spectra, on a 21-103C and a 21-110 (CEC). Gas chromatography was carried out on an A90P3 and a 204 (Aerograph) stream splitter and flame-ionized detector.

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Cocoon Surrounding Desert-Dwelling Frogs

Abstract. *A cocoon formed from a single cell layer of shed* stratum corneum *may reduce water loss from the skin of desert-dwelling frogs while these aestivate in soil-filled burrows. In several Australian examples, the cocoon is a single layer of cells, and thus differs from the multilayered structure obtained from an American species,* Scaphiopus couchi.

Many terrestrial frogs avoid dehydration by burrowing (*1, 2*). Frogs in burrows probably experience cool and moist conditions, and may remain there, except for brief visits to the surface during and immediately following rain. Mayhew (*3*) found that the spade-foot toad, *Scaphiopus couchi*, emerged from burrows after rain with fragments of skin-like material covering the back. These he suggested were remnants of a semi-impervious cocoon formed by the frog while underground. Similar cocoons are developed by a number of Australian burrowing frogs. Here we describe the structure of the intact cocoon, and assess its function as a barrier to water loss.

Cocoons have been found surrounding the frogs, *Cyclorana alboguttatus, C. platycephalus* and *C. australis, Limnodynastes spenceri*, and *Neobatrachus pictus*. In all instances the transparent cocoon completely surrounded the frog (Fig. 1); it was complete across the eyes and the cloaca, but there were small tubular inserts into the external nares. In some instances, the cocoon was sealed around the mouth.

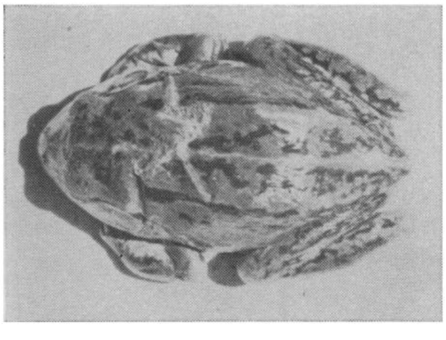

Fig. 1. Cocoon surrounding a specimen of *Cyclorana alboguttatus.*

All of the frogs with cocoons were removed from soil-filled burrows in dry clay. Cocoons surrounding frogs which had been underground for from 3 to 5 months were barely separated from the skin, and were still soft and pliable. Those from two frogs, 7 and 10 months underground, were free of the cocoon except about the head and limb extremities, and were dry but flexible. Beneath the cocoon the skin appeared moist.

Electron micrographs of sections cut tangential to the surface of the cocoon of *N. pictus* show that the cocoon is a single layer of flattened cells with the nucleus still obvious in many of the cells, and the cell membranes well-defined (Fig. 2). The intracellular spaces are filled with sheaves of fine fibrils of keratin. The intercellular contacts between the cells are well-marked, strong, and persistent. A comparison with the structure of the intact skin of the same frog (Fig. 3) shows that the cocoon is identical to the *stratum corneum* of the epidermis, and is derived by sloughing this layer as a compact unit, as occurs in snakes. The separation occurs intercellularly; the contents of numerous small vesicles, which gather beneath the cell membranes before separation and are absent afterwards, appear to enter the intercellular space and may facilitate separation. These cocoons appear to differ from that described for *Scaphiopus couchi*, where there is a keratinization and darkening of the skin after 1 month underground (*4*) with the formation of a black membrane of several layers (*3*).

The rate of passage of water vapor through cocoons of *C. alboguttatus* and *N. pictus* was measured by placing pieces of a cocoon between a saturated atmosphere (against the surface proximal to the frog) and silica gel, both at 25°C. The weight gained by the silica gel represented the weight of water absorbed through the membrane. For comparison, rates of dehydration of four *N. pictus* and eight *C. alboguttatus* were obtained under identical conditions. As activity influenced the weight lost by the frogs, only those hourly periods during which the frogs remained still were used to estimate the rate of dehydration.

The average rate of passage of water vapor through the cocoons of eight *C. alboguttatus* was 0.65 (S.D. ± 0.22) mg of water per square centimeter per hour and through a single cocoon from *N. pictus* it was 0.51 mg. These values are similar, and both are significantly lower ($P < .01$) than the average rates of water loss [expressed in terms of body surface where $S = 6\sqrt[3]{(W)^2}$ (*5*)] from frogs of the same species in an identical atmosphere. In eight *C. alboguttatus*, the rate of water loss from the whole frog was 4.90 (± 0.08) $\text{mg cm}^{-2}\ \text{hr}^{-1}$ and for four *N. pictus* it was 9.0 (± 0.10) mg. However, a direct comparison with cutaneous water loss is not possible since water is lost from the lungs as well as the skin in these frogs.

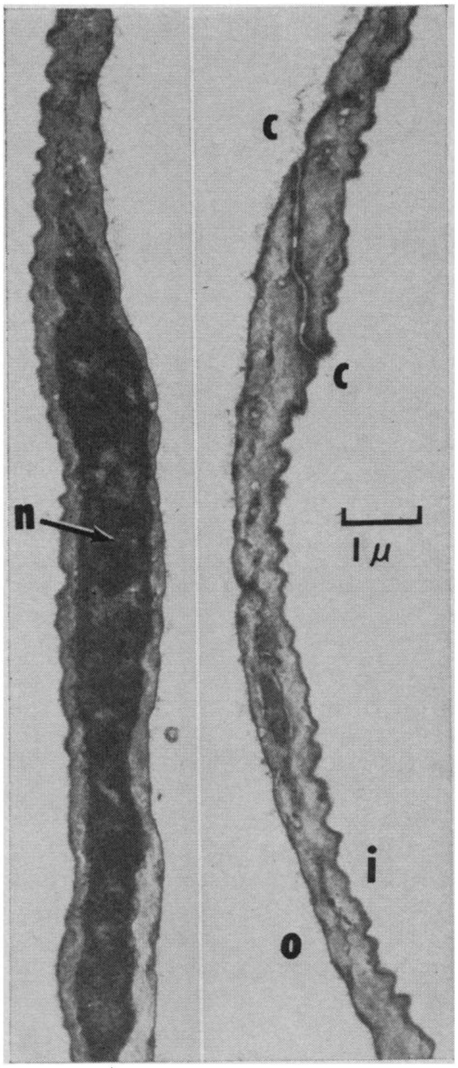

Fig. 2. Electron micrographs of a cross section of two portions of a fold in a cocoon from *Neobatrachus pictus*. The material was fixed in osmium fixative and stained with uranyl acetate and a lead preparation. Details of cellular structure are still evident: *n*, nuclear remnant; *o*, outer cellular surface; *i*, inner cellular surface; *c*, persistent intercellular contact.

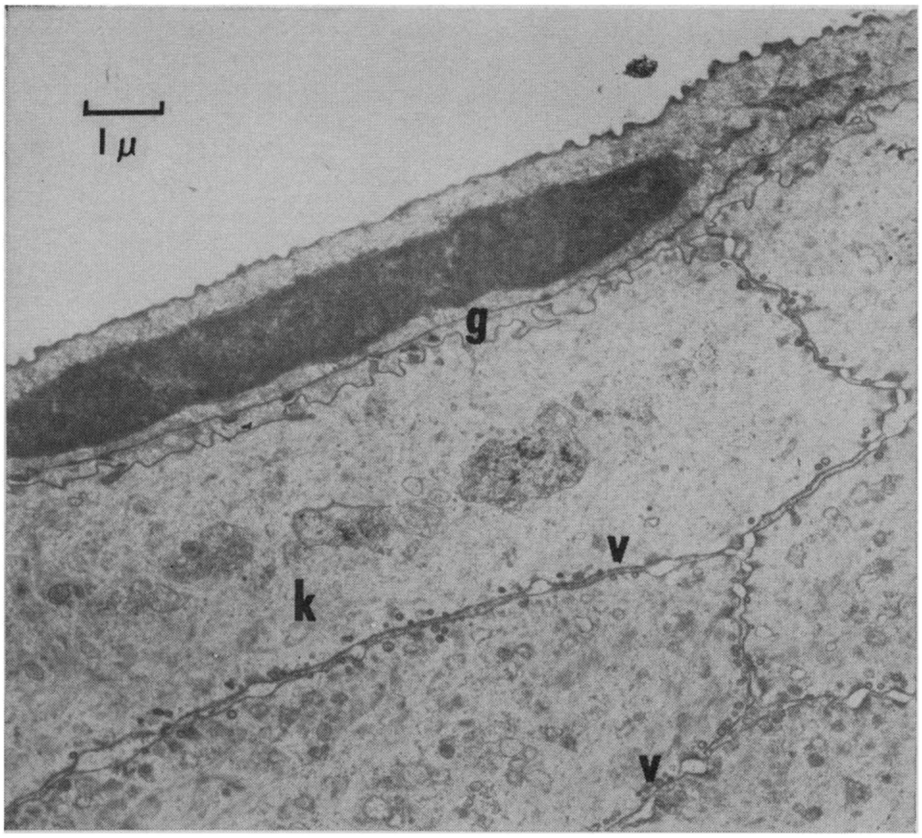

Fig. 3. Outer layers of the epidermis of *N. pictus*, fixed in glutaraldehyde and osmium and stained in uranyl acetate and a lead preparation. The outer keratinized layer of cells (*stratum corneum*) is shown separating from the underlying layer (*g* is intercellular gap) to form the cocoon. Filaments of keratin (*k*) and remnants of intracellular organelles are visible. Notice the small vacuoles (*v*) close to the intercellular gap.

The lethal limits of water loss of previously hydrated desert-dwelling frogs lies between 40 and 50 percent of the body weight (*2*). Thus a *C. alboguttatus* weighing 18 g can afford to lose approximately 7.2 g of water, and an *N. pictus* weighing 9 g, 3.6 g of water. These estimates exclude the water stored in the urinary bladder which may be reabsorbed to replace water lost, and which in desert-dwelling frogs may amount to 50 percent of the body weight (*2*).

Therefore the total amounts of water these frogs can afford to lose is 16.2 g and 8.1 g, respectively. Should conditions as vigorous as those in the experiments prevail, and if the surface area of the cocoons is assumed to be the same as that estimated for the frogs, then the rate of water loss from the cocoons would be 0.027 g/hr for a *C. alboguttatus* weighing 18 g and 0.013 g/hr for an *N. pictus* weighing 9 g. With the exception of water lost from the lungs, *C. alboguttatus* would reach the lethal limit of water loss after 600 hours, and *N. pictus* after 623 hours. Thus, the cocoons contribute significantly to the water economy of the frogs, but some additional factor must suppress evaporative water loss as these frogs may spend periods of 3 months or more underground. This additional factor is presumably moisture in the soil (*2*).

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References and Notes

1. A. R. Main, M. J. Littlejohn, A. K. Lee, in *Biogeography and Ecology in Australia*, A. Keast, R. L. Crocker and C. S. Christian, Eds. (Junk, The Hague, 1959), pp. 404–405.
2. P. J. Bentley, *Science* **152**, 619 (1966).
3. W. W. Mayhew, *Amer. Midland Naturalist* **74**, 97 (1965).
4. L. McClanahan, *Comp. Biochem. Physiol.* **20**, 73 (1967).
5. P. Rey, *Ann. Physiol. Physicochim. Biol.* **13**, 1081 (1937).
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Frequency Sensitivity of Single Auditory Neurons in the Gecko Coleonyx variegatus

Abstract. *Although acoustic communication is not pronounced in reptiles, analysis of single auditory neurons in the medulla oblongata shows that the cochlea is a frequency analyser. Auditory neurons of the lizard* Coleonyx variegatus *respond to acoustic stimuli over a range of less than 0.1 to 17 kilohertz and are maximally responsive between 0.8 and 2.0 kilohertz. The frequencies to which they are most sensitive differ from neuron to neuron, ranging from 0.11 to 4 kilohertz. Some neurons have an inhibitory area which greatly overlaps the response area, so that inhibitory areas do not seem to sharply tune the response area at this level of the auditory tract. The inhibitory area is responsible for producing in some neurons a phasic response and nonmonotonic relation between sound intensity and number of impulses. The response pattern shows a tendency to change from tonic to phasic in more advanced auditory centers. This may serve to code rapid changes in the acoustic stimuli.*

Acoustic communication is a prominent feature in many amphibians (*1*). Frishkopf and Goldstein (*2*) have demonstrated in the eighth nerve of frogs two types of neurons distinguished by different frequency sensitivities. In the midbrain auditory system of frogs, however, Potter (*3*) has demonstrated units with various shapes of response areas (area above threshold curve of a single neuron). Compared to amphibians, the reptilian inner ear is anatomically more specialized for hearing. The basilar membrane is elongated and differentiated in width along its length. The hair cells also show considerable differentiation in number and distribution along the basilar membrane (*4*). Despite these developments, acoustic communication is considered to be rudimentary or absent in most reptiles except for the crocodilians, lizards of the family Gekkonidae, and a few turtles (*1, 5*). The central auditory physiology of reptiles, even the vocal forms, has received almost no attention. The majority of the studies of reptilian hearing have used cochlear microphonics or behavioral responses as indexes of sensitivity. We now report the properties, in terms of frequency sensitivity, of auditory neurons in the medulla oblongata of a gecko.