corresponds to the expected empirical formula of $C_{24}H_{33}NO_4$ (calculated 399.2319).
7. E. X. Albuquerque, J. W. Daly, B. Witkop, *Science* **172**, 995 (1971).
8. C. W. Myers, J. W. Daly, B. Malkin, *Bull. Am. Mus. Nat. Hist.* **161**, 307 (1978).
9. J. W. Daly, C. W. Myers, J. E. Warnick, E. X. Albuquerque, *Science* **208**, 1383 (1980).
10. T. Guilford, *Am. Nat.* **131**, S7 (1988).
11. R. R. Baker and G. A. Parker, *Philos. Trans. R. Soc. London Ser. B* **287**, 63 (1979).
12. T. Guilford, *Anim. Behav.* **34**, 286 (1986).
13. W. Schuler and E. Hesse, *Behav. Ecol. Sociobiol.* **16**, 249 (1985).
14. It could be argued that, if variable and hooded pitohuis are sister taxa, the mimetic resemblances may be historical artifacts due to shared primitive traits. This possibility does not refute that mimicry has selective value for pitohuis, but it offers an alternative explanation for the original evolution of Müllerian mimicry in this group. A phylogenetic analysis of *Pitohui* and the interracial variation in the variable pitohui will clarify this issue.
15. B. M. Beehler, T. K. Pratt, D. A. Zimmerman, *Birds of New Guinea* (Princeton Univ. Press, Princeton, NJ, 1986).
16. We thank N. Wahlberg, B. Iova, and the many other workers who contributed to our field efforts, N. Whittaker for the mass spectral determinations, and S. Arnold, J. Diamond, H. Fales, K. Kelley, K. Kubzdela, H. Landel, and S. Pruett-Jones for comments on earlier drafts of this manuscript. Fieldwork was supported by National Geographic Society grant 4026-89 and Sigma Xi, the Scientific Research Society.

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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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ments from 0.5 to 1.65 cm (23 of 28 ranging from 0.8 to 1.65 cm in ≤24 hours) separated by 2 to 28 days of stasis (Fig. 1). The daily data suggest that many of the weekly and semiweekly increments may have occurred during individual 24-hour (or shorter) intervals. The amplitude of these saltations is 2.5 to 10 times greater than errors of measurement.

Our findings generate the hypothesis that human length growth during the first 2 years occurs during short (≤24 hours) intervals that punctuate a background of stasis. Contrary to the previous assumption that the absence of growth in developing organisms is necessarily pathological (*1*, *15*), we postulate that stasis may be part of the normal temporal structure of growth and development. The validity of this model is supported by two observations: (i) the sum of individual growth saltuses accounts for the entire growth of individual infants during the course of their serial documentation (within measurement error) and (ii) the measurements at the end of each stasis interval are within measurement error of those from the first day of the same plateau. This constancy would not be the case if there were growth during the proposed stasis intervals.

A pattern of saltation and stasis is also found for growth in head circumference in the sample infants and thus may not occur solely in linear bone growth (*16*). Furthermore, this model is not constrained to infancy because daily data on growth in height during adolescence show the same discontinuous saltus and stasis profile (*16*).

The unavailability of long-term, time-intensive data limits further explication of the time structure and amplitude characteristics of growth saltuses. Developmental age changes in growth rates as well as individual differences in size (for example, length and height) and growth rate, may reflect variability in the amplitude, frequency, or both of discrete saltations. This pattern for pulsatile characteristics of growth hormone was observed in (*17*). In the present study, the mean amplitude (but not frequency) of weekly length growth episodes correlated with growth rate (but not size) in a subsample of 14 infants [$P < 0.018$ (*18*)].

Although saltatory growth has been documented for synchronized cell cultures in vitro (*19*), saltation and stasis have not been previously demonstrated at the level of whole animal linear growth. These results may have implications for research into long bone growth factors (*20*), cell kinetics (*21*), and normal and abnormal growth of other cell types in general (*22*).

Our inferences are not inconsistent with recent cell-cycle research [for example, (*23*)]. The plateau periods of no apparent linear growth suggest that periods of growth stasis, possibly reflecting the operation of cellular growth inhibitory mechanisms, constitute normal physiology and that growth itself is a saltatory, highly time-constrained event. Thus, both a suppressive (or inactive) phase and a growth phase seem to be at the basis of the normal pattern of human growth at the organismic level. The gross assessment of human linear growth as described here suggests coordination of multiple cellular processes and perhaps synchronization of cellular growth. The exact mechanisms responsible for generating short-lived growth increments in healthy growing individuals are not known.

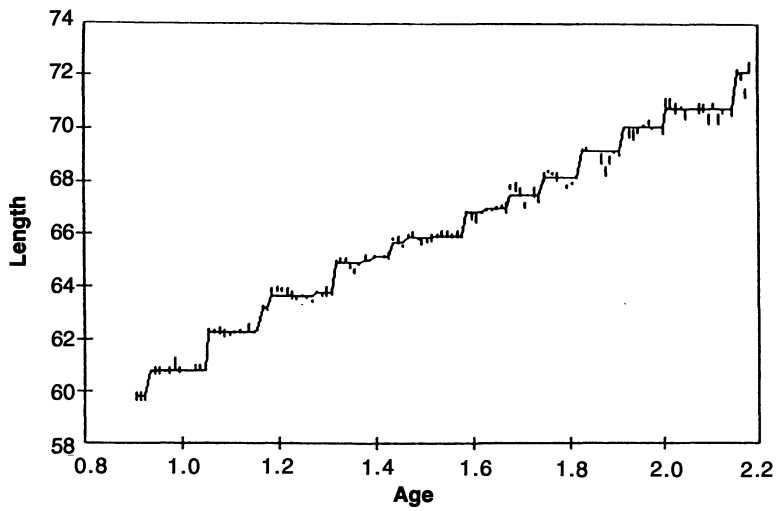

Fig. 1. Daily length measurements of a male infant from 90 to 218 days of age (with the exception of 11 days). Data are plotted by length (centimeters) and age (days × 10^2). Measurements are plotted as vertical bars representing the average of replicate measurements ± a technical error of measurement range (0.08 cm, based on 100 replicate measures). The saltatory model identified 13 significant increments at the $P < 0.05$ level (mean, 0.91 cm; SD, 0.32; SEM, 0.08; median, 0.9 cm; range, 0.53 to 1.67 cm) separated by 2 to 15 days of stasis.

Table 1. Sample summary.

Measurement protocol	Daily	Semiweekly	Weekly
Number of subjects	3	18	10
Age range (days of age)	90–433	2–530	7–602
Number of measurements	372	1172	393
Amplitude of saltations (cm)			
Mean	0.95	1.05	1.3
SD	0.3	0.4	0.4
SEM	0.062	0.02	0.04
Median	0.9	0.99	1.2
Range	0.5–1.65	0.5–2.5	0.5–2.5
Duration of stasis (number of days)			
Mean	11.9	17	24.5
SD	6.5	11.2	13.5
SEM	1.3	0.78	1.28
Median	10	14	21
Range	2–28	3–60	7–63

REFERENCES AND NOTES

1. J. M. Tanner, *Fetus into Man* (Harvard Univ. Press Cambridge, MA, ed. 2, 1990).
2. _____, R. H. Whitehouse, M. Takaishi, *Arch. Dis. Child.* **41** 613 (1966); P. V. Hamill *et al.*, *Vital Health Stat. Ser. 11* **165**, 1 (1977); A. F. Roche and J. H. Himes, *Am. J. Clin. Nutr.* **33**, 2041 (1980); J. M. Tanner and P. S. W. Davies, *J. Pediatr.* **107**, 317 (1985); J. Karlberg, *Stat. Med.* **6**, 185 (1987).
3. R. E. Scammon, *Am. J. Phys. Anthropol.* **10**, 329 (1927).
4. H. V. Meredith, *Child Dev.* **7**, 262 (1936); M. J. R. Healy, M. Yang, J. M. Tanner, F. Y. Zumrawi, in *Linear Growth Retardation in Less Developed Countries*, J. C. Waterlow, Ed., vol. 14 of *Nestlé Nutrition Workshop Series* (Raven, New York, 1988), pp. 41–55.
5. D. A. Scholl, in *Dynamics of Growth Processes*, E. J. Boell, Ed. (Princeton Univ. Press, Princeton, NJ, 1954), pp. 224–241; F. P. G. M. van der Linden, W. J. Hirschfeld, R. L. Miller, *Growth* **34**, 385 (1970).
6. D. W. Smith *et al.*, *J. Pediatr.* **89**, 225 (1976); C. Polychronakos, H. Abu-Srair, H. J. Guyda, *Eur. J. Pediatr.* **147**, 582 (1988); G. E. Butler, M. McKie, S. G. Ratcliffe, *Ann. Hum. Biol.* **17**, 177 (1990).
7. M. Togo and T. Togo, *Ann. Hum. Biol.* **9**, 425 (1982); M. Lampl and R. N. Emde, in *Levels and Transitions in Children's Development*, K. W. Fischer, Ed., vol. 21 of *New Directions for Child Development* (Josey-Bass, San Francisco, 1983), pp. 21–36, I. M. Valk *et al.*, *Growth* **47**, 53 (1983); M. Hermanussen, K. Geiger-Benoit, J. Burmeister,

W. G. Sippell, *Ann. Hum. Biol.* **15**, 103 (1988).
8. W. A. Marshall, *Arch. Dis. Child.* **46**, 414 (1971); J. K. H. Wales and R. D. G. Milner, *ibid.* **62**, 166 (1987); J. M. Wit, D. M. Teunissen, J. J. J. Waelkens, W. J. Gerver, *Acta Paediatr. Scand. Suppl.* **337**, 40 (1987).
9. The sample infants were characterized by birth weights ≥2500 g, 38 to 42 weeks gestational age, uncomplicated pregnancy and delivery, 1- and 5-min Apgar scores between 8 and 10, and no subsequently diagnosed medical problems during the course of the study.
10. N. Cameron, in *Human Growth: A Comprehensive Treatise*, F. Falkner and J. M. Tanner, Eds. (Plenum, New York, ed. 2, 1986), vol. 3, pp. 3–46.
11. The infant measuring board was specially designed after the Harpenden-Holtain infant length board (*10*), equipped with a fixed headboard and mobile footboard. One observer fixes the infant's head as the second applies gentle pressure to the body to ensure that the legs are straight and the ankles are at right angles. The footboard is brought into firm contact with the subject's feet. A final check on the proper alignment of the head and body is made before length assessment. Ninety percent of the measurements were taken within 3 hours of the same time on each visit to control for diurnal variation. Replicate examinations were conducted within 1 hour, and the observer and recorder were the same for each replicate.
12. The technical error of measurement (tem) is the square root of the sum of squared differences between replicates divided by twice the number of paired observations (*10*).
13. G. A. Harrison, G. Brush, A. Almedom, T. Jewell, *Ann. Hum. Biol.* **17**, 407 (1990). The tem of measurements here is slightly less than that of field studies previously reported: R. Martorell, J.-P. Habicht, C. Yarbrough, G. Guzman, R. E. Klein, *Am. J. Phys. Anthropol.* **43**, 347 (1975); C. C. Gordon, W. C. Chumlea, A. F. Roche, in *Anthropometric Standardization Reference Manual*, T. B. Lohman, A. F. Roche, R. Martorell, Eds. (Human Kinetics, Champaign, IL, 1988), pp. 3–9. The technical errors in the present study are not biased by the design or any other known factor except the particular care given to measurement technique by both the anthropometrist (M.L.) and the assistant. The conditions of this study were unusual in that all measurements were taken in the home with mothers as assistants, conditions substantially different from those of previous studies in clinical environments. The mothers of the three children measured daily are all professional anthropometrists. In addition, 250 measurements were taken with an independent rater to verify measurement reliability.
14. J. D. Veldhuis and M. L. Johnson, *Methods Enzymol.* **210**, 539 (1992). This method fits the actual serial growth data by the use of a weighted, nonlinear least-squares procedure of parameter estimation. The analytical method is designed to compare the actual experimental data to a series of models with increasing numbers of pulses. From an initial model with no pulses, subsequent models are generated through the addition of pulses at points of greatest decrement in variance, until no statistically significant improvement of fit is gained from pulse addition.
15. M. Hermanussen, K. Geiger-Benoit, W. G. Sippell, *Acta Paediatr. Scand. Suppl.* **75**, 601 (1987). A comparison was made between the growth episodes, the stasis intervals, and all illnesses of the subjects. There was no positive association between growth stasis and illness (Pearson chi-square).
16. M. Lampl, unpublished data.
17. N. Mauras *et al.*, *J. Clin. Endocrinol. Metab.* **64**, 596 (1987); K. Albertsson-Wikland and S. Rosberg, *ibid.* **67**, 493 (1988); P. M. Martha *et al.*, *ibid.* **69**, 563 (1989).
18. The maximum sample size available for this comparison consists of weekly data from 14 infants between 3 and 9 months of age, assessed as standard deviation scores for age and sex.
19. D. Lloyd and S. W. Edwards, in *Cell Cycle Clocks*, L. Edmunds, Ed. (Plenum, New York, 1986), pp. 27–46.
20. E. A. Wang *et al.*, *Proc. Natl. Acad. Sci. U.S.A.* **87**, 2220 (1990); R. Bortell, L. M. Barone, M. S. Tassinari, J. B. Lian, G. S. Stein, *J. Cell. Biochem.* **44**, 81 (1990); B. A. A. Scheven and N. J. Hamilton, *Acta Endocrinol. Copenhagen* **124**, 602 (1991).
21. E. B. Hunziker and R. K. Schenk, *J. Physiol. London* **414**, 55 (1989); G. J. Breur, B. A. VanEnkevort, C. E. Farnum, N. J. Wilsman, *J. Orthop. Res.* **9**, 348 (1991); S. Stevenson, E. B. Hunziker, W. Herrmann, R. K. Schenk, *ibid.* **8**, 132 (1990).
22. R. A. Weinberg, *Science* **254**, 1138 (1991).
23. J. B. Ghiara *et al.*, *Cell* **65**, 163 (1991); H. Matsushime, M. F. Roussel, R. A. Ashmun, C. J. Sherr, *ibid.*, p. 701; T. Motokura, *Nature* **350**, 512 (1991); U. Surana *et al.*, *Cell* **65**, 145 (1991); T. Chittenden, D. M. Livingston, W. G. Kaelin, Jr., *ibid.*, p. 1073; S. P. Chellappan, S. Hiebert, M. Mudryj, J. M. Horowitz, J. R. Nevins, *ibid.*, p. 1053; L. R. Bandara and N. B. La Thangue, *Nature* **351**, 494 (1991).
24. We thank the parents who participated in this study, L. Hileman for measuring assistance, R. N. Emde and M. Reite for their support of earlier versions of this work, and K. Ryan for technical assistance with the manuscript (M.L.). Supported by the Developmental Psychobiology Research Group, the Grant Foundation, and the Wenner-Gren Foundation (M.L.); National Institute of Child Health and Human Development Research Career Development Award KO4HD00634, National Institutes of Health (J.D.V.); National Science Foundation Science Center for Biological Timing (J.D.V. and M.L.J.); Diabetes and Endocrine Research Center grant DK 38942; and the University of Virginia Pratt Foundation and Educational Enhancement Fund (J.D.V. and M.L.J.).

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Antibody-Catalyzed Rearrangement of the Peptide Bond

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The generation of antibodies from a bifunctional cyclic phosphinate transition-state analog provided agents capable of efficiently catalyzing both steps of the overall conversion of a substrate containing an asparaginyl-glycyl sequence through a succinimide intermediate to the products aspartyl-glycyl and the rearranged isoaspartyl-glycyl sequence. This reaction provides a potential means in addition to amide cleavage for the deactivation of protein or peptide biological functions in vivo.

A major goal in the field of catalytic antibodies is the inactivation of proteins or peptides. There are two principal means by which this may be achieved. One is through the cleavage of the amide bond by hydrolytic or oxidative methods, and a second is through the modification of side-chain residues or through the rearrangement of the main peptidic chain (*1*). Examples of the latter include the deamidation of Asn or Gln residues and the related β-aspartyl shift mechanism. One can generally prevent these processes from rapidly occurring by having the protein chains adopt conformations that exclude the favorable reactive distances and bond angles required for these processes (*2*, *3*). The deamidation of Asn residues has been implicated in protein denaturation (*4*), in the initiation of proteolytic processes leading to protein degradation in vivo (*5*), and in the loss of enzymic activity (*6*).

We were particularly interested in the possibility that properly selected antibodies might restrain the side-chain amide carbonyl and the $n + 1$ amide nitrogen in an alignment and distance favorable for reaction to form a succinimide intermediate. That in turn would hydrolyze to the Asp deamidation and the isoaspartyl (IsoAsp) β-shift mechanism products (Fig. 1). In addition to providing a favorable ground-state conformation, the antibody binding site must also complement the metastable tetrahedral intermediates **1** and **3** (Fig. 1) and their associated transition states, which presumably are involved in the two-step reaction sequence. Differential ground- and transition-state complementarity are necessary for efficient catalysis to occur.

We chose the *N*-acetylasparginylglycyl (*N*-phenethyl)amide, **4**, as representative of the Asn-Gly linkage in proteins and the cyclic phosphinate, **5**, to mimic the intermediate species **1** to **3**. **5** possesses two tetrahedral moieties—the phosphinate and secondary alcohol—so that it mimics both transition state **2** and **3**. The synthesis of **4** and **5** are outlined in Fig. 2. The synthesis of **5** begins with the previously described chloride **6** (*7*) and leads to a racemic product ultimately formed from trans addition of azide to the racemic precursor epoxide. The final product **5** exhibits a single ^{31}P resonance at 49.8 ppm, consistent with values reported for other cyclic phosphinates (*8*, *9*). An immunogenic conjugate was prepared from **5** through its linkage to a carrier protein (keyhole limpet hemocyanin). Monoclonal antibodies were obtained by standard protocols (*10*) and purified to

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