 14, 10, 5 and 1 μm. Once on the filters, the spheres were left to air dry overnight and the filters were placed on aluminum scanning electron microscope stubs and coated with carbon before analysis (Fig. 1).

Elemental Analysis

The samples were examined on a Philips CM12 STEM microscope and inorganic ion content was determined by energy dispersive x-ray microanalysis (EDAX, PV9900) (12). All samples were examined at an accelerated voltage (kV) of 20. To ensure that only the elements from a single sphere were detected, the analyses were conducted on individual isolated spheres (Fig. 1).

To determine whether the ion content of the spheres varied with size, four size categories were selected: <5 μm, 5–8 μm, 9–12 μm and >12 μm. Within each of these categories, five spheres were analyzed from each bird. This strategy gave a sample size of 20 spheres per bird for each of six birds.

A qualitative element spectrum was collected for each sphere (Fig. 2). The running time for each analysis was 100 sec with an average count rate of 1000 cps. A semi-quantitative analysis was performed on the data, and the results were presented as percent dry weight of element for each sphere.

The results were analyzed using a two-way ANOVA. To test for differences in ion composition between individual animals and in different size spheres, the data were analyzed using a Student-Newmans-Keuls test. Statistical significance was set at the 95% confidence interval.

FIG. 1. Urine spheres on a polycarbonate filter. Note the size of the filter pore and the passing of a sphere (see arrow) through a pore.

RESULTS

Energy dispersive x-ray microanalysis showed that the spheres contained predominately calcium and potassium

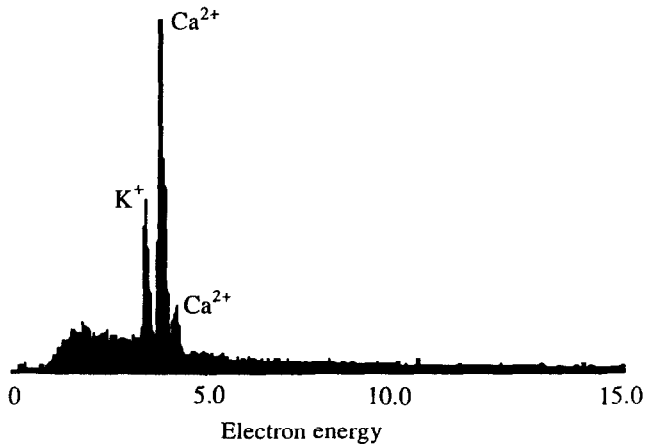

FIG. 2. A typical qualitative spectrum of a urate-containing sphere, collected by x-ray microanalysis. The peaks represent detection of calcium and potassium. Magnesium and chloride were not detected in this particular spectrum.

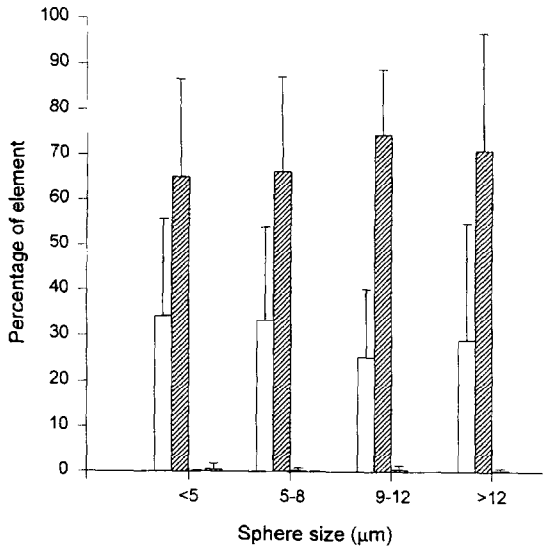

FIG. 3. The percent (±SD) of calcium (▨), potassium (□), magnesium (▧) and chloride (▩) in spheres of different sizes.

and small amounts of magnesium and chloride (Fig. 3). Among five birds, there were no significant differences in the amount of calcium and potassium in the spheres; however, the relationship was reversed in one bird. In five birds, calcium represented approximately 68 ± 17.3% of the total ionic content of the spheres and potassium 31 ± 17.1%, whereas in one bird, the amounts of calcium and potassium was 36% and 63%, respectively. In all birds, magnesium and chloride accounted on average for 0.2% and 0.1% of the total inorganic ion content of the spheres (Fig. 3). There were no significant differences in the inorganic ion composition of spheres of different sizes (Fig. 3). In the two largest size categories (9–12 and >12 μm), no chloride was detected.

DISCUSSION

The primary source of the nitrogen that must be removed from birds is the deamination of amino acids that occurs during gluconeogenesis. This nitrogen is generated intramitochondrially within the liver and passes into the cytosol as glutamate and eventually is converted into urate. The urate then enters the bloodstream and is carried to the kidney for excretion. As mentioned in the introduction, conditions in the plasma prevent the urate from precipitating from solution; hence, no spheres are present within the plasma. The conditions should be the same in the very early segments of the proximal tubule because urate and the electrolytes are freely filtered. However, because of urate secretion and water reabsorption along the proximal tubule, the concentration of urate exceeds its solubility limit; hence, spheres are formed in later segments of the proximal tubule (8) (Fig. 4). In our study, by measuring the inorganic ion composition of the spheres, we hoped to gain a better understanding of how the spheres are formed and how urate is bound within them.

Our microprobe analyses showed calcium and potassium are the predominate inorganic ions within the urate spheres of the ureteral urine of G. *gallus*. Previous chemical analysis of the spheres has shown that they also contain protein (2,3). Because the urate and protein are both anionic, the divalent cationic element calcium may bind the anions to produce the spheres (10,14,19).

We also found a relatively small amount of magnesium and chloride in the spheres. Two other studies using G. *gallus* (14) and 25 species of reptiles (16) also found virtually no chloride in urate spheres. In our study, magnesium (which is divalent) may be acting like the calcium and binding to the protein.

Several investigators have suggested that urinary spheres eliminate excess inorganic ions with a minimal loss of water (13,15–17). However, the question remains whether birds ingest an excess amount of inorganic ions in their diet. Birds reabsorb from 60 to 80% of all potassium and 96 to 99% of all calcium entering into the nephron (1,6,21,22). Hence, comparatively few inorganic ions are present in the ureteral urine. This poses the question: Why develop such an elaborate mechanism to excrete few ions? We favor the hypothesis that the primary purpose of the urate-containing spheres is to protect the nephrons from damage or blockage by crystals of uric acid or urate salts. The production of spheres does lead to the excretion of ions with minimal water loss, but this may be as a consequence of forming the spheres.

The final pH of avian ureteral urine can vary a great deal, extreme values being 5 at the most acidic and 8 for the most alkaline urine. However, the final pH of the urine may have little bearing on the formation of the urate spheres. As

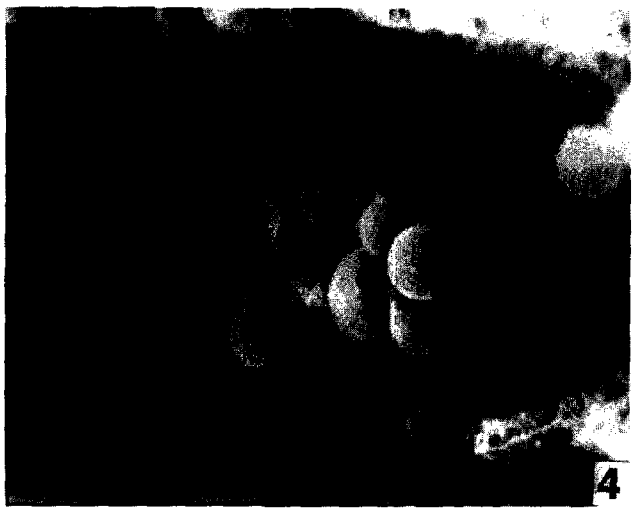

FIG. 4. Spheres present in the proximal tubule of the looped nephron of the domestic fowl *Gallus gallus*.

pointed out above, spheres begin to form in the proximal tubule where the pH of the fluid has changed very little or not at all (7). Although it may be important that the spheres form because of the more acidic conditions later in the nephron or urine, hydrogen ion concentration itself may not be a factor in the events or conditions that initiate the formation of the spheres. One factor or event that causes predictable changes in urine pH is egg laying. However, this probably is not a causative factor in the formation of the spheres.

All animals that excrete excess dietary nitrogen as uric acid form spherical concretions containing urate. The urine of reptiles and the excretory products of many invertebrates contain small spherical structures. The spheres in reptilian urine have a urate content of 88% with potassium being the most abundant cation (16). More information is available for several invertebrates. For the cockroach, *Periplaneta americana*, 68% of the sphere composition has been identified with urate, accounting for 58% and protein about 4% of this total (18). These values are remarkably similar to those measured for the domestic fowl in the present study. Very similar data have been obtained for the spheres found in the excreta of the tobacco hornworm (*Manduca sexta*) where they are 75–78% uric acid and 4–5% protein (5,18). Furthermore, the spheres of both species contain significant amounts of potassium (5,18). Thus available data would suggest the urate sphere formation is not uniquely an avian phenomenon but one common to urate-excreting animals, vertebrate and invertebrate.

In summary, our results show that the inorganic ion content of the urate-containing spheres is mostly calcium and potassium. These cations, together with the urate and protein, associate to form the spheres. This process may enable the removal of the urate from the kidney and may prevent formation of kidney stones that could damage or block the nephron tubules.

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References

1. Braun, E.J.; Dantzler, W.H. Effects of water loading on renal glomerular and tubular function in desert quail. Am. J. Physiol. 229:222–227;1975.
2. Braun, E.J.; Pacelli, M.M. The packaging of uric acid in avian urine. FASEB J. 5:A1408;1991.
3. Braun, E.J.; Reimer, P.R.; Pacelli, M. The nature of uric acid in avian urine. The Physiologist 30:120;1987.
4. Brokl, O.H.; Braun, E.J.; Dantzler, W.H. Transport of PAH, urate, TEA, and fluid by isolated perfused and nonperfused avian renal proximal tubules. Am. J. Physiol. 266:R1085–R1094;1994.
5. Buckner, J.S.; Henderson, T.A.; Ehresmann, D.D.; Graf, G. Structure and composition of urate storage granules from the fat body of *Manduca sexta*. Insect Biochem. 20:203–214;1990.
6. Clark, N.B.; Braun, E.J.; Wideman, R.F., Jr. Parathyroid hormone and renal excretion of phosphate and calcium in normal starlings. Am. J. Physiol. 231:1152–1158;1976.
7. Laverty, G.; Alberici, M. Micropuncture study of proximal tubules pH in avian kidney. Am. J. Physiol. 253:R587–R591; 1987.
8. Laverty, G.; Dantzler, W.H. Micropuncture study of urate transport by superficial nephrons in avian (*Sturnus vulgaris*) kidney. Pflügers Arch. 397:232–236;1983.
9. Levine, R.; Wolson, W.Q.; Lenel, R. Concentration and transport of true urate in the plasma of the azotemic chicken. J. Clin. Invest. 24:186–191;1947.
10. Long, S.; Skadhauge, E. The role of urinary precipitates in the excretion of electrolytes and urate in the domestic fowl. J. Exp. Biol. 104:41–50;1983.
11. Kawashiro, T.; Scheid, P. Arterial blood gases in undisturbed resting birds: measurements in chicken and duck. Respir. Physiol. 23:337–349;1982.
12. Marshall, A.T. Principals and instrumentation. In: Hayat, M.A. (ed). X-ray microanalysis in biology. Baltimore: University Park Press; 1980:1–64.
13. McNabb, R.A. Urate and cation interactions in the liquid and precipitated fractions of avian urine, and speculations on their physico-chemical state. Comp. Biochem. Physiol. 48A:45–54; 1974.
14. McNabb, R.A.; McNabb, F.M.A. Avian urinary precipitates: their physical analysis, and their differential inclusion of cations (Ca, Mg) and anions (Cl). Comp. Biochem. Physiol. 56A:621–625;1977.
15. Minnich, J.E. Water and electrolyte balance of the desert iguana, *Dipsosaurus dorsalis*, in its natural habitat. Comp. Biochem. Physiol. 35:921–933;1970.
16. Minnich, J.E. Excretion of urate salts by reptiles. Comp. Biochem. Physiol. 41A:535–549;1972.
17. Minnich, J.E.; Piehl, P.A. Spherical precipitates in the urine of reptiles. Comp. Biochem. Physiol. 41A:551–554;1972.
18. Mullins, D.E. Isolation and partial characterization of uric acid spherules obtained from cockroach tissues (Dictyopteria). Comp. Biochem. Physiol. 62A:699–705;1979.
19. Porter, P. Physico-chemical factors involved in urate calculus formation II. Colloidal flocculation. Res. Vet. Sci. 4:592–602; 1963.
20. Shannon, J.A. The excretion of uric acid by the chicken. J. Cell. Comp. Physiol. 11:135–148;1938.
21. Wideman, R.F.; Braun, E.J. Stimulation of avian renal phosphate secretion by parathyroid hormone. Am. J. Physiol. 241: F263–F272;1981.
22. Wideman, R.F.; Clark, N.B.; Braun, E.J. Effects of phosphate loading and parathyroid hormone on starling renal phosphate secretion. Am. J. Physiol. 239:F233–F243;1980.