

Uric Acid: The Main Nitrogenous Excretory Product of Birds

We would like to raise several questions about Folk's (1) conclusion that bird urine does not contain much, if any, uric acid.

The biological literature contains abundant evidence that most excretory nitrogen of birds is in the form of uric acid or its salts (2). Most of Folk's data on the white part (the urine) of bird droppings are not inconsistent with this generalization. (i) Folk finds that bird droppings exhibit only part of the x-ray analysis pattern typical of uric acid or two of its salts. We would like to know why Folk emphasizes the heterogeneous composition of the droppings that he analyzed but rejects the idea that the x-ray peaks seen are for as yet uncharacterized salts of uric acid or obscure some of the peaks typical of uric acid, or both. Folk himself says that ultraviolet spectrophotometric analyses demonstrate the presence of the urate radical in his samples. (ii) Folk argues that bird droppings dissolve in water or weak acids and therefore cannot be uric acid. Even so, the original dried droppings could have been some urate salt that dissolved and then recrystallized as uric acid. Furthermore, it seems odd for Folk to feel that the addition of water alters the composition of bird urine, when one considers that he used "air-dried" excrement that itself is quite modified from the condition in which it entered the cloaca from the ureters and passed retrograde into the intestine, mixing with the feces, before it was voided; also, freshly voided excrement has a considerable water content. Even the driest bird excrement known to date has a water content of 40 to 50 percent (3).

Folk claims that many articles and texts merely "parrot" the generalization that bird urine consists largely of uric acid. On the contrary, in many references cited by such texts as Sturkie (4) and Marshall (5), uric acid has been determined by acceptable biochemical methods with the use of fresh excreta (2, 6, 7). The most accurate determinations of uric acid in bird urine utilized uricase digestion to abolish ultraviolet absorbance of the purine ring of uric acid or its salts (7, 8). Only about 10 percent of the total ultraviolet absorbance of bird urine is not abolished by uricase (8). Hence only about

10 percent of ultraviolet absorbance is not due to uric acid or its salts. Therefore, even colorimetric methods that do not employ uricase digestion are not seriously in error.

Folk is also critical of "spurious" evolutionary arguments concerning uric acid excretion. It seems to us that the presence of enzymes specific for uric acid synthesis argues for its significance in birds (2, 9). For the evolution of the shelled amniote egg, the low solubility and inertness of urates serves as well as that of uric acid per se. These points, rather than measurement of uric acid in urine, are the crux of Needham's argument (10). It can also be argued that the excretory system of birds and reptiles is structurally consistent with the elimination of precipitated materials such as uric acid and its more insoluble salts (8, 11).

In summary, Folk's observations are interesting re uric acid chemistry but do not refute the generalization that uric acid (or its salts) are the predominant nitrogenous excretory product of birds.

THOMAS L. POULSON
F. M. ANNE McNABB

Departments of Biology,
University of Notre Dame,
Notre Dame, Indiana 46556, and
Virginia Polytechnic University,
Blacksburg 24061

References and Notes

1. R. L. Folk, *Science* **166**, 1516 (1969); *Texas J. Sci.* **21**, 117 (1969).
2. Biochemically, urates and uric acid are not different. Uricase, acting over a moderately large pH range, breaks the purine ring and thus converts either uric acid or urates to allantoin. And, as P. Porter points out [*Res. Vet. Sci.* **4**, 592 (1963)], urate salts, especially ammonium urates, are nearly as insoluble as uric acid itself.
3. T. J. Cade and J. A. Dybas, Jr., *Auk* **79**, 345 (1962); G. A. Bartholomew and M. Smyth, *Condor* **68**, 447 (1966); E. J. Willoughby, *Comp. Biochem. Physiol.* **27**, 723 (1968).
4. P. D. Sturkie, *Avian Physiology* (Comstock, Ithaca, New York, ed. 2, 1965).
5. A. J. Marshall, Ed., *Biology and Comparative Physiology of Birds* (Academic Press, New York, 1960).
6. R. E. Davis, *J. Biol. Chem.* **74**, 509 (1927); W. H. Edwards and W. O. Wilson, *Amer. J. Physiol.* **179**, 76 (1954); W. M. Hart and H. E. Essex, *ibid.* **136**, 657 (1942); J. H. Krakhoff and R. L. Meyer, *J. Pharmacol. Exp. Ther.* **149**, 417 (1965); R. Levine, W. O. Wolfson, R. Lenel, *Amer. J. Physiol.* **151**, 186 (1947); E. B. Mayrs, *J. Physiol.* **58**, 276 (1924); B. R. Nechay and L. Nechay, *J. Pharmacol. Exp. Ther.* **126**, 291 (1959); J. A. Shannon, *J. Cell. Comp. Physiol.* **11**, 135 (1938); A. H. Sykes, *Res. Vet. Sci.* **1**, 308 (1960).
7. G. L. O'Dell, W. D. Woods, D. A. Laerdal,

- A. M. Jeffay, J. E. Savage, *Poultry Sci.* **39**, 426 (1960).
8. F. M. A. McNabb and T. L. Poulson, *Comp. Biochem. Physiol.* **33**, 933 (1970); *Amer. Zool.* **8**, 777 (1968).
9. N. L. Edson, H. A. Krebs, A. Model, *Biochem. J.* **36**, 1380 (1936); T. H. Milroy, *J. Physiol.* **30**, 47 (1904).
10. J. Needham, *Chemical Embryology* (Macmillan, New York, 1931), vol. 2.
11. T. L. Poulson, *Science* **148**, 389 (1965); *Amer. Zool.*, in press.

5 February 1970

Poulson and McNabb have rejected my contention that bird urine (the white, splashy excretion) is not mainly uric acid, a dogma that has enthralled biologists for generations (1). My own experiments embrace only the two extremes of the analytical spectrum: from microscopic examination during treatment of bird urine with household vinegar, tap water, and weak HCl, on the one hand, to x-ray diffraction analysis at the other extreme. Both techniques, the absurdly simple and electronically sophisticated, demonstrate that the material cannot be mainly uric acid. Because I am an ordinary geologist and not a biochemist, I have not undertaken the vast range of intermediate-level techniques such as enzyme digestion and colorimetric or manometric analysis. No one doubts that the material is mainly some combination of C, H, N, and O; the question is whether it is actually excreted as insoluble uric acid, or whether it is excreted as some other material, such as soluble urates, and unwittingly transformed into uric acid by the chemist during preparation for analysis.

Poulson and McNabb criticize my x-ray findings. The droppings I have examined exhibit only one diffraction peak that matches with the uric acid pattern, and it is only the sixth highest peak. If the white material is indeed mainly uric acid, there is no way to mix in a number of extraneous compounds (such as unidentified urates) and suppress the five more intense uric acid peaks while shooting the sixth-ranking peak up to tremendous magnitude. Because the particles are themselves spheres, and the tiny crystallites are arranged tangentially or spirally on the spheres, there is no way one could get a preferred orientation of crystals on the slide to give only a one-peak pattern. The x-ray diffraction analyses show conclusively that the white material may contain only a small quantity of uric acid per se, and perhaps may contain none; but the analyses do not rule out the possibility that a whole

spectrum of different complex urates or other nitrogen compounds is present (2), all combining to give one very strong peak, perhaps corresponding to some spacing between rings of atoms. If uric acid is present at all, it is merely one among many compounds.

There also can be no doubt that the white, crystal-aggregate (not "amorphous") spheres that comprise bird urine go into true solution, although there is much variation in the amount of solubility from one bird to another. If you place a tiny speck of white material on a glass slide and add weak acid, you can watch under the microscope as a sphere shrinks and totally disappears, and elsewhere on the slide a new, much larger, bladed, perfect crystal will start to grow where no solid was visible before. I believe this is commonly accepted as evidence of true solution and reprecipitation. This process goes on in a matter of a few seconds to a few minutes, before any water or acid has evaporated. Of course, after the acid has stood for an hour or so and totally evaporated, many more crystals form. Biologists have not realized this, because they have never watched the material under the microscope.

I do not simply "feel" that the addition of water or acid alters the nature of bird droppings; anyone can "see" for himself if he will take parrot's excrement and mix it with vinegar, and then watch the spectacular microscopic display.

The "acceptable" biochemical methods cited by Poulson and McNabb involve mixing the bird urine with various chemicals, adjusting the pH (3), attacking it with enzymes, and measuring the surviving chemical wreckage colorimetrically, manometrically, spectrophotometrically, and so forth. In my opinion, the drastic wet chemical method of analysis converts the material from whatever it is originally into uric acid before the identification part of the analysis. Although, indeed, most of my studies have been on air-dried excrement of uncertain history (from bottoms of bird cages, chicken coops, and others), I have also looked at freshly excreted material and found no essential difference (except in the case of Australian eagle droppings). But the biochemists have been equally vague about the time elapsing between urination and eventual analysis, a rare exception being Nechay and Nechay (4) who analyzed the material the day of

excretion. In view of the bacteriological changes that may go on in the material (5), possible changes in pH, oxidation-reduction potential, and rapid loss of water as the material is excreted, falls through the air, and dries, it behooves the biochemist to analyze it as rapidly as possible by x-ray diffraction, as that is the only way to conclusively discover what the material really is without altering it by the very method of analysis.

The two papers that Poulson and McNabb cite identifying the presence of uric-acid secreting enzymes in birds are dated 1904 and 1936, and hopefully the identification of urine components was much cruder then than it is even today. Needham (6) stresses again and again that it is the *insoluble* nature of uric acid that allowed the development of the terrestrial egg—a "closed box" in which embryonic waste products had to be retained, but kept in inert form; for example, he says (p. 1144), "the only solution to the problem of getting rid of nitrogen by the embryo is through uric acid." The whole evolutionary theory of development of terrestrial oviparous vertebrates is based on this purported insolubility; I claim it is not uric acid, but is mainly acid-soluble compounds, probably largely urates.

Poulson and McNabb's final structural argument is not pertinent, because obviously the material is a solid pre-

cipitate, whatever its composition, or I could not have seen crystalline spheres with the microscope.

Anyone must conclude that this is a subject that needs to be studied by non-alterative methods (x-ray) on many kinds of birds; the whole subject of urine diagenesis with time, from embryo, liver, kidney, and cloaca to wet splashes and "lithified" hard white crusts, is a wide-open field. To the multitude who have a vested interest in the presumptive predominance of uric acid in bird urine: I challenge you to prove it by x-ray diffraction if you are that confident of being right!

ROBERT L. FOLK

Geology Department,
University of Texas,
Austin 78712

References and Notes

1. For example, a recent confident statement by I. H. Krakhoff and R. L. Meyer [*J. Pharmacol. Exp. Ther.* **149**, 417 (1965)]: "Birds are known to excrete uric acid as the principal end-product of nitrogen metabolism."
2. R. L. Folk, *Texas J. Sci.* **21**, 125 (1969).
3. For example, G. L. O'Dell, W. D. Woods, D. A. Laerdal, A. M. Jeffay, J. E. Savage [*Poultry Sci.* **39**, 426 (1960)] added HCl to bring the pH to between 1 and 2 before analyzing for uric acid.
4. B. R. Nechay and L. Nechay, *J. Pharmacol. Exp. Ther.* **126**, 291 (1959).
5. F. Liebert, *Koninkl. Akad. Wetenschap. Amsterdam Proc. Sect. Sci.* **12**, 54 (1909).
6. J. Needham, *Chemical Embryology* (Macmillan, New York, 1931), vol. 2.
7. I acknowledge Professors L. S. Land and E. C. Jonas (geology department, Univ. of Texas) and W. F. Bradley (chemical engineering) for discussion of chemical questions.
- 10 March 1970

Obsidian Hydration Rates

Johnson (1) reports a series of obsidian hydration readings from a site in northern California which are explained in terms of the Friedman hydration formula:

$$x^2 = kt$$

where x is the thickness of hydration band in microns, k is a constant for a given temperature, and t is the time in years. These readings convinced Johnson that the Friedman rate formula is universally valid: "It is important to underline, for archeologists, the universality of Friedman's equation." I must dissent, because, regardless of the universality of the equation, archeologists who rely on it for determining the age of obsidian specimens will get wrong answers some of the time. Empirical evidence that not all obsidian

forms hydration bands as explained by Friedman has been presented by Clark for California (2) and Meighan *et al.* for western Mexico (3). Johnson does not discuss Clark's evidence, even though it applies to obsidian from other California sites (4), and he doubts the evidence from west Mexico. The same kind of criticism was presented by Friedman and Evans (5) against the data from west Mexico, namely, that the archeology had been incorrectly interpreted and that a proper interpretation would bring the dating into line with the Friedman formula.

Figure 1 shows the differences in interpretation between the linear rate for west Mexico and two rates proposed for temperate areas by Friedman. Table 1 presents two new groups of hydration readings for which the linear rate