ing a fluid which is similar, if not identical, to cerebrospinal fluid produced by the nonplexectomized animal.

Ventricular perfusion studies were performed on five bilaterally plexectomized animals and nine normal rhesus monkeys with $C^{14}$-labeled inulin according to the technique of Pappenheimer *et al.* (*7*). Production of cerebrospinal fluid by both lateral ventricles, third ventricle, and aqueduct of Sylvius (determined by ventriculo-aqueductal perfusion) averaged $15.6 \pm 4.5$ μl/min in normal animals (*8*) and $11.6 \pm 3.2$ μl/min in plexectomized animals (*9*). This represented an overall decrease in production of cerebrospinal fluid of only 26 percent after removal of the choroid plexuses from both lateral ventricles. Even if the choroid plexus of the third ventricle produces fluid in proportion to its size and weight (*10*), the total contribution of the combined plexuses was less than one-third of the newly formed cerebrospinal fluid rostral to the fourth ventricle.

It seems reasonable to conclude that the plexus contributes to the formation of cerebrospinal fluid, but there is even stronger evidence to indicate that it is not the sole or even major source within the primate ventricular system. It is possible, of course, that some functions of the plexus are assumed by other structures after choroid plexectomy.

Bering and Sato (*11*) have concluded that considerable cerebrospinal fluid is produced outside the ventricular system (in the subarachnoid space). However, the consensus view is that, within the ventricular system, cerebrospinal fluid is elaborated primarily by the choroid plexuses. Current data indicate that this is not the case and that most of the cerebrospinal fluid formed within the ventricles is either a specific secretion of the ependymal epithelium or represents a product of cerebral metabolism which enters the ventricular system across the ependymal lining, or both.

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19 August 1969; revised 24 September 1969

## Spherical Urine in Birds: Petrography

Abstract. *The white part of bird droppings (the urine) consists of microscopically uniform spheres 2 to 8 micrometers in diameter. Strange behavior of the spheres in polarized light indicates that they are often made of a spiraling arrangement of crystals that are particularly well revealed by the electron microscope. Bird urine has a varied composition, and x-ray analysis shows that it does not consist largely of uric acid, as has frequently been parroted.*

During an investigation of the geology of Recent sediments, I accidentally discovered that the white part of bird droppings (the urine) consits of minute spheres with extraordinary optical behavior. At the same time, a student was working on Arctic muds and found abundant quartz spheres of similar size. The present investigation was begun in order to study a possible link between the two occurrences. A few simple experiments showed that statements on the composition of bird urine in standard texts are erroneous, and I report these findings in the hope that further investigations may be made by others in the fields concerned.

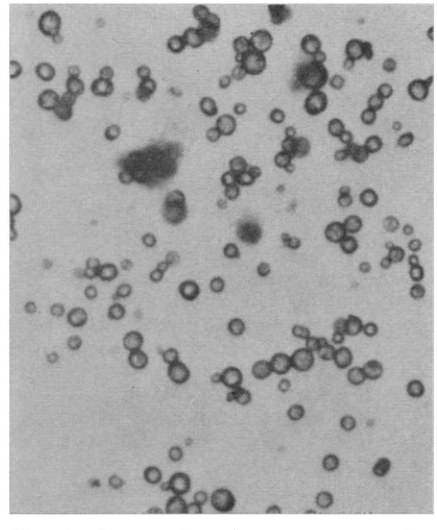


Fig. 1. Pigeon droppings as seen under a light microscope. Largest spheres measure about 5 μm. [Swallow Hall, University of Missouri; C. M. Hoskin, collector]

It is not customary for biologists to use high-powered polarizing microscopes on excrement; hence in reading more than 50 references on excretion, I found only two that say anything more specific than that bird urine is a whitish, pasty, semisolid mass of microcrystalline uric acid crystals. Kaupp (*1*) showed a poor photograph of hen urine with rhombs, which were said to be uric acid, and exceedingly minute dots, said to be sodium urate. Steel (*2*) described the white part of fowl excrement as consisting "almost entirely of minute crystalline spheroids of ammonium urate and uric acid, constituting the urinary secretion of the fowl," and said the same applies to other birds. Steel apparently did not look at the material in polarized light, and his is the only published microscopic description I have encountered; even Hutchinson's (*3*) massive volume on guano mentions no microscopic study of modern bird droppings.

In America, I examined the white part of the droppings of chicken, goose, turkey, pigeon, sea gull, sparrow, starling, and parakeet, in addition to many "unknowns" scraped up from various sidewalks, fence posts, or automobiles. In Australia, I examined urine of pigeons, sparrows, magpies, galahs (a type of parrot), parakeets, white and black cockatoos, ravens, eagles, emus, and sea gulls. Without exception, the white part of the excretion consists almost entirely of spheres (or aggregates of spheres) with identical optical behavior and general appearance, and there appears to be only one solid phase present (Fig. 1). In any one specimen, the spheres appear to be of rather uniform size, although the average size of the spheres varies from about 2 to 8 μm in various droppings. Under highest magnification, some of the larger spheres show an apparent radial-fibrous structure, and rare broken ones divide along radial fractures as if they also had a similar structure.

When the Nicol prisms were crossed (without any interference-figure apparatus in use), the spheres showed pseudointerference figures and appeared to

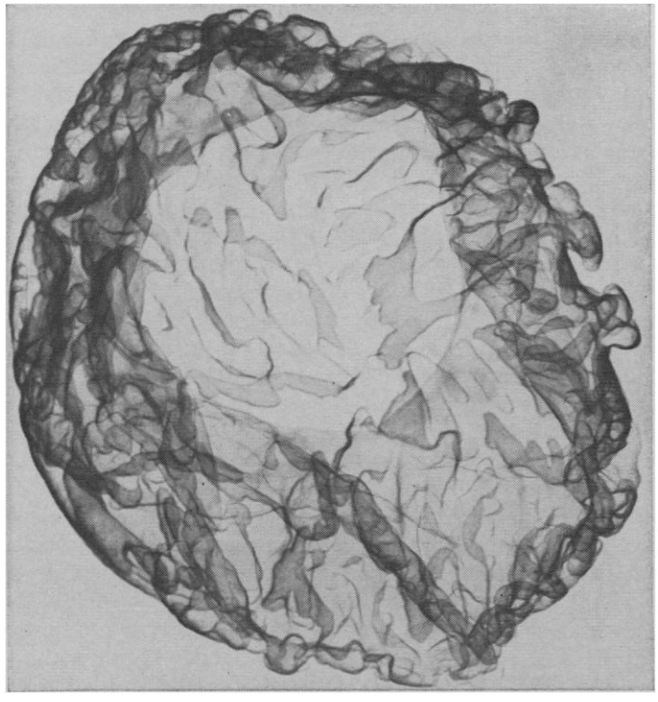
have very high birefringence and high index resembling that of calcite. All small spheres (1 to 3 $\mu$m) showed strong white to pale yellow interference colors and pseudointerference figures. Many of these showed a pseudouniaxial cross that either was inclined up to about 20° from the vertical or had twisted arms. As the stage was rotated, some spheres showed a movement of the dark extinction bands that simulates exactly the movement of isogyres in a biaxial interference figure (acute bisectrix) meeting in the center and retreating hyperbolically toward the margins. A few of the tiniest ones showed only one dark equatorial band that appeared to rotate in the same direction as the stage was rotated. Many of the larger ones that showed the pseudouniaxial cross did so only at certain positions of the stage; with further rotation, the cross dissolved into a high-birefringent blur, and then the cross re-formed when the stage was rotated 90° from its starting point.

If a large sphere was turned over (pushing the cover glass in thick oil mount), it could be made to change from the pseudouniaxial or biaxial figure with low birefringence to a confused tangle of high birefringence, which shows that these two types of optical appearances are really caused by different orientations of the same grain. One would assume, because of the apparent radial-fibrous structure in ordinary light, that the spheres should show in all orientations a rotating cross similar to that of an oolite. That this is not the case was shown by revolving the sphere to various orientations. It is evident that the spheres must have one, and only one, axis of radial symmetry. When this polar axis was vertical, the low colors and uniaxial pseudointerference figures were seen; when the polar axis was horizontal, the biaxial pseudofigures showed up; when seen at an oblique angle, high interference colors and confused bands were seen.

The larger spheres (5 to 10 $\mu$m) generally showed a tangle of confused $2d$ to $3d$ order birefringence; they seemed to be aggregates, made up of a group of two to six or more small spheres packed together and molded to each other to form a nearly spherical larger mass (Fig. 2). As the grain drifted by and rotated in thick index oil, one could see the brief flash of a uniaxial pseudofigure in various parts of the megasphere.

According to the biological literature, the white part of a bird dropping consists largely of uric acid, and uric acid is almost insoluble in water and dilute mineral acids (1 part in 10,000). However, I found that the spheres were very rapidly soluble even in household vinegar, recrystallizing immediately to tiny platelike crystals, rectangular or lozenge-shaped, with a spectacular first-order birefringence in ordinary polarized light. Spheres were also readily soluble in weak HCl. The proportion of spheres converted to platy crystals by acid treatment varied from about 20 to 90 percent, and the crystals produced by acidization had the optical properties of uric acid. Thus the textbooks on avian physiology are in error, and the soluble spheres in bird urine cannot be uric acid when they are excreted.

It was even more intriguing to find that, in the droppings of galahs and other members of the parrot family, from about half to nearly all the spheres were rapidly soluble in water, and separate pinhead-size crumbs from the same dropping yielded different crystal



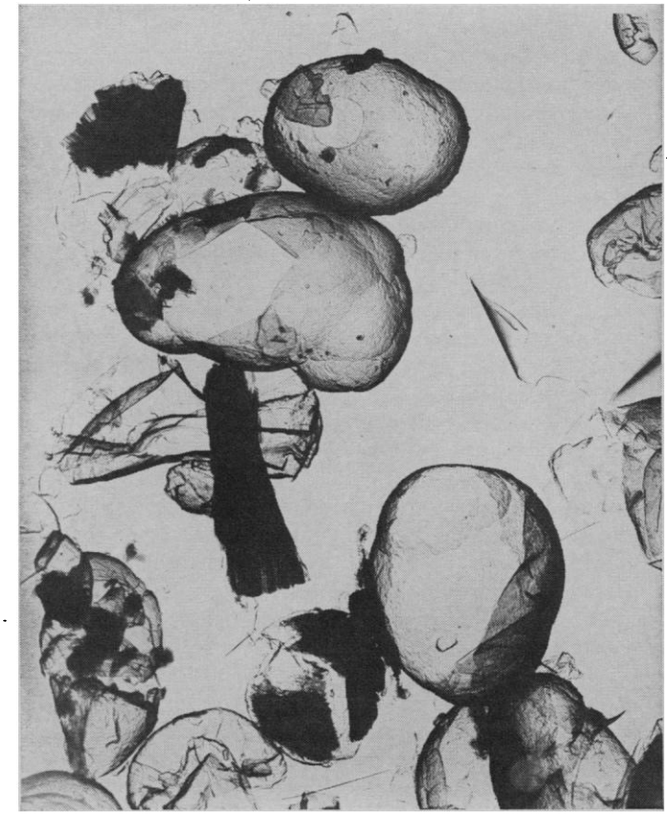


Fig. 2 (left). Electronmicrograph of one sphere approximately 4 $\mu$m in diameter, viewed almost down the polar axis. Note crude spiraling arrangement of tiny crystals. [Parakeet dropping: Horn-Felsher specimen] Fig. 3 (right). Bird urine may occur as single spheres or as aggregates made of several spheres mashed together. The largest composites here are about 5 $\mu$m long. Some individual spheres show an indistinct polar-fibrous pattern. [Dropping from unidentified bird, Oregon; Jane Gray, collector]

forms when attacked by water. Australian eagle droppings were still more unstable, for in the course of a few days, while the material was kept dry in a vial, it changed from spheres into large-bladed crystals.

Obviously, bird droppings, because of their rapid solubility in weak acid (some even being soluble in water), cannot consist mostly of uric acid. The exact determination of composition is a complex matter of organic chemistry and far outside my field. Suffice it to say that the material, although excreted by all birds as microscopically similar spheres, must consist of several things: (i) substances that, even in the dry state, are unstable and change from spheres to large crystals (for example, eagle droppings); (ii) substances that are rapidly soluble in water and immediately reprecipitate as crystals (for example, droppings from members of the parrot family); (iii) substances that are stable in water but are rapidly soluble in weak acids; and (iv) substances that are stable under acid treatment. Even in one bird dropping that is apparently homogeneous (as seen under the microscope) many different substances may be present, judging by the radically different behavior of separate tiny crumbs from the same dropping.

Past misidentification of the droppings as being mostly uric acid is probably due to the fact that the chemist who analyzes the material naturally suspends it in water and acidizes it when he starts his analysis. To the naked eye, the material would appear to be insoluble in water and in acids because it forms a superficially similar white microcrystalline powder both before and after acid attack; only the microscope reveals the radical change that has taken place as it changes from spheres to platy crystals.

In regard to the reliability of standard wet techniques used in chemical analysis for identifying such highly reactive and ephemeral substances as the organic compounds in bird excrement, I quote Prien and Frondel (*4*) who, in advocating the use of the x-ray spectrometer and petrographic microscope, state: "The analysis of urinary calculi by chemical methods is unsatisfactory. Many supposed constituents of calculi recorded in the medical literature are found to be nonexistent when sought by modern physical methods of investigation." Among the reasons they cite for these unknown reactions that may take place during the chemical procedures is the interference by other compounds. These remarks apply equally well to bird urine.

No x-ray studies have previously been made of the droppings, and this apparently is the only way to analyze the material satisfactorily. If possible, x-ray analysis should be done while the substance is fresh from the cloaca, and care should be taken that no water is added to it. In my first x-ray studies, the droppings were mounted on a glass slide with water; but after the accidental discovery that some of the droppings were instantly soluble in water, further studies were made with the dropping being held on the slide with Vaseline. All bird droppings tested showed a single very sharp, very intense peak at 3.20 to 3.23 Å, with no other peaks consistently above noise level.

Uric acid has an "8" intensity peak at 3.22 Å (*4*); but none of the other standard uric acid peaks stronger than these (such as those at 6.69, 4.98, 3.91, 3.12, or 2.90 Å) showed up on my patterns. None of the peaks of uric acid dihydrate (*5*) appeared except the one at 3.20 Å. Sodium acid urate has a "6" intensity peak at 3.22 Å (*4*), but, again, none of the other higher-intensity peaks of this compound showed up on my pattern. Sodium urate does form radiating bundles of needles, soluble in acids, and would be a logical candidate except for the poor showing on the x-ray. It is possible that the odd single-peaked patterns were caused by a mixture of several substances, all of which have peaks in the 3.20- to 3.23-Å region, and that their various other peaks are suppressed for some reason. However, one can safely conclude that x-ray analysis shows that the material is not mainly uric acid, as has been stated so often.

An ultraviolet spectrophotometer analysis (*6*) showed that the droppings contained the urate radical but did not reveal the mode of combination. Thus the chemical composition of the white product of the bird's cloaca remains unknown; my contribution is restricted to the petrography.

Electron microscopy was done in order to distinguish the bird droppings from coccoliths, which they resemble very closely under the light microscope, and also in order to arrive at an explanation of the peculiar interference behavior. Here again, it is evident that optically identical spheres have several types of surface morphology under the electron microscope. One type of sphere consists of fibers spiraling out from a polar axis like a ball of yarn (Fig. 2). This would explain the rotating interference cross with inclined arms (when seen in the polar position) and the pseudobiaxial figure (when seen in oblique view). Another type is made of elongate needles stuck at random tangentially on the surface, like straw on sheep dung; still another type is relatively smooth, with an indistinctly pole-seeking fibrous pattern (Fig. 3).

In 1811, Fourcroy and Vauqelin first reported that bird urine consisted mainly of uric acid. Since then, the idea that uric acid is the chief end product of nitrogen metabolism in birds (and reptiles) has been repeated in numerous research papers and physiology texts (*2, 3, 7*). Uric acid is stated as comprising between 50 and 80 percent of the urine, and the material is described as a white semisolid mass of microcrystalline uric acid crystals and urates.

Elaborate evolutionary theories have been erected on this spurious foundation. Needham (*8*) was the strongest advocate of the importance of uric acid excretion in the evolution of terrestrial birds and reptiles and states that terrestrial oviparous animals would have been impossible without the development of secretion of insoluble uric acid by the embryo, a physiologic process which is continued into adult life. Welty (*9*) claims "The great advantage of excreting uric acid is that it is relatively insoluble in water, so that once it is formed . . . water may be reabsorbed from it until the uric acid is nearly dry and can be discharged from the body with very little water loss. . . . [This] makes it possible for desert birds . . . to exist solely on the water they obtain from the insects they eat. . . . Natural selection, making an adult virtue of embryonic necessity, has preserved the uric-acid excreting machinery in birds and reptiles to promote their conquest of air and habitation of dry land." Now, if this "semisolid mass of white crystals" is indeed largely not insoluble uric acid, as shown by the x-ray data presented here, then these theories must be reevaluated.

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3 June 1969

## Afferent Discharges from Osmoreceptors in the Liver of the Guinea Pig


Abstract. *Afferent activtiy from vagal nerve filament of guinea pig liver was recorded. Perfusion of liver by hypertonic solution caused an increase in firing rate. There may be osmoreceptors in the liver.*


Osmoregulation is generally explained by a feedback loop between the hypothalamo-pituitary system and the kidney. The existence of osmoreceptors in the superior nucleus was established (*1*) in 1947. Haberich *et al.* (*2*) concluded that there are osmoreceptors in the liver because the volume of the urine changes after hyper- or hypotonic solutions are injected into the portal venous system. I now present neurophysiological evidence suggesting that there are osmoreceptors in the liver.

Experiments were performed on the livers of 60 guinea pigs. The animals were anesthetized with urethane, and the liver with the hepatic branch of the vagal nerve was excised from the body and perfused with standard Ringer solution or test solutions through a catheter inserted into the portal vein. The solution entered through the portal vein, circulated in the liver, and exited through the hepatic vein. The solution was saturated with 95 percent oxygen and 5 percent carbon dioxide and maintained at about 30°C. The perfusion pressure was about 50 cm-$H_2O$, and the perfusion rate was about 50 ml/min. An electrometer recorded the afferent impulse discharges from a fine filament of a nerve dissected from the vagal nerve branch of the liver; the records were preserved on magnetic tape. The activity of single or several nerve fibers was converted into standard pulses by a discriminator and analyzed by a digital computer. When a small filament was placed on recording electrodes, spontaneous afferent discharges were usually observed.

In the first experiment, hypertonic Ringer solution which contained 165 m*M* NaCl and had a total osmolarity of 346 milliosmole/liter was used as a test solution. As the range of osmolarity in mammalian systemic blood is supposedly ± 10 milliosmole, the osmolarity of this test solution must equal the maximum possible value expected in the portal venous blood after the absorption of the salt from the intestine. When the system was switched from standard Ringer solution to a test solution, an increase in the discharge rate was observed (Fig. 1). Thus, an increase in NaCl content in Ringer solution causes an increase in the discharge rate of vagal afferents from the liver.

In the next experiment, the relation between the afferent discharge rate and the concentration of NaCl in the test solutions was observed. Four different concentrations of Ringer solution which contained different amounts of NaCl were used. The higher the NaCl concentration, the higher the discharge rate (Fig. 2). Because the increase of 20 milliosmoles in the perfusion solution caused an increase in discharge rate, test solutions which differed by only 17 milliosmoles from

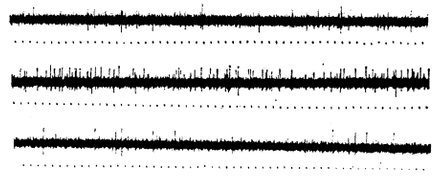

Fig. 1 (above). Afferent impulse discharges recorded from hepatic vagal nerve filament. (Top) Perfusion by standard Ringer solution (326 milliosmole/liter). (Middle) After change to hypertonic Ringer solution (346 milliosmole/liter). (Bottom) After changing back to standard Ringer solution (326 milliosmole/liter). Time mark: 0.1 second.

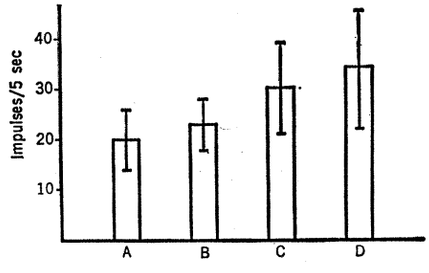


Fig. 2 (right). Relation between afferent discharge rate and concentration of perfusion solution.

| | Concentration | | Impulses/5sec. | |
|---|---|---|---|---|
| | NaCl (mM) | Osmolarity (mOsm) | Mean S.D. N=10 | |
| A | 150.2 | 316 | 20 ± 6 | A<C (0.05>P>0.01) |
| B | 155.2 | 326 | 23 ± 5 | B<D (0.05>P>0.01) |
| C | 160.2 | 336 | 30 ± 9 | A<D (0.01>P) |
| D | 165.2 | 346 | 34 ± 11 | |

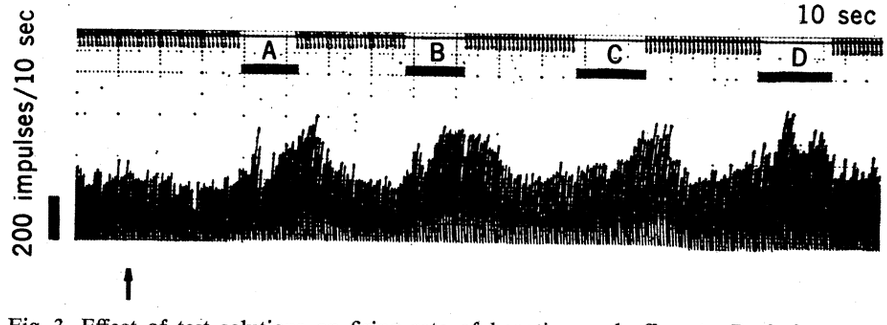


Fig. 3. Effect of test solutions on firing rate of hepatic vagal afferents. Perfusion pressure was increased by 20 cm-$H_2O$ at the time indicated by the arrow. Horizontal bars indicate the time of perfusion by test solution. (A) Hypertonic mannose Ringer solution. (B) Hypertonic NaCl Ringer solution. (C) Hypertonic glucose Ringer solution. (D) Hypertonic sucrose Ringer solution.