RENAL MUCOID MATERIALS IN PIGEONS FED HIGH AND LOW PROTEIN DIETS

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AT low rates of flow, avian urine can be described as viscous and mucoid in consistency. Mucoid materials in bird urine may function in uric acid excretion by acting (1) as protective colloids that serve to stabilize colloidal dispersions of uric acid and urate salts, or (2) as physical lubricants to aid in the movement of precipitated material through the kidneys and ureters. Longley et al. (1963) used histochemical techniques to demonstrate that mucoids are present in the distal parts of nephrons and in the ureters of chickens and canaries.

We determined the types of mucoids and the sites where mucoids are present in the pigeon kidney, and we tested the hypothesis that an increase in uric acid excretion, produced by feeding a high protein diet, is accompanied by an increase in the amount of mucus in the urinary system.

METHODS

Feral pigeons (*Columba livia*), weighing 225–300 g, were captured in Christiansburg, Virginia. They were accustomed to laboratory conditions for several weeks, then divided into two groups. Three birds received a low protein wheat diet (about 11 percent protein), and three received a high protein diet of ground soybeans (about 44 percent protein). Within 12 days or less on the experimental diets, all birds had achieved stable body weights.

On the 16th day the birds were decapitated and bled. The kidneys were removed immediately and fixed for 48 hours in neutral buffered formalin, embedded in paraffin, and sectioned at 5 micra. Staining characteristics were identical in the right and left kidneys. Data were taken from eight sections cut from the center of each of the three lobes of the left kidney from each bird.

Slides were stained for general structure with Heidenhain's Azan and with three stains specific for mucoid and polysaccharide material: (1) Periodic Acid Schiff (PAS)—haematoxylin which stains primarily muco- and glycoprotein. Digestion with testicular diastase showed that none of the PAS-positive material in these kidneys was glycogen; (2) Alcian blue, which selectively stains acid mucopolysaccharides; (3) Toluidine blue, pH 2.6, which stains specifically for acid mucopolysaccharides and shows purple gamma-metachromasia in the presence of sulphur-bearing carbohydrates.

For each stain, all slides were stained simultaneously, and three slides per bird (one from each kidney lobe) were examined. The intensity of the stain was rated on an arbitrary five-point scale: 0, absent; 1, light; 2, medium; 3, intense; 4, very intense. All sections on each slide were scanned before rating the staining intensity for that slide.

Results

Drinking rates and urine production were not measured, but we did note that birds eating the high protein diet drank more water and produced more copious wet droppings than birds eating the low protein diet. In addition, the droppings of birds on the high protein diet contained large amounts of a white precipitate—presumably uric acid or urates.

Mucoid materials, stained by Alcian blue, Toluidine blue, and PAS were present in the distal portions of the nephrons (primarily in the medullary portions of the collecting ducts) and in the ureters (Table 1). When present, mucoids showed similar stain intensity in the cytoplasm of epithelial cells and in the lumen of the tubule. Stain intensity was greatest in the most distal parts of the system. For example, stain intensity was ranked: ureters > ureteral branches > large collecting ducts > small collecting ducts.

The mucoids present were muco- and glycoproteins, and acid mucopolysaccharides, including sulphur-bearing carbohydrates.

A comparison of staining in the kidney tissues from birds fed high and low protein diets showed no consistent differences in the distribution, types, or amounts of mucoids between the two groups (Table 1).

Discussion

The distribution of mucoid substances in the avian urinary system is consistent with the idea that mucoids function in removing uric acid from the kidneys and ureters. As the avian kidney concentrates urine by a countercurrent multiplier system (Poulson, 1965; Emery, 1967), concentration of the urine will occur in the distal parts of the nephron, especially in the collecting ducts. The consequent increases in uric acid and electrolyte concentrations both will promote uric acid or urate precipitation. Thus the distribution of mucoid materials in the distal parts of the nephrons and in the ureters of chickens, canaries (Longley et al., 1963), and pigeons (this study) points to mucoid function in uric acid elimination.

Mucoids from dog and human urine are capable of stabilizing colloidal urates at concentrations where precipitation otherwise would occur (Porter, 1966). The protein and polysaccharide materials comprising mucoids in bird kidneys are the same types as those in dog and human urine Porter (1966) used. Although colloidal urate accounts for only a small part of the total urate in pigeon urine (McNabb and Poulson, 1970), mucoids probably do exert some protective effect. Porter's (1966) data suggest that at an ammonium ion concentration typical of pigeon urine (mean ammonium ion concentration, 77.6 mg percent in

TABLE 1

THE LOCATION AND INTENSITY OF STAINING MUCOID MATERIALS IN PIGEON KIDNEYS[1]

	Toluidine blue		Alcian blue		PAS-haematoxylin	
	Low protein	High protein	Low protein	High protein	Low protein	High protein
Cortex						
Bowman's capsule	0	0	0	0	0	0
Proximal tubule cells	0	0	0	0	0	0
Proximal tubule lumina	0	0	0	0	0	0
Distal tubule cells	0	0	0	0	0	0
Distal tubule lumina	0	0	?	?	0	0
Collecting duct cells	?	?	2.0 (2.0–2.0)	2.0 (2.0–2.0)	1.0 (1.0–1.0)	1.1 (1.0–1.3)
Collecting duct lumina	0	0	1.7 (1.0–2.0)	1.7 (1.0–2.0)	0	0
Medulla						
Loops of Henle cells	0	0	?	?	0	0
Loops of Henle lumina	0	0	?	?	0	0
Collecting duct cells	1.0 (0.7–1.3)	1.1 (1.0–1.3)	2.1 (2.0-2.3)	2.3 (2.0–2.7)	1.6 (1.0–2.0)	2.0 (1.7–2.3)
Collecting duct lumina	1.4 (1.3–1.7)	1.7 (1.7–1.7)	2.5 (2.0–2.7)	2.1 (1.3–2.7)	1.7 (1.0–2.0)	1.9 (1.7–2.0)
Ureter						
Wall epithelium	3.1 (1.7–4.0)	3.3 (3.0–4.0)	3.1 (2.7–3.5)	3.3 (3.0–4.0)	2.8 (2.3–3.0)	2.8 (2.5–3.0)
Lumen	2.7 (1.7–4.0)	3.7 (3.0–4.0)	2.6 (2.0–3.0)	2.3 (2.0–3.0)	2.5 (2.0–3.0)	2.0 (2.0–2.0)

[1] Stain intensity: 0, absent; 1, light; 2, medium; 3, intense; 4, very intense. Spaces marked ? indicate trace amounts of stain in some tubule sections, but no consistent pattern of staining. Values shown are $\bar{X}$ (range) for three birds. The value used for each bird was a mean of the ratings for each of the three kidney lobes. Means were calculated to one decimal place to aid in expressing the data although estimates of intensity were only whole numbers.

22 samples of pigeon urine; McNabb and Poulson, MS) not more than 125 mg percent urate can exist in the ionic plus colloidal states. However, McNabb and Poulson (1970) found supernatant (ionic + colloidal) urate concentrations of 124–588 mg percent indicating some protective effect, probably attributable to mucoid substances.

An additional function of mucoids may be as physical lubricants and binding agents for precipitated uric acid. The distribution of mucoids within the urinary tract corresponds to those parts handling the greatest amount of precipitated uric acid.

Dietary protein content (and the amount of uric acid excreted) affect neither the sites of mucoid secretion nor the types of mucoids secreted. Our results are equivocal regarding the amount of mucoids produced by birds on these diets. Histological criteria indicate no differences in the amounts of mucoids present in the kidneys of birds fed diets of different protein content. However as histological data give only a static picture, physiological data are also necessary for any inference about rates of mucoid production. Ward et al. (1971) found a nearly fourfold increase in *ad libitum* water consumption when domestic pigeons fed a wheat diet were switched to a diet of soy bean meal (a fourfold increase in dietary protein content). Urine flow rates are four times higher in pigeons fed the high protein diet than in those fed the low protein diet, but urinary uric acid concentrations do not differ significantly between these two groups (unpublished data, our laboratory). If mucoid secretion is related to urinary uric acid concentration, the constant urine uric acid concentrations may explain the similar amounts of mucoids present in histological material from the two groups. The total daily excretion of mucoids probably parallels the large increase in urine production (and thus, uric acid and water excretion) on a high protein diet. High urine flow rates would prevent accumulation of mucoids even with high rates of mucoid production.

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SUMMARY

Glyco- and mucoproteins and acid mucopolysaccharides are present in the distal portions of the nephrons and in the ureters in pigeons. The distribution and types of these mucoids are consistent with the idea that they stabilize colloidal urates and act as physical lubricants in facilitating uric acid elimination.

Dietary protein content does not affect the sites of mucoid secretion or the types of mucoids secreted. High dietary protein content is not associated with mucus accumulation in the renal tubules, but physiological data suggest that total renal mucoid production is increased under these conditions.

LITERATURE CITED

EMERY, N. 1967. A microcryoscopic study of the concentrating mechanism in avian kidneys. Ph.D. dissertation, Syracuse, New York, Syracuse Univ. (University Microfilms, Ann Arbor, Michigan, Univ. Michigan.)

LONGLEY, J. B., H. J. BURTNER, AND B. MONIS. 1963. Mucous substances of excretory organs: A comparative study. Ann. New York Acad. Sci., 106: 493–501.

McNABB, F. M. A., AND T. L. POULSON. 1970. Uric acid excretion in pigeons, *Columba livia*. Comp. Biochem. Physiol., 33: 933–939.

PORTER, P. 1966. Colloidal properties of urates in relation to calculus formation. Res. Vet. Sci., 7: 128–137.

POULSON, T. L. 1965. Countercurrent multipliers in avian kidneys. Science, 148: 389–391.

WARD, J. M., JR., F. M. A. McNABB, AND R. A. McNABB. 1971. The effects of dietary protein content on water consumption and ammonia excretion in the pigeon, *Columba livia*. Virginia J. Sci., 22: 103.

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