of the method. However, in cell-free systems, unnatural amino acid incorporation has been used not only to modify side chains, but also to substitute nonpeptide linkages for the peptide bond and to incorporate fluorescent, photolabile, and spin-labeled moieties (*2*). These tactics can now be applied to many questions concerning structural and functional aspects of ion channels, receptors, and transporters.

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4. The gene for tRNA-MN3, flanked by an upstream T7 promoter and a downstream Fok I restriction site, was constructed from eight overlapping DNA oligonucleotides and cloned into pUC19. Digestion of the resulting plasmid (pMN3) with Fok I gave a linearized DNA template corresponding to the tRNA transcript, tRNA-MN3, shown in Fig. 1B minus the CA at positions 75 and 76. In vitro transcription of Fok I–linearized pMN3 was done as described [J. R. Sampson and O. C. Uhlenbeck, *Proc. Natl. Acad. Sci. U.S.A.* **85,** 1033 (1988)]. The 74-nucleotide tRNA transcript, tRNA-MN3 (minus CA), was purified to single-nucleotide resolution by denaturing polyacrylamide electrophoresis and then quantitated by ultraviolet absorption [J. R. Sampson and M. E. Saks, *Nucleic Acids Res.* **21,** 4467 (1993)]. Digestion of pMN3 with Bst NI gave the template for transcription of the full-length tRNA.
5. Most unnatural amino acids were purchased from commercial sources. For the preparation of 4-COOH-Phe, an appropriately protected (NVOC = nitroveratryloxycarbonyl) Tyr [NVOC-Tyr(OH)-OtBu] was converted to the triflate [NVOC-Tyr(OTf)-OtBu]. A mixture of the trislate, KOAc, $Pd(OAc)_2$, 1,1′-bis-(diphenylphosphino)ferrocene (dppf), and dimethylsulfoxide (DMSO) was reacted at 85°C by means of a Pd-catalyzed carbonylation sequence that was quenched with HCl [see S. Cacchi and A. Lupi, *Tetrahedron Lett.* **33**, 3939 (1992)]. The synthesis of dCA and the procedure for coupling the amino acid are described in (*2*).
6. The dCA-amino acids were coupled to tRNA-MN3 (minus CA) according to the method of T. E. England, A. G. Bruce, and O. C. Uhlenbeck [*Methods Enzymol.* **65**, 65 (1980)]. Before ligation with T4 RNA ligase, tRNA-MN3 (minus CA) was heated to 90°C in 6 mM Hepes-KOH (pH 7.5) for 3 min and slowly cooled to room temperature. Gel-shift analysis indicated approximately 75% efficiency in ligation, which was independent of the identity of the dCA–amino acid donor.
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16. A rabbit reticulocyte lysate system (Promega, Madison, WI) was used with incorporation of [^{35}S]methionine.
17. Site-directed mutagenesis was carried out with the Clontech Transformer site-directed mutagenesis kit (Palo Alto, CA). The α, β, γ, and δ subunits of AChR were subcloned into pAMV-PA, a modified pBS (S/K$^+$) vector containing an alfalfa mosaic virus (AMV) region directly upstream from the coding region of the insert and an A_{50} sequence downstream from the insert. The AMV region binds ribosomes tightly and increases expression [S. A. Jobling and L. Gehrke, *Nature* **325**, 622 (1987)], and we find that it is effective in both the in vitro translation and oocyte expression systems. Capped transcripts were prepared in vitro.
18. Before in vitro translation or microinjection, the NVOC–aminoacyl–tRNA-MN3 was renatured by incubation at 65°C for 3 min. The NVOC protecting group was subsequently removed by irradiation of the sample for 5 min at 23°C with a 1-kW Xenon lamp using WG-335 and UG-11 filters (Schott, Duryea, PA). The deprotected aminoacyl–tRNA-MN3 was immediately mixed with the desired mRNA and either added to the in vitro translation reaction or microinjected into *Xenopus* oocytes.
19. In the δ subunit of mouse AChR, TAG is the stop codon. To prevent the aminoacylated tRNA from inserting an amino acid at this position, this sequence was mutated to TGA.
20. Deprotected aminoacylated tRNAs were mixed with the desired AChR αTyr93TAG, αTyr190TAG, or αTyr198TAG mRNA (10:1:1:1 to 100:1:1:1) and microinjected into *Xenopus* oocytes (50 nl per oocyte) [M. W. Quick and H. A. Lester, in *Ion Channels of Excitable Cells*, T. Narahashi, Ed. (Academic Press, San Diego, CA, 1994), pp. 261–279]. Injected tRNA and mRNA concentrations were 0.4 ng/nl and 0.30 ng/nl, respectively. Wild-type AChR, AChR αTyr190Phe, and AChR αTyr198Phe (4:1:1:1) mRNAs were injected at concentrations of 0.035 mg/ml and 0.35 mg/ml. Electrophysiological recordings were carried out 12 to 24 hours after injection with the use of a two-electrode voltage clamp circuit. Electrode resistance was 0.5 to 1.0 megohm. Bath solutions contained 96 mM NaCl, 2 mM KCl, 1 mM $MgCl_2$ and 5 mM Hepes (pH 7.5). To prevent activation of the endogenous Ca^{2+}-activated Cl^- channel by muscarinic receptors, atropine (1 μM) was included in the bath solution and Ca^{2+} was omitted. Wild-type mRNA synthesized from the AMV vector generally gave EC_{50}values approximately two times greater than those obtained with mRNA synthesized from pBluescript. We have not systematically studied this effect.
21. Abbreviations for the amino acid residues are as follows: A, Ala; C, Cys; D, Asp; E, Glu; F, Phe; G, Gly; H, His; I, Ile; K, Lys; L, Leu; M, Met; N, Asn; P, Pro; Q, Gln; R, Arg; S, Ser; T, Thr; V, Val; W, Trp; and Y, Tyr.
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32. We thank V. Cornish, P. Deshpande, O. Uhlenbeck, and Y. Zhang for suggestions; E. Chapman for help with synthesis; and J. Jankowski for help with the measurements. Sponsored by grants from NIH, the Office of Naval Research, the Howard Hughes Medical Institute, the University of California Tobacco-Related Disease Research Project, and the Beckman Institute at Caltech.

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TECHNICAL COMMENTS

Patterns of Human Growth

Human growth has generally been assumed to be a continuous process, the cumulative sum of millions of unsynchronized cell replications. In 1978, J. M. Tanner wrote, "Growth is in general a very regular process. Contrary to opinions still sometimes met, growth in height does not proceed by stops and starts . . ." (*1*). Previous studies of human and animal growth support this concept (*2*).

In their report (*3*), M. Lampl *et al.* challenge this view, concluding instead that human linear growth occurs in sudden spurts, separated by long periods with no measurable growth. They measured crown-heel length weekly, semiweekly, or daily in healthy infants and describe brief aperiodic bursts of growth, up to 1.65 cm in a single day, separated by long intervals, up to 63 days, with no measurable growth. Thus, the human infant was proposed to alternate between two states, one with a growth velocity of zero, the other with a mean velocity of 1 cm per day, which would correspond to an annualized velocity of 365 cm per year.

These observations, if correct, would require fundamental revisions in our understanding of growth (*1*, *2*). For example, the model of saltation and stasis proposed by Lampl *et al.* (*3*) implies the existence of previously unsuspected synchronizing mechanisms, presumably hormonal, able to switch cell division on or off simultaneously throughout the organism, but none of the known endocrine regulators of growth fluctuate in such a manner.

We attempted to confirm the hypothesis of saltatory growth with an experimental

design that avoids some of the limitations in the study by Lampl *et al.* Five healthy infants (three males and two females), aged 1.6 to 4.2 months (mean 2.5 months), were studied. A single observer measured knee-heel length, crown-heel length, head circumference, and weight at the same hour of every day for one month. Knee-heel length was measured by creating 10 photographic images of each lower leg. To prevent measurement bias, the observer was "blinded" to the subject and to the date of the picture (*4*). Crown-heel length was measured in triplicate, with the use of a Harpenden-Holtain infantometer. The observer was blinded by covering the digital counter with opaque paper until the mobile footboard was locked in place. Weight and head circumference were measured by standard techniques (*5*). Within-day measurement errors, similar in magnitude to mean daily growth (Table 1), probably underestimated the true between-day measurement error (*6*).

First, individual growth curves were examined visually to assess the growth pattern (Fig. 1). Saltations of the magnitude described by Lampl *et al.* (*3*) were not observed, nor were the many lengthy periods of stasis that they described (mean duration 12 days).

Second, to analyze the growth patterns statistically, we determined the frequency distribution of daily growth velocities and compared this observed distribution with those predicted by the saltatory and continuous growth models (*7*). The proposed model of stasis and saltation (*3*) predicts a majority (~92%) of daily growth velocities clustered around zero (during stasis), and a minority (~8%) of high daily growth velocities (during saltation), that is, a bimodal or composite distribution (Fig. 2, A and B, dashed curves). The distribution predicted by a continuous growth model is a single cluster about the median growth velocity (Fig. 2, A and B, solid curves). The observed frequency distributions (Fig. 2, A and B, bars) for all four types of measurements were significantly different from the predictions of the saltatory model ($P < 0.01$, χ^2 test), under the assumption of a mean duration of stasis of 12 days, as previously described (*3*, *8*). Instead, the distribution of the growth velocities for the crown-heel length (Fig. 2A), the knee-heel length (Fig. 2B), and weight (not shown) was approximately Gaussian (*9*). The distribution of the growth velocities for the head circumference, although platykurtic, also appeared unimodal (not shown). Thus, the data were inconsistent with the proposed saltatory model and supported instead a continuous model. The cumulative probability plots of daily growth velocities also support this conclusion (Fig. 2, C and D).

Table 1. Daily growth (in centimeters or kilograms) and measurement errors (centimeters).

	Crown-heel length	Knee-heel length	Head circum-ference	Weight
Mean daily growth	0.102	0.020	0.046	0.021
Total number of daily measurements	148	234	148	148
Within-day measurement error (SEM)	0.193	0.031	0.078	NA*

*Only one measurement was taken each day

The bimodal or composite frequency distribution predicted by the saltatory growth model is skewed to the right (predicted skewness coefficient, $g_1 \sim 0.8$), whereas the predicted unimodal frequency distribution predicted by the continuous model is not skewed (predicted skewness coefficient, $g_1 \sim 0$) (*10*). The observed skewness coefficient did not differ significantly from zero for any of the four growth parameters (data not shown), which again favors a continuous rather than the saltatory growth model.

As a third test of the saltatory model, we asked whether, in a given infant on a given day, the knee-heel growth velocity correlated with the crown-heel growth velocity. Since the knee-heel length is a component of the crown-heel length, stasis in crown-heel length would necessarily require stasis in knee-heel length. Similarly, saltations in knee-heel length would necessarily produce saltations in crown-heel length. Thus, the saltatory model predicts a strong positive correlation between the two velocities. On the basis of described magnitude of the sal-

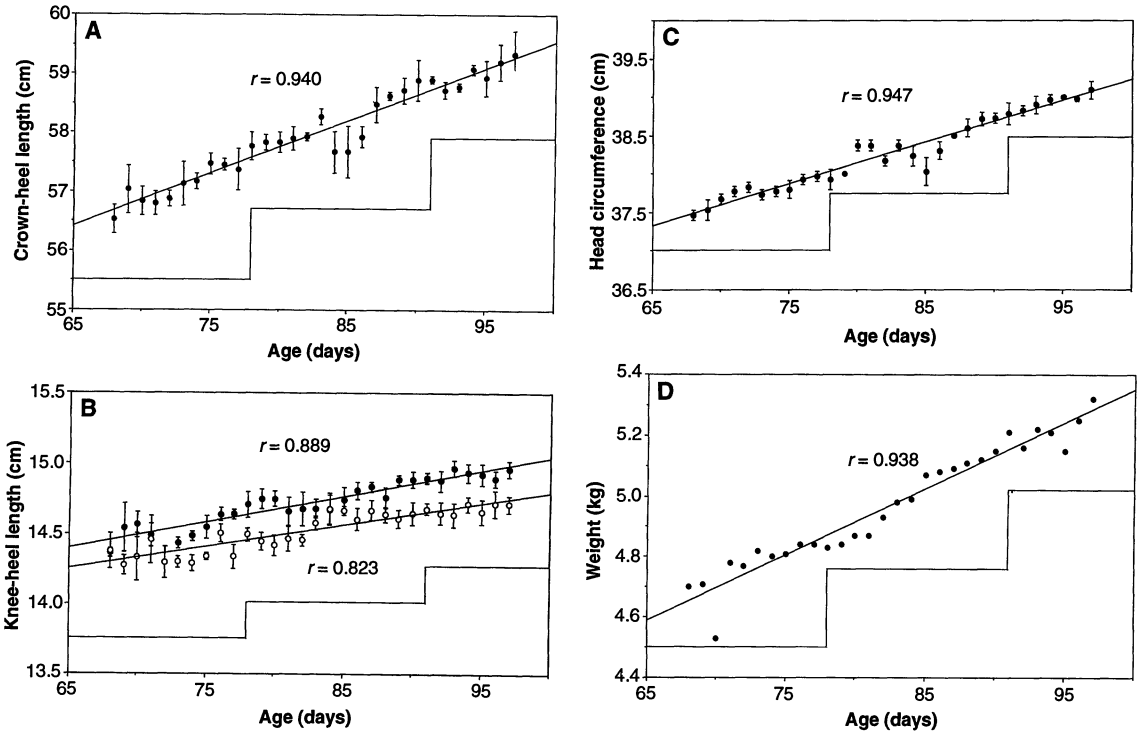

Fig. 1. Growth in a single infant. (**A**) Crown-heel length, (**B**) knee-heel length (● : right; ○ : left), (**C**) head circumference, (**D**) weight. Error bars represent the mean ± 2 SEM. Linear regression line predicted by the continuous model and representative growth curve predicted by the saltatory model (duration of stasis, 12 days).

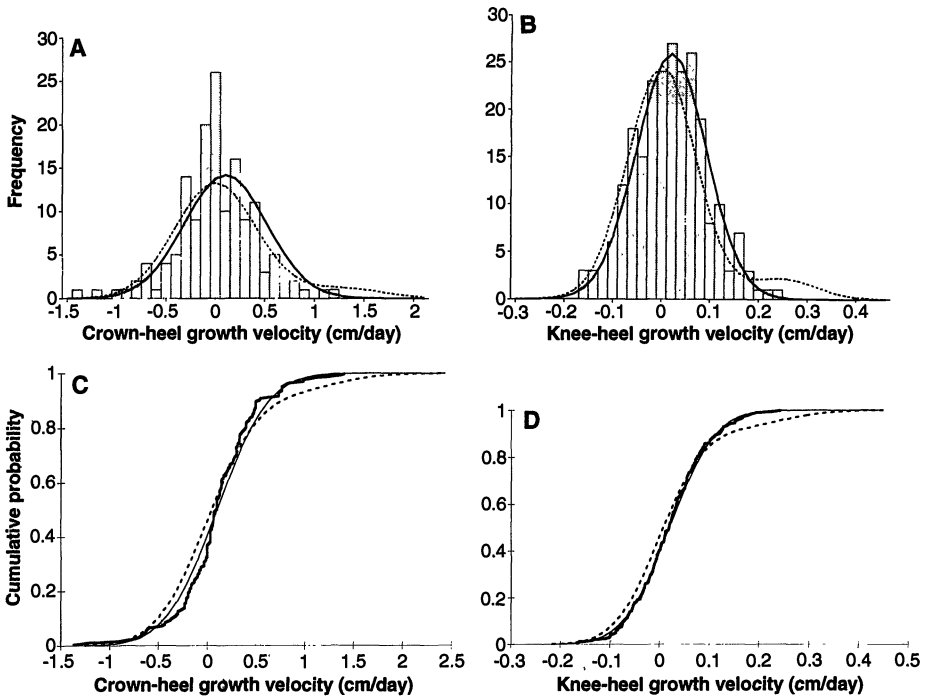

Fig. 2. Frequency distributions of individual daily growth velocities. Daily growth velocities were calculated for (**A** and **C**) crown-heel length and (**B** and **D**) knee-heel length for each infant. For each growth measure, the histogram (A) and (B) shows the observed frequency of velocities for the combined data from all infants. Solid curves, single Gaussian distributions predicted by the continuous growth model. The mean and SD of these curves were set equal to the mean and SD of the data sets. Dashed curves, representative bimodal or composite distributions predicted by the saltatory model. Overall means of the composite distributions were set equal to the means of the data sets. (C and D) Cumulative probability distributions for the same data as in (A) and (B). Ordinate represents the proportion of days with growth velocity less than or equal to the abscissa. Actual data are shown by the heavy solid curve. Lighter solid curve represents the single Gaussian distribution predicted by the continuous growth model. The dashed curve shows a representative bimodal or composite distribution predicted by the saltatory model with growth on 8% of the days.

tations (*3*) and the magnitude of our measurement errors, the saltatory model predicts $r^2 \sim 0.5$. In contrast, the continuous model predicts only a weak correlation between the crown-heel and the knee-heel growth velocities as a result of the gradual decline in growth velocity with age. As this decline is smaller than the measurement error by a factor of approximately 10 to 20, the predicted correlation under the continuous model, $r^2 \sim 0.001$, is negligible. The observed correlation between daily knee-heel and crown-heel growth velocities was weak ($r^2 = 0.03$), which is consistent with a continuous, but not the saltatory growth model.

As a fourth test, we fit each 1-month growth curve first with a saltatory model having up to three saltations separated by stases of variable length, and second with a continuous growth curve comprised of up to three linear segments (a linear spline). The continuous growth model fit the overall data set significantly better (as measured by the residual sum of squares) than did the comparable saltatory model ($P < 0.01$, t test).

Thus, by all four statistical approaches, the data were incompatible with the saltatory model. The discrepancy between our findings and the observations of Lampl *et al.* cannot be explained by differences in measurement error. In both studies, the within-day measurement error ranged from one to two times the mean daily growth velocities. The discrepancy could be a result of the different study designs. In contrast to the report by Lampl *et al.* (*3*), our study employed an observer blinded to preclude measurement bias (*11*), rigorous statistical tests to compare the two models, and an analysis of multiple growth measures to distinguish fluctuations due to measurement error from saltatory growth.

An analogous study, which used rabbits and extremely accurate radiological growth measurements, also demonstrated continuous growth (*12*), which suggests that growth in other mammals also occurs continuously.

Thus, our data (*13*) do not support the proposed model of saltatory growth and suggest instead that human growth occurs continuously.

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5. The crown-heel length, read to the nearest 0.05 cm, was measured three times in succession with complete repositioning of the infant between measurements. The weight was measured once daily under nonfasting conditions with the use of an electronic scale (Scale-Tronix Pediatric Scale 4800; accuracy: 0.01 kg), which rounded the weight to the nearest 0.01 kg. The head circumference was measured in triplicate to the nearest 0.05 cm with the use of a cloth measuring tape [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)].
6. Replication measurement error (within-day measurement error) was expressed as the root-mean-square SEM (meaned over all infants for all days of the study), where SEM represents the standard error of the mean of the replicate measurements on a given day. This statistic is equivalent to the technical error of measurment [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] for duplicate measurements, but also applies to higher numbers of replicates.
7. The term continuous is not meant to imply an invariant growth rate, but simply that, under normal circumstances, body size increases progressively without the prolonged periods of stasis and the sudden bursts proposed by Lampl *et al.* (*3*).
8. The applicability of χ^2 analysis to this data set, despite the non-independence of serial daily growth velocities, was confirmed by comparing the predicted and observed rejection rates for 1000 simulated data sets.

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14. We thank K. Oerter Klein and J. A. Yanovski for helpful discussions, H. C. Sachs for assistance with recruiting subjects, J. Crawford for advice about photographic methods, and the parents and children who participated in this study.

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Response: The saltatory model tested by Heinrichs *et al.* is not the aperiodic saltatory model presented in our report (*1*), but is a model they have constructed to assess data in which the number of observations is so small and the error of measurement so great that meaningful conclusions are not supportable.

We reported (*1*) 1973 serial length measurements (*2*) from 31 normal infants (aged 2 to 602 days) who were followed for 4 to 15 months. The data were fit by a mathematical model designed (i) to identify significant differences in serial measurements from background measurement error noise ($P < 0.05$), (ii) to distinguish between a continuous and a discontinuous function, and (iii) to provide a false positive rate for identifying significant increments when they are not present near zero (*3*). Each infant's total growth during the study was accounted for by aperiodic pulses in total body length, separated by intervals of no growth, which could not be accounted for by Gaussian error (*4*). We termed this pattern of bursts "saltatory growth" and found the saltatory descriptor to be significantly better than continuous linear models by tests of autocorrelation and weighted variance ($P \leq 0.05$). The patterns of the amplitude (amount of growth at each episode) and frequency of growth events (number of days between growth events) were unique and variable within and between individual infants.

We presented (*1*) a figure of one infant's growth and a table of sample descriptive statistics (*5*); Henrichs *et al.* apply our sample averages to individual patterns and use the specific growth pattern of a single infant as a model for growth in all children. This approach produced results contrary to the unique individual growth pattern that we documented (*1*).

Heinrichs *et al.* analyze month-long data sets by a "saltatory model having up to three saltations separated by stases of variable length." This is a similar but substantively different method from ours (*1*). The a priori assumption that 1 month of growth data would be described by zero to three significant growth increments if growth were saltatory is neither an assumption nor a prediction of our study (*1*). The 12-day average stasis interval reported by us (*1*) is not identical to the increment expected in an individual's growth pattern.

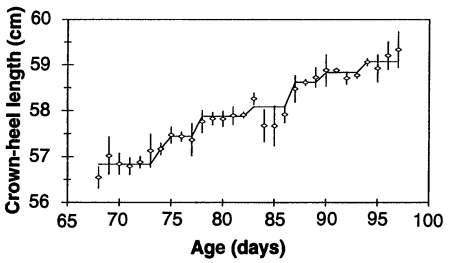

Fig. 1. Six significant saltations ($P < 0.05$) in figure 1 of the comment by Heinrichs *et al.* (at 74, 75, 78, 87, 90, and 94 days of age), as identified with the use of our saltatory algorithm (*1*). Error bars indicate the mean ± 2 SEM, as per Heinrichs *et al.*

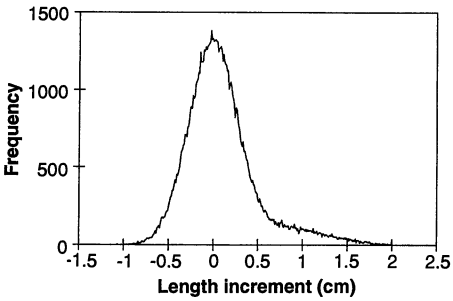

Fig. 2. Expected frequency distribution of daily growth increments taken from figure 1 of our report (*1*).

When our mathematical model (*1*) is applied to the length data represented in figure 1A of Heinrichs *et al.*, six significant saltations are identified, separated by 1- to 9-day intervals of no significant growth (Fig. 1). The original saltatory model fits the data of Heinrichs *et al.* better (but not with statistical significance) than the continuous models used in their comment (*6*). Thus, these data neither support nor detract from the proposition of saltatory growth. The growth pattern is unresolvable, as both a linear and saltatory pattern are statistically equally likely.

The second method of Heinrichs *et al.* assumes that a frequency distribution of daily growth velocities can be used to distinguish continuous from saltatory growth. The data in our report do not support this and do not follow the criteria used by Heinrichs *et al.* (Fig. 2). Instead, our data produce a skewed and platykurtic curve (*7*), as does the curve produced by the head circumference data reported by Heinrichs *et al.* The use of velocity histograms as an analytic method (*8*) is problematic: The saltatory model has two basic parts, the saltations and the times of stasis. A frequency distribution of growth increments preserves information about the magnitudes of saltations, but removes the temporal information pertaining to the sequence of the growth increments and thus contains no information about stasis periods. One cannot re-create a unique growth pattern, or temporal ordering of growth episodes, from a frequency distribution of the size of daily changes. Because these daily changes could occur in any order, there are actually n factorial ($n!$) different growth patterns (where n is the number of serial increments) that will generate the same distribution function. There are 5×10^{194} (or 118!) different growth patterns (two are shown in Fig. 3) that generate a frequency distribution identical to the saltatory growth pattern revealed in the experimental data in our report ($n = 118$ serial velocities) (*9*). Two of these computer-generated nonsaltatory, continuous functions (Fig. 3) have non-Gaussian, nonbimodal distributions identical to those of the experimental data (Fig. 2), and all three have a skewness of 1.265, predicted as necessarily saltatory by Heinrichs *et al.* (*10*). Thus, frequency distributions of increments, and the associated cumulative probability plots and skewness coefficients, are nonspecific and insensitive diagnostic tools for identifying continuous as opposed to saltatory processes.

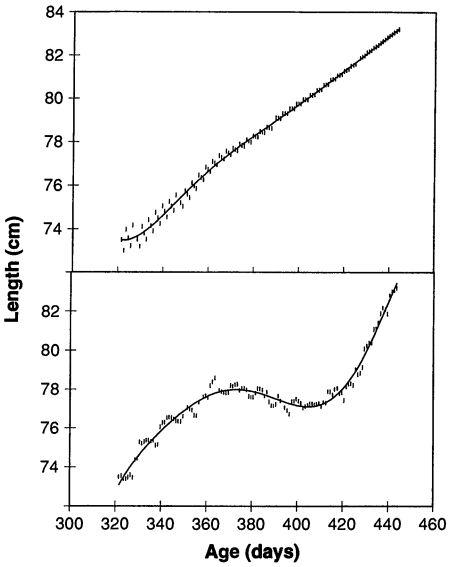

Fig. 3. Two simulated growth patterns that generate the identical frequency distribution, cumulative probability plot, and skewness coefficient as the saltatory experimental data shown in Fig. 2.

The third statistical method used by Heinrichs *et al.* raises the question: What are the relationships between body parts in growth? Research employing direct measurement of the body has documented

strong correlations between short-term growth spurts in the lower leg (assessed by direct instrumentation) and total body height (*11*), findings not corroborated by Heinrichs *et al.*, perhaps because they use different techniques, relying on indirect photographic assessments. To our knowledge, this technique has not been previously validated for short-term measurement accuracy on infants (*12*). Knee-heel length as measured by Heinrichs *et al.* is not the same measurement as the length of the anatomical leg that contributes to crown-heel length, but includes soft tissue on the superior surface of the knee, which may confound correlations between knee-heel length and crown-heel length. Recent studies that use measurements of the boney lower leg with the knemometer (*13*), a blind-measurement technique that avoids the pitfalls of soft-tissue error (*14*), have shown a saltatory growth process during infancy (*15*).

Data on head circumference and weight are shown in the comment by Heinrichs *et al.* As Scammon noted in 1930 (*16*), different body parts grow at different rates: Head circumference does not mimic body length data. Weights shown by Heinrichs *et al.* appear to have been collected when the infants were not under fasting conditions: Small babies' significant intra-daily weight changes resulting from eating and output negate the usefulness of these data to document daily growth (*17*).

It is unlikely that the differences in results reported by us and by Heinrichs *et al.* are primarily a result of data collection techniques. The effect of observer bias was similarly minimized in both studies, and our study (*1*) documented that measurements taken by an independent observer provided identical growth patterns of saltation and stasis at precisely the same time intervals (*19*). This would be unlikely to occur by chance. However, other sources for the differences in the conclusions of the two studies include methods of analysis, as outlined above, and aspects of data related to (i) levels of measurement error and (ii) total number of observations. Heinrichs *et al.* describe their "within-day measurement error (SEM)" as being "similar in magnitude to the mean daily growth," but their 95% crown-heel length "measurement error" is actually 0.378 cm, which is 270% greater than their proposed daily linear growth rates (*20*). Consecutive differences of less than 0.378 cm are not statistically identifiable from error in their data, and thus the linear growth model of Heinrichs *et al.* cannot be documented through daily measurements. The error level reported by Heinrichs *et al.* is 56% greater than that reported by us (*1*), and the total study length only 6 to 25% of the duration of ours.

Heinrichs *et al.* incorrectly present the concept of saltation and stasis when they state that the saltatory model predicts growth to occur at "an annualized velocity of 365 cm per year." They state that our model of saltatory growth proposed an alternation between two states, "one with a growth velocity of zero, the other with a mean velocity of 1 cm per day." It is important to point out that this mean daily velocity derives from the growth pattern of the children in our sample (middle class, well-fed Northwestern Europeans), and it is likely that genetic and environmental influences contribute to saltus amplitude and frequency. Our saltatory model postulates that normal growth proceeds by time-constrained growth episodes that punctuate durations when no growth occurs; our model does not specify either the amount of growth at each episode or the frequency of growth episodes. Heinrichs *et al.*, in their annualized calculations, apparently did not account for the presence of the stasis intervals observed in our data: Our experimental data documented growth on only 33 out of 372 days. The mean of the growth increments that occurred on these 33 days was 0.95 cm. Thus, our experimental observations could be summarized as illustrating saltatory growth that occurred at an annualized growth rate of 31 cm per year in our sample infants. This is the growth velocity of the average to above average child according to growth standards developed for American infants during the first year of life (*21*). Our data suggest that this normal annual velocity was accrued as 33 episodic, time-constrained pulses (within 24 hours), not by continuous daily acquisition.

Heinrichs *et al.* cite, as an authoritative opinion against a saltatory growth model, a statement in a book by J. M. Tanner (1978) [their reference (*1*)]. However, in the most recent edition (1990) of the same book, Tanner has dropped the phrase "growth does not proceed by stops and starts . . ." and notes that "In some short periods growth arrest or even shrinkage seems to occur . . . " (*22*).

The model of saltation and stasis we propose does challenge scientific views of how humans grow. However, recent work by other researchers appears to support our observations of saltatory growth (*15*, *23*), and a pattern of saltation and stasis reflects well-documented normal cell cycle controls (*24*). Our data suggest that saltation and stasis, as a growth process, reflects the cell cycle at the organismic level. Several studies emphasize that our understanding of abnormal growth, including malignancy, may be enhanced by an appreciation of normal growth inhibitory mechanisms (*25*) like those that control normal stasis intervals in growing organs. Saltatory human growth patterns reflect complex interplays of transcription factors, signal transduction, and receptor-mediated processes (*26*), for the coordinated release of stasis intervals during normal growth. Although postulated endocrine-signaling mechanisms that might synchronize growth may not have been elucidated yet, our observations of normal growth saltation and stasis challenge researchers to investigate "previously unsuspected mechanisms" that bring together normal growth processes at the level of the cell and whole organism.

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1. M. Lampl, J. D. Veldhuis, M. L. Johnson, *Science* **258,** 801 (1992).
2. Data on head circumference, weight, or lower leg length measurements were not included in our report (*1*).
3. The saltatory algorithm approximates a smooth continuous curve if significant saltations are not distinguished from error. The false positive rate was established by Monte Carlo simulations.
4. This would not be the case in all individuals if the saltations were random error components.
5. See figure 1 and table 1 of (*1*). The average daily growth increment, or saltation, was 0.95 cm (SD = 0.3; SEM = 0.062; range = 0.5 to 1.65 cm), and the average of all stasis intervals was 11.9 days (SD 6.5; SEM 1.3; range = 2 to 28).
6. The weighted variance of fit using the error bars presented in Fig. 1A as weighting factors for the saltatory model is 0.82 and for the triple linear spline model with variable knee points, 1.19 (the latter is their linear model). The F statistic to demonstrate that the saltatory model is a better description of the data is approximately 2.4 at $P < 0.05$. Here, the ratio of the continuous function and the saltatory model is 1.44. One factor contributing to the large F ratio factor required for statistical significance is the small sample size of the data series of Heinrichs *et al.*
7. With increasingly large data sets, the significance of platykurtic characteristics will increase; small data sets like those of Heinrichs *et al.* are unlikely to contain sufficient data points, if saltatory, to result in significant skewness.
8. K. E. Oerter, P. J. Munson, J. D. Bacher, G. B. Cutler, J. Baron, *Endocrinology* **134,** 1317 (1994).
9. These data appear in figure 1 in our report (*1*).
10. The basis of the calculated skewness coefficient for saltatory growth of approximately 0.8 is said to be the assumption that growth occurs on 8% of days.

This assumption seems to be based on the growth pattern of a single infant, aged 4 to 7 months, who grew in 11 saltuses during 118 days of observation, or 8% of the time [figure 1 of (*1*)]. The actual skewness coefficient calculated from our experimental data for that infant is 1.265 (Fig. 2).
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14. A. T. Gibson, R. G. Pearse, J. K. H. Wales, *Arch. Dis. Child.* **69,** 498 (1993).
15. A. T. Gibson and J. K. H. Wales, *Human Biol. Budapest* **24,** 34 (1994).
16. R. E. Scammon, in *The Measurement of Man*, J. A. Harris *et al.*, Eds. (Univ. of Minnesota Press, Minneapolis, MN, 1930).
17. For studies assessing short-term changes in weight, assessments must be standardized by ingestion, micturition, and defecation. [C. C. Gordon, W. C. Chumlea, A. F. Roche, in *Anthropometric Standardization Reference Manual*, T. G. Lohman, A. F. Roche, R. Martorell, Eds. (Human Kinetics, Champaign, IL, 1988), pp. 3–8.
18. When using the original instrumentation, it is not possible to simultaneously read the length measurement and position the child. One's attention is focused on proper positioning and, after the leg is released, the length measurement is read. Thus, the effect is similar to the methods cited here. While blind measurement is a protocol to be recommended for future studies, clinical studies have documented significant errors introduced from the locking mechanism on the Harpenden-Holtain instrument itself, a mechanism designed for portability, not blind read-out accuracy.
19. M. Lampl, *Am. J. Hum. Biol.* **5,** 641 (1993).
20. Heinrichs *et al.* appear to be in error when describing their "[W]ithin-day measurement error (SEM)" as being 0.193, 0.031, and 0.078 cm for crown-heel, knee-heel, and head circumference, respectively [table 1, now (*3*) in their comment]. In fact, 0.378, 0.061, and 0.153 cm are the correct 95% "measurement errors." Neither these revised estimates nor the original estimates are "similar in magnitude" to the mean daily growth rates; the original estimates are 89%, 55%, and 70% greater than the daily growth rates, and the revised 95% errors are 270%, 205%, and 233% greater than the daily growth rates, respectively. Thus, continuous growth cannot be documented by these data [N. Cameron, *The Measurement of Human Growth* (Croom-Helm, London, 1984)].
21. A. F. Roche and J. H. Himes, *Am. J. Clin. Nutr.* **33,** 2041 (1980).
22. J. M. Tanner, *Foetus into Man* (Harvard Univ. Press, Cambridge, MA, 1990), p. 8.
23. M. Kobayashi and M. Togo, *Am. J. Hum. Biol.* **5,** 193 (1993); M. Hermanussen, K. Geiger-Benoit, J. Burmeister, W. G. Sippell, *Ann. Hum. Biol.* **15,** 103 (1988); M. Hermanussen, *Am. J. Hum. Biol.* **5,** 653 (1993); I. M. Bernstein, K. Blake, G. Badger, *Am. J. Obstet. Gynecol.* **172,** 323 (1995). L. Greco, A. Capasso, C. De Fusco, R. Paludetto *Arch. Dis. Child.* **65,** 373 (1990); A. Guala, G. Pastore, M. Barbaglia, J. Buffa, unpublished data; The applicability of 24-hour measurements in small mammals is of questionable relevance, as daily long bone growth has been documented to consist of shorter circadian rhythms in several animal species (including rabbits), which is more in line with an underlying saltatory growth mechanism than a continuous growth mechanism. [D. A. Simmons, L. A. Whiteside, S. W. Whitson *Metab. Bone Dis. Relat. Res.* **2,** 49 (1979); L. I. Hansson, A. Stenstrom, K. G. Thorngren *Acta Orthop. Scand.* **45,** 499 (1974)]. Nonmammalian species have been documented to grow episodically. (W. B. Roush, G. F. Barbato, T. L. Cravener, *Poultry Sci.* **73,** 1183, 1994). Heinrichs *et al.* apparently assume that the control mechanisms for a continuous model of growth are understood, but this is not the case.
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27. We thank E. Frongillo, A. Kellermann, and R. Martorell for helpful comments.

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Variability Among Human Umbilical Vein Endothelial Cultures

Endothelial cell (EC) cultures have been extensively used in models of different vascular phenomena such as inflammation and angiogenesis. One of the most widely used sources for cultured ECs is human umbilical veins. Until recently, most investigators have isolated human umbilical vein endothelial cells (HUVECs) in their own laboratories and propagated the cultures for brief periods (that is, several weeks) under standardized (STD) conditions using Medium 199 supplemented with 10 to 20% serum (usually fetal calf or human) plus heparin-binding growth factor 1 [also called acidic fibroblast growth factor or endothelial cell growth factor (ECGF)] stabilized by heparin as originally described by Levine and colleagues (*1*). Recently, several commercial sources of HUVECs have become available, often provided with variant (VAR) conditions of media and growth factors that may differ significantly from STD conditions. Few, if any, data are available that compare cultures of HUVECs propagated in these different conditions with cells from STD cultures.

In 1992 A. E. Koch *et al.* reported, in part, that HUVECs obtained from a commercial source responded to human interleukin-8 (IL-8) as a mitogen (*2*). We had been unable to reproduce this finding using HUVECs isolated and cultured in our laboratory (*3*). We decided to investigate whether differences in the culture systems used could contribute to this discrepancy. We isolated HUVECs in the standard manner and split the cells into two groups: a STD group cultured in Medium 199, 20% fetal calf serum (FCS), 50 μg ml ECGF, 100 μg ml heparin, and a VAR group cultured in the medium used by Koch *et al.* (*2*). In multiple experiments, involving three different paired isolates, we found that cells propagated under STD conditions did not proliferate to IL-8 whereas those cultured under VAR conditions did (Table 1). Cells switched from STD to VAR conditions did not acquire responsiveness, but cells switched from VAR to STD conditions showed diminished responsiveness to IL-8 (Table 1). These data are most consistent with the interpretation that the cultures grown under VAR conditions contain an IL-8 responsive cell type that is lost in STD cultures. Unexpectedly, even cultures under VAR conditions that respond to IL-8 do not appear to display high affinity binding of [^{125}I]-IL-8, using an assay (*3*) easily able to quantify binding to neutrophils (data not shown).

There are several possible explanations for lack of detectable high affinity receptors: (i) IL-8 could be mitogenic for HUVECs

Table 1. Mitogenic actions of IL-8 on HUVEC cultures.

Culture conditions*	Cell number†		Significance‡
	No IL-8	+IL-8	
STD→STD	22.3 ± 9.9	18.7 ± 4.1	n.s.
VAR→VAR	15.3 ± 7.6	34.8 ± 5.5	$P < 0.005$
STD→VAR	31.03 ± 7.3	28.3 ± 5.7	n.s.
VAR→STD	22.8 ± 10.5	31.8 ± 9.8	$P = 0.08$

*HUVEC isolates were serially subcultured in parallel in standard medium (STD = Medium 199, 20% FCS, 50 μg/ml ECGF, 100 μg/ml heparin, 0.4 mg/ml L-glutamine, 100 U/ml penicillin-G, and 100 μg/ml streptomycin sulfate), or variant medium [VAR=Clonetics (San Diego, California)–optimized Endothelial Growth Medium, 2% FCS, 10 ng/ml human epidermal growth factor, 1μg/ml hydrocortisone, bovine brain extract, 40 ng/ml heparin, 50 μg/ml gentamicin, and 50 ng/ml amphotericin B] for two passages. Each culture was then split (1:3) into two groups; one remained in the same medium (STD→STD or VAR→VAR) and one was crossed over to the other medium (STD→VAR or VAR→STD). †10×10^3 cells were plated into 2.0-cm wells containing fresh STD or VAR medium ± 1×10^{-9} M IL-8. Cells were harvested and counted at 48 hours. Each condition was tested in six replicates, and cell number is the mean ± SD of 10^{-3} cells per well. ‡*P* values from paired Student's *t* test. Only cells maintained in VAR show a significant mitogenic response; the response of cells transferred from VAR to STD medium is diminished. n.s., not significant.