activation of the enzyme is not yet established, and reactivation may be an artifact of laboratory manipulation.

Inactivation of the enzyme correlates well with facts about aggregation and differentiation in the cellular slime molds. During the aggregation process, the organism must be able to hydrolyze chemotactic cyclic AMP. Attractant cyclic AMP, if unhydrolyzed, would degrade the signal-to-noise ratio of the chemotactic system. Later, gradual inactivation of the enzyme allows higher concentrations of cyclic AMP to exist. These concentrations trigger phases of differentiation (*3, 23*). Concentrations of cyclic AMP higher than 1 m*M* are inhibitory to differentiation, but the residual activity of the enzyme form with high K_m would be sufficient to prevent the concentration of cyclic AMP from rising to inhibitory levels (*3*). The 5′AMP produced by hydrolysis stimulates the rate of differentiation (*24*).

Thus, cyclic nucleotide phosphodiesterase in the slime mold appears to have the novel property of possessing two kinetically distinct forms for two physiologically distinct roles. More generally, one might ask whether the two forms of the phosphodiesterase derived from brain tissue (*14, 25*) have two functions and are interconvertable, and whether the interconversion is under hormonal control.

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Uricotelism and Low Evaporative Water Loss in a South American Frog

Abstract. *A South American anuran* (Phyllomedusa sauvagii) *produced large amounts of semisolid urate when it was maintained on a diet of insects. Rates of evaporative water loss in* Phyllomedusa sauvagii *were only about 5 to 10 percent of those other anurans tested and were similar to those of lizards of comparable size.*

The ability of some anuran amphibians to survive in arid and semiarid regions has prompted numerous studies of their physiology and behavior. The success of the more terrestrial species is apparently not attributable to major physiological differences related to water, electrolyte, and nitrogen metabolism (*1*). Terrestrial forms generally lose water by evaporation from the skin at high rates when they are exposed to dry air, and they differ from more aquatic species primarily by greater tolerance to large water losses and by avoidance of dehydration by behavioral means. Most anurans excrete nitrogen in the form of ammonia and urea, with the more terrestrial species being ureotelic. When water is not available, these animals can store large amounts of urea in their body fluids. (*2*), but they are unable to excrete nitrogen without considerable expenditure of water. However, an African anuran, *Chiromantis xerampelina,* has recently been found by Loveridge (*3*) to have a relatively impermeable skin and to excrete uric acid. This frog tolerates long periods of exposure to dry air. This discovery by Loveridge is of major significance since it demonstrates physiological mechanisms hitherto unknown and unexpected in amphibians.

While studying a variety of anurans in Argentina, we became aware of *Phyllomedusa sauvagii,* a species that occurs in semiarid regions and appears to be exclusively arboreal. We collected these animals in January 1971 near Ojo de Agua in the province of Santiago del Estero. They were then shipped by air to California where they were kept in a terrarium that contained moist soil and was provided with perches. The animals were offered a variety of insects, but only a few individuals fed voluntarily. After about 6 weeks we began to force-feed the frogs with mealworms (larval *Tenebrio molitor*).

While handling these frogs we observed them to void large masses of white and yellow material enveloped in mucus. After drying this material to constant weight at 100°C, 5 to 10 mg of each sample was dissolved in about 1 ml of 3*N* NaOH and then diluted to 50 ml with distilled water. An additional dilution was made to give a final concentration of 10.0 μg/ml. The ultraviolet absorption spectra of these samples were compared to those of similarly treated uric acid standards. The absorption spectra of all samples were identical to that of uric acid, with maximum absorbance at 293 nm. The absorbances of the yellow and white material were, respectively, 97 and 81 percent of that of uric acid. Incubation of samples and uric acid with uricase (E.C. 1.7.3.3) in borate buffer (*p*H 8.5) abolished absorption at 293 nm.

To determine the relation between food intake and urate production, four *P. sauvagii* were kept without water in individual gallon jars and were fed known amounts of mealworms. Each jar was sealed except for a small hole in the lid and contained a perch made from a length of glass tubing. Animals were in a windowless room at 26°C and 30 percent relative humidity and were kept on a 12-hour photoperiod. Initially, frogs were allowed to hydrate by sitting on a wet synthetic sponge and were weighed after their bladders were emptied. Then, they were force-fed about 20 mg of fresh mealworms per gram of body weight every 2 days. Animals were checked

Table 1. Output of urate and feces in *Phyllomedusa sauvagii* fed mealworm larvae. For the first feeding trial, frogs were maintained without access to water; and in the second feeding trial, water was available. Values for mealworms (M), urate (U), and feces (F) are dry weights.

Animal number	Days	Mealworms fed (g)	Urate excreted (g)	U/M ratio	Feces (g)	F/M ratio
			No water			
1	37	2.78	0.366	0.132	0.342	0.122
2	39	4.92	1.02	0.207	0.760	0.154
3	39	4.80	0.914	0.190	0.415	0.086
4	26	1.98	0.344	0.173	0.284	0.143
			Water available			
1	16	1.52	0.222	0.146	0.146	0.096
2	16	2.18	0.344	0.157	0.322	0.148
3	16	2.27	0.532	0.235	0.182	0.082
4	16	1.46	0.189	0.130	0.231	0.157

daily, and any voided material was collected and frozen. They voided feces regularly, but no urate or liquid urine was voided. Under these conditions the frogs remained very close to their initial weights. After 26 days, the cloaca of one individual was cannulated, and a large mass of mucus-enveloped urate was obtained along with about 0.05 ml of liquid urine. The other frogs were left for an additional 11 to 13 days, during which no urate or urine was spontaneously voided. They were then placed in water, where they began to expel the contents of their bladders, usually within a few minutes. After their bladders were emptied, they weighed only 85 percent of their initial weight, with most of the weight loss being attributable to the voided mucus and urate. The frogs were left in water for several hours, and their weights (with bladder empty) stabilized at about 110 percent of the initial weight. Thus the frogs had apparently incurred a water deficit during the feeding trial.

To determine the effect of the availability of water on urate production, we conducted a second feeding trial, which lasted 16 days and was identical to the first trial except that a petri dish containing a wet synthetic sponge was placed in each jar. Animals frequently sat on the sponges. In addition to feces, liquid urine and mucus-enveloped urate were spontaneously voided. All voided materials except liquid urine were collected quantitatively and frozen. Samples of liquid urine were periodically collected by cannulation of the cloaca, and were analyzed for ammonia and urea by the Conway microdiffusion method.

The urate and associated mucus collected in each feeding trial were pooled by animal, dried, and weighed. Fecal samples were similarly pooled, and their dry weights were determined. About 10 mg of each pooled urate sample was dissolved in 1 ml of 3*N* NaOH and diluted with water to a final concentration of 10.0 μg/ml. The ultraviolet absorption spectrum was always identical to that of similarly treated uric acid, with the absorbance at 293 nm being about 96 percent of that of pure uric acid, and the dry weight of the urate was corrected accordingly. All of the urate solids were yellow except for a small white portion in each of the samples collected at the end of the dry feeding trial. A sample of mealworms contained 367 mg of solids per gram (fresh weight), and this factor was used to calculate the dry weights of mealworms consumed.

Table 2. Rates of evaporative water loss in *Phyllomedusa sauvagii,* other anurans, and desert lizards under comparable conditions (dry air, 26°C, 12 cm/min).

Species and animal number	Weight (g)	Evaporative water loss (mg g^{-1} hr^{-1})
	Anurans	
Phyllomedusa sauvagii		
1	22.5	0.68
2	34.5	0.68
3	35.4	0.33
4	25.3	1.61
Bufo cognatus		
1	33.1	12.2
2	40.0	11.8
Scaphiopus couchii		
1	23.8	14.2
2	27.9	13.6
Rana temporaria		
1	32.7	14.2
2	24.4	18.9
	Lizards	
Dipsosaurus dorsalis		
1	28.0	0.39
2	21.5	0.36
3	27.3	0.30
Uma scoparia		
1	11.3	0.61
2	29.5	0.42

The amount of urate produced was the same whether or not the frogs had water available and ranged from 0.12 to 0.23 g (average, 0.17 g) per gram of mealworm (dry weight). Thus the major difference between the two situations was that urate was retained in the bladder and little or no fluid urine was voided when water was not provided, whereas urate was voided with liquid urine when water was available. Urine produced in the latter situation contained about 40 mmole of urea and 20 mmole of ammonia per liter, but the amount of nitrogen excreted in these substances did not significantly alter the rate of uric acid excretion. The uric acid content of mealworms was about 0.005 g per gram of mealworm (dry weight), and thus the amount of uric acid excreted was far in excess of the amount in the diet. Frogs generally produced less feces than urate (Table 1), and the rate of feces production averaged about 0.12 g of feces (dry weight) per gram of mealworm (dry weight).

Although our results imply that *P. sauvagii* excretes nitrogen primarily as uric acid even when water is available, the degree to which uricotelism is facultative in this species has not been determined. In fact, *P. sauvagii* has been reported to be ureotelic (*4*), although the conditions under which these determinations were made were not specified. Activities of the enzymes arginase (E.C. 3.5.3.1), xanthine oxidase (E.C. 1.2.3.2), and uricase in liver and kidney of *P. sauvagii* resemble those of a lizard, *Dipsosaurus dorsalis,* and differ markedly from those of *Rana pipiens* (*5*).

During the first feeding trial the relative humidity in the jars was high after the animal had been handled during feeding, but the value then dropped to about 60 percent within a day. This led us to suspect that rates of water loss were unusually low in *P. sauvagii,* so we compared rates of evaporative water loss with those of several other anurans and with those of desert lizards. Evaporative water losses were determined by passing 0.5 liter of dry air per minute through a cylindrical chamber 7.5 cm in diameter and 20 cm long. The chamber contained a wire cage to confine the animal and sieve plates at each end to distribute air flow. The relative humidity and temperature of air from the chamber was monitored with a hygrometer (*6*) that was fitted with narrow-range sensors and coupled to a strip chart recorder. This system provided a means by which evap-

oration rates could be monitored continuously, and 1 hour or more was required for stable and minimal rates to be achieved. For all frogs except *P. sauvagii,* rates of water loss were calculated from the lowest stable relative humidity measured. Activity of the animal greatly increased the rate of evaporation, but this method permitted determination of minimal rates during periods in which the animals were quiet. For lizards and for *P. sauvagii*, the relative humidity fell to less than 1.5 percent—below the usable range of the sensors—when the animals rested quietly in the chamber. Their evaporative water losses were then measured from the increase in weight of tubes of Drierite (anhydrous $CaSO_4$) which were connected to the outflow from the chamber for 6 to 18 hours.

Rates of water loss in an aquatic anuran, *Rana temporaria,* were similar to those of two terrestrial fossorial desert species, *Scaphiopus couchii* and *Bufo cognatus*. These results agree with earlier studies that showed a lack of correlation between habitat and evaporative water loss. However, *P. sauvagii* lost water at rates that were only 5 to 10 percent of those measured for other anurans of comparable size; these rates were similar to those of the desert lizards *D. dorsalis* and *Uma scoparia* (Table 2). This result parallels the observation made by Loveridge (*3*) on the African anuran *C. xerampelina*. These animals survived well without water in open jars and lost weight at rates comparable to those of the lizard *Chameleo dilepis,* whereas *Rana angolensis, Bufo regularis,* and *Xenopus laevis* dehydrated rapidly under the same conditions. The nature of the cutaneous barrier in *P. sauvagii* has not yet been determined. The skin appears dry and shiny when the animals are kept out of water, but when the animals are handled their skin becomes moist and evaporative water loss is increased.

P. sauvagii and *C. xerampelina* show similar and unusual physiological and ecological adaptations even though these species are considered to be phylogenetically distinct. The anuran *C. xerampelina* is a member of the family Rhacophoridae, which is restricted to the Old World, and *P. sauvagii* belongs to the Hylidae, which is thought to have originated in the New World (*7, 8*). Nevertheless the species resemble each other in body form, and both are arboreal and lay eggs in vegetation over water (*7*). Although relatively little is known of the ecology of *P. sauvagii,* this species is known to live in semiarid regions. Similarly, *C. xerampelina* is reported to remain exposed in hot, dry areas (*8*). Although terrestrial fossorial anurans from arid regions are not markedly different physiologically from aquatic forms, the exploitation of arid regions by arboreal forms has apparently required novel adaptations for the conservation of water.

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Mapping of Interactions in the Pitch Memory Store

Abstract. *A technique obtaining a precise mapping of interactive effects in the pitch memory store is described. Subjects were required to compare two tones for pitch when these were separated by a 5-second interval during which six other tones were played. In the second serial position of the intervening sequence there was placed a tone whose pitch bore a critical relationship to the pitch of the first test tone. When the critical intervening tone was identical in pitch to the first test tone, memory facilitation was produced. As the separation in pitch between these two tones increased, errors rose progressively, peaked at a separation of 2/3 tone, and declined roughly to baseline at a whole tone separation. It is concluded that the pitch memory store is arranged logarithmically in a highly ordered and specific fashion.*

Investigations into human memory storage have traditionally emphasized the use of verbal materials; in contrast little is known about the retention of unlabeled sensory stimuli. Yet in exploring a memory store there are obvious advantages to the use of stimuli which can be precisely specified and controlled, and which can be varied along one dimension at a time. Tonal pitch represents an example of such a stimulus. The experiment reported here demonstrates that the pitch memory store is laid out in a highly ordered and specific fashion, and that it is possible to map very precisely both facilitatory and disruptive interactions taking place within it.

Previous studies have shown that pitch information decays spontaneously though slowly in the absence of intervening stimulation (*1*). The incorporation of other tones during the retention interval produces considerable memory disruption (*2, 3*). This interference cannot be explained in such general terms as a limitation in general short-term memory capacity, or a distraction of attention, since the incorporation during the retention interval of spoken numbers (which the subjects are required later to recall) produces only a minimal decrement in the same pitch recognition task that is severely disrupted by the interpolation of other tones (*3*). It is clear, therefore, that interactive effects must take place within the pitch memory store itself. The following study represents a mapping of such effects.

Subjects were required to make a series of judgments of the following nature. A 200-msec test tone was played, which was followed 5 seconds later by a second 200-msec test tone. During the retention interval six other tones were played. These were also 200 msec in duration, and were separated by 300-msec intervals, leaving a 2-second pause before the second test tone. The subjects were instructed to ignore the intervening tones, and simply to indicate whether the test tones were the same or different in pitch by writing "S" or "D."

For the test tone stimuli 12 tonal pitches were used. These were taken