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30. We thank S. Uyeda, T. Hilde, W. Sager, L. Stokking, and D. Merrill for reading the earlier version of the manuscript; J. Watkins and the Institute for Rock Magnetism, University of Minnesota, for support; and two anonymous reviewers for helpful comments and suggestions that improved the paper.

15 June 1992; accepted 25 August 1992

Homobatrachotoxin in the Genus *Pitohui*: Chemical Defense in Birds?

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Three passerine species in the genus *Pitohui*, endemic to the New Guinea subregion, contain the steroidal alkaloid homobatrachotoxin, apparently as a chemical defense. Toxin concentrations varied among species but were always highest in the skin and feathers. Homobatrachotoxin is a member of a class of compounds collectively called batrachotoxins that were previously considered to be restricted to neotropical poison-dart frogs of the genus *Phyllobates*. The occurrence of homobatrachotoxin in pitohuis suggests that birds and frogs independently evolved this class of alkaloids.

A variety of organisms are known to produce or sequester noxious compounds that can be used for defensive purposes. Until recently, no examples of chemical defense were known among birds although there are many examples in all other vertebrate classes. In 1990 we discovered that the hooded pitohui, *Pitohui dichrous*, contained in its feathers and muscle tissue a toxic substance that could function as a defensive chemical. The toxin caused numbness, burning, and sneezing on contact with human buccal and nasal tissues during collection and preparation of specimens. Local New Guineans referred to the bird as a "rubbish bird" that should not be eaten unless it was skinned and specially prepared (*1*). We have since collected tissue of three species of the genus, and we report the results of bioassays of toxicity and the identification of the toxin as a steroidal alkaloid.

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Feathers, skin, striated muscle, uropygial gland, heart-liver (combined), and stomach with contents were separated from individual hooded pitohuis, variable pitohuis (*P. kirhocephalus*), and rusty pitohuis (*P. ferrugineus*) (*2*). Each tissue was stored separately in 100% ethanol and later was macerated and washed with 100% ethanol. These crude ethanol extracts were concentrated so that 100 μl of extract were equivalent to 100 mg of tissue.

We conducted bioassays by injecting ethanol extracts subcutaneously into the hindquarters of mice (*3*). The effect of the injection was monitored for 3 hours or until death. These assays showed that in all three species the skin and feathers of the pitohuis were most toxic, the striated muscle was much less toxic, and the heart-liver, stomach, intestines, and uropygial gland were least toxic (Table 1). Concentrations of the toxin varied interspecifically, and of the three *Pitohui* species the hooded pitohui was most toxic, the variable pitohui was less toxic, and the rusty pitohui was least toxic (Table 1). In the variable pitohui, tissues of an adult were more toxic than tissues of an immature bird.

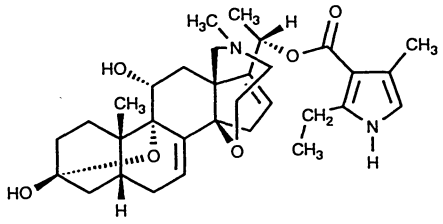
After fractionation of extracts of feathers, skin, or muscle by acid-base partitioning (*4*), only the alkaloid fraction was toxic to mice. Alkaloid fractions from skin and muscle were then examined by gas chromatographic–mass spectral analysis, thin-layer chromatography, and direct probe mass spectrometry. Thin-layer chromatography revealed the presence in skin of a single alkaloid that gave a blue color reaction with modified Ehrlich's reagent identical to that of homobatrachotoxin (*5*). That alkaloid cochromatographed with homobatrachotoxin (R_f 0.50), and the mass spectrum (*6*) was also identical to that of homobatrachotoxin; these results confirmed the identity of the major *Pitohui* toxin in skin. Toxic effects of the alkaloid fractions from skin were virtually identical to those of homobatrachotoxin, causing partial paralysis of hind limbs, locomotor difficulties, and prostration at low dosages (<0.01 μg of homobatrachotoxin) and tonic convulsions and death at higher doses (>0.03 μg of homobatrachotoxin).

Homobatrachotoxin (Fig. 1) is a member of a family of steroidal alkaloids collectively called batrachotoxins. Batrachotoxin (R_f 0.45), a closely related member of the same family of toxins, was not present. Homobatrachotoxin was also present in muscle tissue but at much lower concentrations, consonant with the lower toxicity of muscle extracts. Batrachotoxins depolarize nerve and muscle cells by activating Na^+ channels (*7*) and thus irritate sensory neurons in buccal tissue (*1*).

Table 1. Toxic effects in laboratory mice of extracts from tissues of three species of pitohuis.

Species	Equivalents of tissue injected (mg)	Toxicity*
Hooded pitohui (*P. dichrous*)		
Skin	10	Convulsions and death in 18 to 19 min
Feathers	25	Convulsions and death in 15 to 19 min
Striated muscle (breast)	100	Convulsions and death in 35 to 70 min
Heart and liver	300	No convulsions or death
Stomach	300	No convulsions or death
Intestines	300	Minimal or no effects
Uropygial gland	100	Minimal or no effects
Variable pitohui (*P. kirhocephalus*)†		
Skin	20	Convulsions and death in 16 to 18 min
Feathers	50	Convulsions and death in 19 to 27 min
Striated muscle (breast)	200	No convulsions or death
Heart and liver	300	No convulsions or death
Stomach	300	No convulsions or death
Rusty pitohui (*P. ferrugineus*)		
Skin	40	Convulsions and death in 30 to 40 min
Feathers	100	No convulsions or death
Striated muscle (breast)	200	No convulsions or death

*At the indicated dosage all extracts except those marked "minimal or no effects" induced hind limb paralysis, marked locomotor difficulties, and then profound prostration. Some extracts induced permanent paralysis in a hind limb. These effects are similar to those of 0.005 to 0.01 μg of homobatrachotoxin [compare with (*3*)]. Additional toxic sequelae are noted, indicating higher concentrations of toxin. Death in 18 to 19 min is commensurate with the presence of about 0.05 μg of homobatrachotoxin. †Extracts of tissues from a juvenile appeared somewhat less toxic.



Homobatrachotoxin

Fig. 1. Structure of homobatrachotoxin (*3*).

Homobatrachotoxin was previously known only from the genus of poison-dart frogs, *Phyllobates* (Dendrobatidae), in which it occurs with nearly equal concentrations of batrachotoxin and with the less toxic, probable precursor, batrachotoxinin A (*3*, *8*). The genus *Phyllobates* contains the three toxic Colombian frogs (*P. aurotaenia*, *P. bicolor*, and *P. terribilis*) whose skins are used for poisoning blowgun darts (*8*). In *Phyllobates* frogs, Na^+ channels are insensitive to the effects of batrachotoxins (*9*); the frog is thus protected from toxins that are released from storage sites in skin. It is not yet known how pitohuis tolerate homobatrachotoxin in their own muscle tissue. *Pitohui* birds and *Phyllobates* frogs are phylogenetically and geographically separated and are the only known organisms that contain batrachotoxins, suggesting independent evolutionary acquisition of this alkaloid in birds and frogs.

On the basis of bioassays of toxicity, we estimate that a 65-g hooded pitohui contains 15 to 20 μg of homobatrachotoxin in the skin and another 2 to 3 μg in the feathers, whereas muscle and other tissues contribute less than 1 μg. Skin of an adult variable pitohui (85 to 95 g) is estimated to contain 6 to 10 μg of homobatrachotoxin, whereas skin of a 100-g rusty pitohui is estimated to contain 1 to 2 μg of homobatrachotoxin. In contrast, the total amount of batrachotoxins in the skin of Colombian poison-dart frogs of the genus *Phyllobates* ranges from about 100 μg in two species (*P. aurotaenia* and *P. bicolor*) to 1000 μg in a third species (*P. terribilis*) (*8*). The two Central American frog species (*P. vittatus* and *P. lugubris*) have much lower levels in the skin, ranging from undetectable to about 3 μg. Levels of homobatrachotoxin in pitohuis are thus low compared to levels in the frogs, particularly when the great difference in size is considered. Skin from a pitohui has a mass of 4 to 5 g, whereas skin from the poison-dart frogs has a mass of 0.2 to 0.5 g, depending on the species.

Batrachotoxins have been shown to depolarize electrogenic membranes in a range of vertebrates and invertebrates (*7*). The most likely natural predators of pitohuis are snakes, raptors, and potentially some arboreal marsupials. All are expected to be sensitive to batrachotoxins. We therefore believe that homobatrachotoxin provides the hooded, variable, and perhaps rusty pitohuis some protection against predators.

Poisonous animals are often conspicuous (*10*, *11*), and several studies have argued that conspicuousness enhances the effectiveness of educating predators about prey noxiousness (*10*, *12*), that conspicuous color patterns startle or cause hesitation in predators (*11*), and that some colors or color patterns are avoided by an instinctive or unlearned mechanism in predators (*13*). Both hooded and variable pitohuis emit a strong sour odor and are brightly colored. The wings, tail, and head of the hooded pitohui and of some races of the variable pitohui are black, and the remaining portions of the body are a sharply contrasting orange-brown.

Although the exact function of homobatrachotoxin in pitohuis remains unknown, the discovery of this toxic alkaloid in pitohuis expands the known possible antipredator adaptations in birds to include chemical defense and perhaps mimicry. When a toxic species (a model) is avoided by predators, other species (mimics) may avoid predation by resembling the model. Four races of the variable pitohui resemble the coloration of the hooded pitohui, and both races of the variable pitohuis that we sampled (one resembles the coloration of the hooded pitohui, and one does not) have the same sour odor as the hooded pitohui (*14*). In some regions, immature greater melampittas, *Melampitta gigantea*, also look similar to the hooded pitohui (*15*).

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5. Silica gel thin-layer chromatoplates were used with chloroform-methanol (9:1) as the developing solvent, and modified Ehrlich's reagent (0.1% *p*-dimethylaminocinnamaldehyde in 0.1 N HCl) was used for detection.
6. Mass spectrometer VG7070F· Electron impact spectrum, direct probe (numbers in parentheses are intensities relative to the base peak set equal to 100): mass-to-charge ratios (*m/z*) of 399 (25), 370 (20), 312 (43), 294 (17), 286 (15), 184 (54), 153 (17), 138 (24), and 88 (100). Batrachotoxin and homobatrachotoxin do not give a significant molecular ion because of thermal decomposition. The exact mass of *m/z* 399 (measured 399.2410)

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5 May 1992; accepted 30 July 1992

Saltation and Stasis: A Model of Human Growth

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Human growth has been viewed as a continuous process characterized by changing velocity with age. Serial length measurements of normal infants were assessed weekly (n = 10), semiweekly (n = 18), and daily (n = 3) (19 females and 12 males) during their first 21 months. Data show that growth in length occurs by discontinuous, aperiodic saltatory spurts. These bursts were 0.5 to 2.5 centimeters in amplitude during intervals separated by no measurable growth (2 to 63 days duration). These data suggest that 90 to 95 percent of normal development during infancy is growth-free and length accretion is a distinctly saltatory process of incremental bursts punctuating background stasis.

The present assumptions regarding the biology of human growth are based primarily on height and weight data collected in auxological studies. Individuals have been traditionally measured at quarterly intervals during infancy, and annually or biannually during childhood and adolescence. Physiological data are mathematically smoothed and growth is represented as a continuous curve of three sequential stages: infancy, with growth progressing at a rapidly decelerating rate from birth; childhood, as growth approaches a relatively constant but slow rate; and adolescence, when the pubertal growth spurt propels the body toward final adult form with a sharp increase and final rapid decrease in growth velocity (*1*, *2*).

Although undulations in growth velocity patterns have been described in individual data as early as the 18th century (*3*), they have most often been assumed to reflect measurement error (*4*). The consensus for most of the century has been that a focus on the structure of the individual time course of growth is unprofitable. Sporadic reports suggest that traditional studies may overlook important aspects of individual growth patterns because undulations in growth rates shorter than the period of measurement go undetected (*5*). Descriptive studies support this conclusion with data on nonlinearity (*6*) or short-term velocity oscillations in serial height as well as total body or lower leg length (*7*). The general dictum, however, is that while some oscillation occurs in the growth rate of some children, growth is a continuous and generally constant process (*1*), and that the most satisfactory assessment of children's growth is still considered to be made over annual intervals (*8*).

The availability of human growth hormone and the resulting clinical potential for treatment of growth disorders, as well as advances in molecular biology describing normal cellular growth control mechanisms, underscore the importance of clarifying normal growth dynamics.

The present study further investigates the nature of normal infant growth with time-intensive data and an analytic descriptor. Thirty-one clinically normal (*9*) Caucasian American infants (19 females and 12 males) were studied between the ages of 3 days and 21 months after parental informed consent of an institutionally approved human subjects protocol. Ten of these infants were measured weekly for periods of 4 to 12 months, 18 were measured semiweekly for 4 to 18 months, and 3 infants were measured daily for 4 months.

Recumbent length, weight, and head circumference were assessed according to standard techniques (*10*); the serial length measurements are the focus of this report. Total recumbent length was measured to the nearest 0.05 cm by two observers with a specially designed infant measuring board (*11*) during home visits; 80% of the measurements were replicates.

Sources of measurement error and variation include the equipment, repeated measurements, the technique of the observer, and the cooperation of individual subjects. The technical errors of repeated measurement (*12*) for length were significantly different between children, reflecting individual variability in cooperation. A pooled intra-observer error of 0.124 cm (1729 replicates) and an inter-observer pooled error of 0.11 cm (with an independent rater), parallel reports of technical errors of replicate measurement (0.114 to 0.145 cm) recently published (*13*). Because the technical error of repeated observations cannot account for all errors of measurement, the quantitative analytic methods were designed to take into account a wider range of possible measurement error inherent in the data.

Analytical methods developed in part for the evaluation of episodic hormonal pulses (*14*) were modified for the analysis of the serial body measurements as an ad hoc first approximation descriptor. Individual serial growth data were modeled as a series of putative, distinct, stepwise (saltatory) increases or jumps separated by variable intervals of no change. Using replicate measurements and an error estimate, we express serial increments as standard normal deviates. These deviates are assessed at an experimentally defined probability (or P value) of falsely rejecting the null hypothesis of no difference in serial length measures.

The growth in length of all subjects in this study occurred by saltatory increments with a mean amplitude of 1.01 cm identified at the $P < 0.05$ level. A plot of this growth punctuates intervals when no statistically significant growth occurred (Table 1). We found that the growth saltations were not identifiably periodic but episodic. Information on the precise temporal structure of a growth saltation is constrained by the measurement interval, the smallest window for incremental growth documentation. When assessed weekly, length increments from 0.5 to 2.5 cm punctuated 7- to 63-day intervals of no growth. Semiweekly assessments showed saltatory length increments of 0.5 to 2.5 cm punctuating 3- to 60-day intervals of no growth. Daily measurements documented length incre-

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