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GROWTH AND CELL PATTERN FORMATION ON AN AXIS: CRITIQUE OF CONCEPTS, TERMINOLOGY, AND MODES OF STUDY

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The processes which generate length and cell pattern down a growing axis have traditionally been termed cell division and cell expansion; they are usually presented as alternate means of generating volume or length. But, because cell division can involve varying amounts of volume increase between mitoses, it can have no fixed units with regard to the volume-generating function. This nonquantitative interlocking of the two processes renders them incapable of either precise description or quantitative prediction of the phenomenology of growth and pattern. A flexible and comprehensive framework for quantitative analysis, put forward by GOODWIN, ERICKSON, and others, employs two independent but superimposable processes (not alternate means to the same end). *Surface extension* is the sole volume (length) generating process, measured as a compound interest rate; *cell partitioning* is the instantaneous act of completing a cross wall, also measured as an interest rate. Change in cell length is a direct function of the difference between the two processes (e.g., if the extension rate, in the sense of doubling, is not fully balanced by an equal partitioning rate, cell length will increase). Consistent independent gradients for extension and partitioning down an axis will generate a consistent pattern for mean cell length down the axis. This forms the basis of a revised growth-zone nomenclature linking histological change to well-defined meaurable processes. Various traditional concepts of cell division reappear as special cases. The cell cycle is the interval between partitioning events. Using the cell-partitioning function down an axis, one can measure cell-cycle duration in two senses: (*a*) its duration at a given distance from the tip, as if the cells could remain at that position in the gradient for one cycle, and (*b*) the duration of the cycle for a given group of cells as they move down the axis. For (*a*), the expected agreement with the label accumulation method is limited but reasonable; the agreement with the metaphase accumulation method is poor. For (*b*), agreement with the labeled mitosis method is to be expected. Since, however, the method appears not to have been applied appropriately (i.e., to a specific group of moving cells), clear comparison cannot be made. The kinetic methods are indirect but intrinsically free of radiation/chemical artifact. They can serve as independent measures of total cycle time where the duration of separate cycle phases is under study by label methods.

Introduction

Growth is defined as increase in volume which, in elongating axes, is nearly proportional to length. Growth in such axes is traditionally discussed as being generated by two processes: cell division and cell elongation. These processes are considered to be responsible for the formation of cell pattern which, in the one-dimensional case, reduces to a characteristic sequence of mean cell lengths down an axis. When the developmental processes and the cell pattern are consistent through time, the growth of the axis is in a "steady state."

It will be argued here that the analysis of growth and pattern in such axes cannot be carried out properly using the traditional concepts where division and elongation are viewed as alternate modes of generating volume. The main difficulty is that, in meristems, cell division can be associated with varying amounts of volume increase between mitoses. Thus, this process can have no usable units and is of no value in quantitative studies. It appears that a concept suitable only for ideal cell cultures, where increases in total cell number and total cell volume are indeed proportional, has been applied to meristems. In meristems, however, the evidence for such a proportionality (constant mean cell length) is not generally found. Despite being quantitatively intractable with regard to volume generation, cell division in growth zones is nonetheless often presented as a valid qualitative alternative to cell expansion to account for the growth of axes. The two processes are frequently described as being mutually exclusive or antagonistic: "The relative contributions of cell division and cell expansion towards longitudinal growth in the apices of roots have been investigated . . ." (CLOWES 1961*a*, p. 3). "This accords with the general belief that active cell-expansion is incompatible with the continuation of a meristematic function" (DORMER 1972, p. 67). "The treated plant showed a remarkable capacity to attain normal gross dimensions by substituting a quite abnormal degree of cell-expansion for the lost alternative of cell-division" (DORMER 1972, p. 80).

The increase in mean cell length in proximal parts of the division region reveals that the supposed mutually exclusive alternatives clearly overlap. There are two ways out of this logical dilemma. One is to adhere to the original concepts and model a growth zone so simplified that, indeed, quantitation is possible. A growth zone can be represented as one region of cells behaving like a cell colony (constant mean cell length, constant cell-cycle time) which contributes its progeny across an abrupt boundary into a second region of cell elongation. This was the starting assumption for early analyses (GRAY and SCHOLES 1951) and has reappeared as a formal model (LÓPEZ-SÁEZ et al. 1975). The other solution is to grant that the nonquantifiable interlocking of the two concepts in meristems forces one to search for a somewhat broader frame of analysis.

In the latter vein, all the above problems disappear

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when the traditional growth-zone duality is viewed as "growth with cell division" on the one hand, and "growth without cell division" on the other (textbook by RAY 1972). Growth is now considered a single process; cell pattern in a meristem is viewed as the result of the two processes operating in combination, not as alternatives. Outside the meristem, growth operates alone. This view does reduce cell division to the momentary act of completing a cross wall, but this is in no way incompatible with the concept of the cell cycle, or the older broad meaning of "cell division," which becomes simply the interval between two such instantaneous division events.

The quantitative application of such concepts has been developed by others (GOODWIN and STEPKA 1945; ERICKSON and SAX 1956*a*, 1956*b*; GOODWIN and AVERS 1956; ERICKSON 1976). The purpose of this article is (*a*) to elaborate, in simple form, this comprehensive mode of analysis, (*b*) to use it to clarify terms relating histology to developmental processes, and (*c*) to point out its utility in a variety of growth-axis studies, particularly those dealing with the cell cycle.

The process related to the traditional concept of growth will be termed surface extension; the process related to cell division will be termed cell partitioning. Surface extension alone generates length and volume. Both partitioning rate and extension rate influence cell pattern (mean cell length). The following points will be made:

A. The two processes are precise and have units. The units are those of compound interest, which allows the process to be measured at individual points down the axis and over very short periods of time.

B. They are completely distinct from each other in terms of their cytology and physical meaning for the generation of cell pattern. Being independent and superimposable, they can occur in virtually any combination at any position along the growing axis.

C. The processes are such that the difference between them, at any position on the axis, precisely determines the changes in histology (cell-length pattern) that will take place at that region. The histological sequences stemming from the various qualitative relations between the two processes are given in figure 1*A*. Note that there is an analogy to financial investment in that value per share ("cell length") is subject to alteration by two independent processes: value gain and stock splitting (extension and partitioning, respectively).

D. They are useful in redefining many loosely used terms such as "growth by cell division," "elongation zone," etc. These become rigorous concepts which can be given more precise names. A diagram relating several such terms to well-defined relations between the two processes is given in figure 1*C*.

E. They are related to experimental design and interpretation in ways that are highly important but generally unrecognized. Both processes operate continuously in a fashion that varies with position down the axis, so that only an integration over both time and/or space can relate the processes to long-term observations (such as cell-cycle time, as also revealed in the appearance of consecutive waves of labeled mitoses in cell-cycle experiments). It is not difficult to predict long-term consequences of the operation of either process, such as cell-cycle times for a given group of cells, if the pertinent process function is known. It is very difficult to perform the reverse operation: deduction of the quantitative nature of the gradient in the two processes on the basis of the consequences of their long-term operation.

A. The nature of the two pertinent processes

Continuous gradients of measurable activities are needed to describe precisely the length- and pattern-generating processes in an axis. To vary continuously along a growing axis, a process must be both instantaneous in time (so that it will not move to a new part of the gradient during its measurement) and "elemental," that is, infinitesimal in space (so that it will not span significant regions of the gradient).

SURFACE EXTENSION.—This process is basically the ability to separate two marks along an axis. Because the axis is a cylinder, the process extends volume as well. The term "surface" is used because the phenomenon is generally measured by following natural or applied marks on a surface. This process is, like all biological activities, carried out by cells. It is, however, an activity that can occur in strong gradients within a single cell, and hence is subcellular. It has been measured over small fractions of a cell length in epidermal cells (CASTLE 1955) and parenchyma cells (WILSON 1957, 1964), as well as in algae (GREEN and KING 1966; CHEN 1973) and fungi (CASTLE 1958). The process is continuous at least down to the level of a micrometer (CASTLE 1940). Since these dimensions are very small relative to an organ axis, the process is sufficiently infinitesimal (or "elemental") for use. The fine-grained quality of this process weighs against its being termed cell elongation, since the latter could imply a process measured in terms of whole cell lengths. That surface extension is, in fact, continuous over significant periods of time is indicated by the smoothness of the trajectories of marks in continuous-marking data experiments (e.g., ERICKSON and SAX 1956*a*; CASTLE 1958).

MEASUREMENT.—The basic assumption is that a small district of surface gains in length by activities taking place within the district. Thus, by analogy with bacterial colony growth, extension for short periods of time, at least, is exponential:

$$L_1 = L_0 e^{rt} \, , \tag{1}$$

where L is the length of the district at times 0 and 1, t is time, and r is rate. The value of r is $(\ln L_1 - \ln L_0)/$time (fig. 2).

The units of r are time^{-1} because the rate reflects

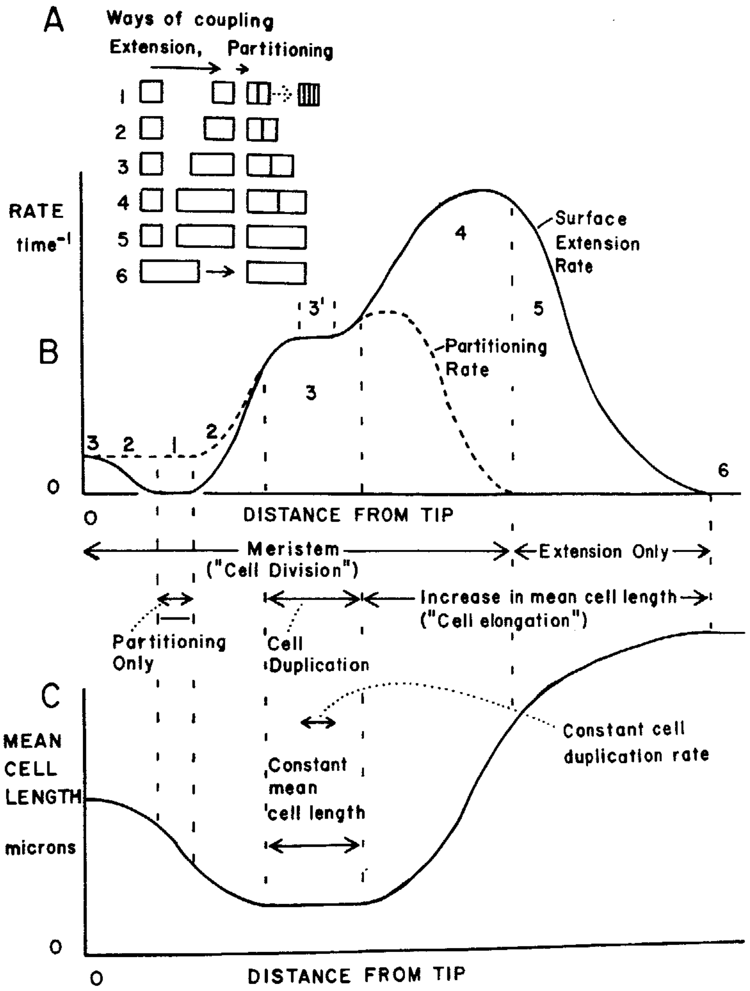

FIG. 1.—An analysis of a hypothetical growing axis (tip is at left) which is characterized by curves of extension rate and partitioning rate. The six qualitative types of histogenetic behavior given in *A* are all-inclusive for cell pattern down a line. Each type of behavior brings on a change (or constancy) in mean cell length. Cross-reference between the curves in *B* and the histogenetic images in *A* is given by the numbers. Cross-reference between the curves of *B* and change in mean cell length, *C*, is given by the vertical dashed lines. *A*, 1: Cell partitioning only. Here the extension rate is zero and the partitioning rate is finite. This behavior is typical for xylem regeneration from parenchyma and is not typical for apices. It is given here for completeness of illustration. Cells in such a zone would be pushed to the right by growth of cells to the left and hence would resume expansion. 2: Both rates finite, partitioning having the higher value. Mean cell length decreases, but less rapidly than before if the partitioning rate is unchanged. This type of behavior is seen in roots (ERICKSON and SAX 1956*b*). 3: Here the rates are equal to each other but not necessarily constant with position or time. There is no change in mean cell length; hence such a zone would be one of cell duplication. In corn this condition exists only at the point where the curves cross. 3′: Here the rates are not only equal but are constant with position and hence, for a while, constant with time. This zone of constant cell-duplication rate is the only one where the presumed characteristics of cell-colony growth obtain: constant exponential production of cells of constant mean dimensions. In the corn root, case 3′ does not exist as the curves are not horizontal while identical. The colony-growth situation, from which traditional concepts relating to axis differentiation have evolved, is clearly a special case in the general frame of analysis being reviewed here. 4: Both partitioning and extension rates are finite, the extension rate predominating. Here mean cell length must increase, even though cell partitioning is occurring. 5: Partitioning rate is zero; extension rate is finite. Mean cell length increases. 6: The trivial case in which both parameters are zero—the "maturation" zone. The major generality is that change in mean cell length in a region is proportional to the difference between the curves in the region. If partitioning predominates, cell length decreases; if extension predominates, cell length increases. If the values are identical, cell length is constant. The generality has application in the definition of zones along the axis. Apparent old names are given in quotes. Zones are all defined by the relative values of the two curves and hence have unambiguous meaning and well-defined boundaries. The names are chosen to imply the correct histogenetic characteristics. To have constant histology at the tip, an axis would have to begin with cells showing no growth (case 6) or cell duplication (case 3). This need be true only over an infinitesimal distance, as shown.

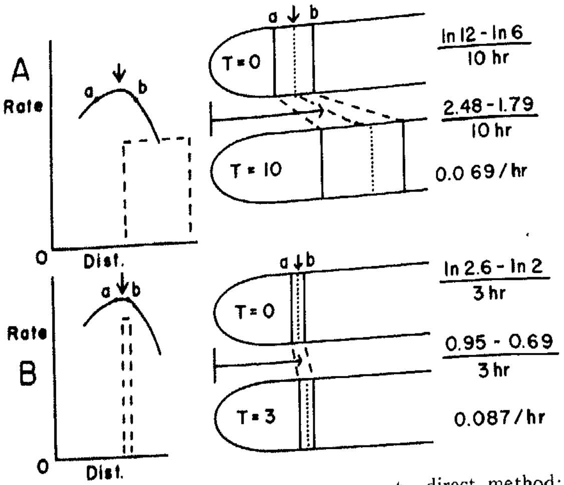

FIG. 2.—Surface extension measurement—direct method: The graphs at the left give the true distribution of the relative (elemental) surface extension rate for the axis. The aim is to measure relative extension rate at a point (the vertical arrow) for a given instant. The compromise is to measure the separation rate of two points, *a* and *b*, straddling the point of interest. In *A* the points are far apart, and the time between measurements of separation is long, 10 h. The estimate of rate is made by the calculations (from eq. [1]) at right. During the time period the points will separate as well as be displaced to the right. Displacement of the midpoint to a new distance from the tip is shown by the horizontal arrow. Because the district's rate values are descending over the time period, the more apical "half" of the district will always have higher rate values and will expand relatively more: the original midline (dotted, vertical) is hence no longer the midline after 10 h. This method gives an average rate over a long distance (dashed bars on rate graph) and underestimates the true value at the position of interest. In *B*, a comparable exercise is carried out but with a much shorter district and a shorter time. The calculations give a higher and more accurate rate. Displacement of the mark pair (*a*-*b*) is reduced, and hence ambiguity as to the "point" associated with the observed rate is reduced (narrow dashed bar below curve at left). This method gains validity as distance and time are reduced. Such reductions always increase variability in measurement, so complete precision cannot be obtained.

length gained per unit length present, and the length units cancel. The rate is therefore relative. Rate measurement by studying the behavior of pairs of marks on a surface suffers from the fact that if the marks are far apart, they may span changing values of the rate gradient on the organ. Also, if a long period of time is taken for the measurement, not only will the point pair span more of the gradient than initially, they both will be displaced away from the tip due to the concurrent expansion of surface between the points and the tip. The changes that inevitably occur during such measurements are diagrammed in figure 2. They can be minimized (fig. 2*B*) by reducing the point separation and the time of observation. When further reduction in these parameters yields only minor changes in rate, acceptable values for rate are being obtained.

There is a problem in identifying the region for which a given value is to be characteristic. The desire is to characterize rate at a point, such as the arrow midway between the two points shown in figure 2. This midpoint is inevitably displaced away from the tip during the measurement. It has no fixed distance from the tip, so its mean position is used. Approximate characterization of a growth zone can be made this way, despite the "indeterminacy principles" operating, if the displacements are small. GREEN and KING (1966) applied this method to the surface of the *Nitella* apical cell. CHEN (1973) applied it to growing rhizoid tips. (A simple version of this method, designed for analysis of the nondividing region of root longitudinal sections when root-elongation rate is known, is given in the Appendix. It is suitable for a class exercise.) The method can be applied periodically to an axis (e.g., a stem internode) on which the extension gradient changes with time. A characteristic family of curves is obtained (e.g., GREEN 1965). A comprehensive plot would be a three-dimensional surface with rate, position, and time axes.

MEANING OF THE RELATIVE RATE.—The rate is a fractional increase expressed in units of finite time. Thus a rate of 0.69 h^{-1} means 69% per hour. Because this rate is continuously compounded during 1 h, there will be more than a 69% increase. The way to find the final length (here, after 1 h) of any district is to take the initial length and multiply it by the anti-ln (inverse of the natural logarithm) of the rate, (e^{r}). Thus, if a district is initially 23 μm long, and the rate is 0.69 h^{-1}, the final length would be $e^{0.69} \times 23$, or 46 μm. The anti-ln of a rate gives the fraction by which the initial value has been changed (in the sense of being multiplied by that fraction) during the time unit of the rate.

Rates can be converted to doubling times as follows: If L_1 is twice L_0 then, after (1),

$$2 = e^{rt} \qquad (2)$$

and

$$\ln\ 2 = rt_d\,, \qquad (3)$$

so

$$(\ln\ 2)/r = t_d \text{ (doubling time)}\,. \qquad (4)$$

In this case the doubling time is obviously 1 h. But if the rate were 1.1 h^{-1}, t_d would be 0.63 h, or about 38 min.

The doubling-time concept can be applied to an infinitesimal district, such as the peak of the relative elemental extension rate in corn (ERICKSON and SAX 1956*b*), where the value is 0.4 h^{-1}. The surface would double in 1 h 44 min, if it could grow continuously under conditions obtaining at the peak. An actual zone would be swept through the peak during 1 h, and hence it would expand somewhat less. The concept is nonetheless valid. It is analogous to saying a sprinter can run at 40 km/h, even though he could not cover 40 km in 1 h. Thus, at the peak of a rate curve, an infinitesimal district grows at a specific rate for an instant of time. This reflects the instantaneous nature of the rate. By the same token, a finite zone of uniform behavior (surface of a *Nitella* internode; GREEN 1954) could show a constant relative growth rate for a finite period.

The study of finite separations on steadily growing organs has the above errors which are, in general, biases. The ability to measure rate of relative extension at a point in space and at an instant in time, the definitive solution to the dilemma above, has been developed in the work of GOODWIN and STEPKA (1945) and ERICKSON and SAX (1956*a*). The rate (termed relative elemental rate of elongation) is conceptually identical with the r in (1) but is measured by studying the velocity by which successive marks on the axis are being displaced from the tip. As elaborated in figure 4 and its legend, the rate is actually obtained from the change in velocity, as a function of position, as successive points along the axis are considered. The appropriate data are derived from an ingenious streak-photograph method (ERICKSON and SAX 1956*a*) or from direct observation of cross walls in living *Phleum* roots (GOODWIN and STEPKA 1945). Noteworthy experimental studies involving the extention-rate curve include those of HEJNOWICZ (1961). He showed that, in roots, auxin reduced the area under the curve (as it must to inhibit growth velocity) but changed the shape of the curve, a small region showing rate stimulation! A very high resolution kinetic study of roots has been made by LIST (1969).

It is important to realize that the elemental extension process is a separation, symbolized by a double-headed arrow. Under close examination, growth is bidirectional. Growth of a whole organ, such as a root, can obviously be characterized by a growth velocity such as 1 mm h^{-1}, symbolized by a unidirectional arrow. The proper connection between these two facts is that the growth rate of the root is the integral over all the infinitesimal growing regions in the root. The integral over distance of the rates we are considering has the units of velocity: mm/h. To impute a unidirectional character to the immediate process of extension is an error. Unidirectional growth (not due to summation of bidirectional processes) generates length in the manner of an inelastic tape being pulled through a slit in a dispenser. In a plant the surface behaves as a tape which generates stretch internally; there is no analogue to the slit in the dispenser, nor the hand pulling the tape.

The cytological basis of surface extension is simply the extension of the sidewalls of the cells in the axis. That this suffices to extend an axis independently of concurrent cell divisions is shown by the work of HABER (1962). A common definition of "cell division," wherein one cell gives rise to two like itself, encompasses surface (and volume) extension. The utility of extracting the extension component out of such concepts, and measuring it independently, is that it can have the appropriate features for continuous variation along an axis. Also, in combination with a parallel concept for partitioning, all possible meanings of "growth by cell division" (cases 2, 3, and 4 in fig. 1) can be clearly stated.

CELL PARTITIONING.—The companion process to the extension of surface (and volume) is partitioning. This is the "rate of cell formation" of ERICKSON and SAX (1956*a*, 1956*b*), but since that term could be taken to imply a process taking place over a whole cell cycle, partitioning is used here. The act of completing a cross wall is instantaneous for all practical purposes, and the wall itself has a thickness essentially infinitesimal compared to the length of an organ growth zone. The rate in question becomes the rate, relative to the number of partitions already present, of adding new cross walls. Since the addition of a cross wall always adds another cell, partition number and cell number vary in parallel. This concept in no way conflicts with the concept of the cell cycle or the fact that a finite amount of cell activity is required prior to the completion of a cross wall. The physiological cell cycle is simply the activity between successive partitioning events. Clearly, when this partitioning is coupled with surface extension, conventional cell-growth cycles ensue (cases 2, 3, and 4 in fig. 1).

The concept of partitioning as divorced from growth is evident in texts: "Cell division does not itself constitute growth" (STEEVES and SUSSEX 1972). "But increase in the number of cells does not itself result in growth" (CLOWES 1961*a*, p. 3). This reflects the fact that partitioning can occur in the absence of growth. In light of the first set of quotations (Introduction), however, the key next step of superimposing division upon growth (as against making it an alternate form of growth) has not been taken generally. The major difference between surface and partitions is that the former is continuous in space and the latter are not.

MEASUREMENT OF THE PARTITIONING RATE.—This could be done for epidermis by direct observation of the organ. A short section of surface could be tallied for cross walls and then, after a short period of time, tallied again and the relative increase per unit time readily calculated (GOODWIN and STEPKA 1945). The same "indeterminacy principle" that plagued the direct analysis of surface extension applies here. It is even more severe because partitions are separated by distances greater than the smallest practical particle separations. Thus the partition number in a sample is seriously reduced as the segment is reduced in size and the time of observation shortened (fig. 3). One could have a section so short that no new partitions formed in it during the period of observation (or that lacked partitions throughout). One has to average over many comparable sections on many comparable axes. The calculations are exactly parallel to those for surface extension, and the units are $time^{-1}$ as before. If the partitions in a short zone went from 20 to 25, or from 4 to 5, in 2 h, the relative rate of partitioning would have the value of .11 h^{-1}.

This variable can be measured in a manner free of the shifting inherent in the segment method (ERICK-

SON and SAX 1956*b*). The rationale involves the evaluation of slopes at points in the structure and at instants of time (fig. 4*II*). Particular slopes that are difficult to measure directly can be arrived at by the multiplication of two other more accessible slopes, terms canceling out to yield the desired variable. (See fig. 4*II* for a model example and ERICKSON and SAX [1956*b*] for actual details.)

MEANING OF THE RELATIVE PARTITIONING RATE FOR A REGION.—The interpretation of this rate is parallel to that for surface expansion. A peak rate of 0.15 h^{-1}, as noted in corn by ERICKSON and SAX (1956*b*), means that the number of partitions (cells) in the short region is altered by a factor of 1.162 during 1 h, provided the rate is constant throughout that hour. The doubling time for cells is $(\ln 2)/r$, and in this case would be 4.62 h. This is the site-specific cell-cycle duration. It is characteristic of a fixed distance from the tip, not a given group of cells. A given cell on the root would pass through this peak rate only momentarily and would have a longer cell cycle. How exactly to predict the length of the cycle for a given group of moving cells (rather than a fixed position) and how to confirm it by the labeled mitosis technique will be discussed in Section E.

Note that because the rates are instantaneous, they can be expressed in various units of time by retaining proportionality: 0.24 day^{-1} = 0.01 h^{-1}.

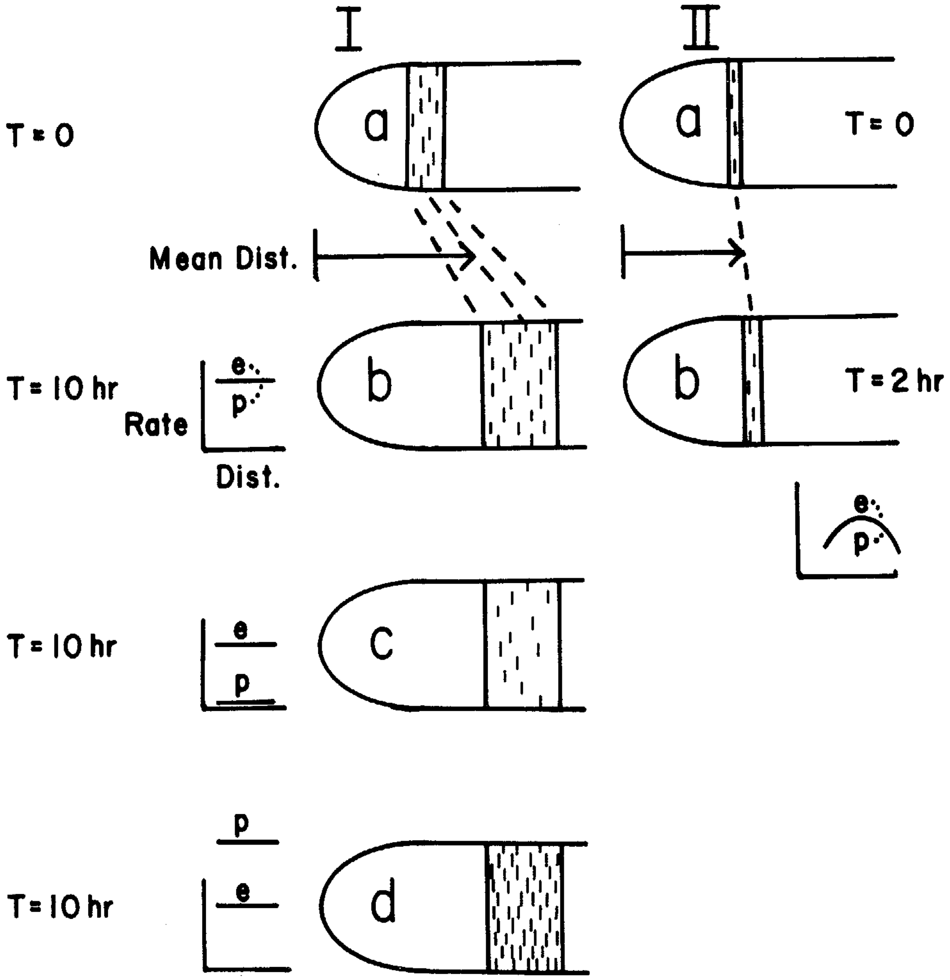

FIG. 3.—This illustrates the joint action of partitioning and extension on histological pattern, *I*, and how precision in the direct estimation of partitioning rate is enhanced by reduction in both length of the zone studied and time between measurements, *II*. *I*, A zone initially with 12 partitions, *a*, is seen, after 10 h, to contain 24 partitions in *b*, the original 12 in *c*, and 48 in *d*. In all cases the length and volume of the zone have doubled. The relative values of the extension, *e*, and partitioning, *p*, functions are given in the small graphs at left. It is seen that mean cell length is constant, *b*, increases, *c*, or decreases, *d*, in accord with the relative values of *e* and *p*. The measurement of *p* from such data is normally complicated by the gross displacement of the zone in question between measurements. Partitioning rate should be characteristic of a point. In *I*, where the rates do not vary with position, the data collected would be valid. *II*, When *p* and *e* are in continuous gradients, a closer approximation to a point measurement must be made. Here both the length of the zone and the time between measurements have been reduced. This reduces the ambiguity of the "position" of the zone because the midpoint moves relatively less. Unfortunately these reductions increase the intrinsic variability in the measurements. When partitions are not frequent, measurements would have to be summed over many comparable zones in comparable roots. Mean position of the midpoint (horizontal arrow) roughly identifies the zone, but rate is not strictly determined for a point. Techniques to calculate the latter are given in figure 4 and its legend.

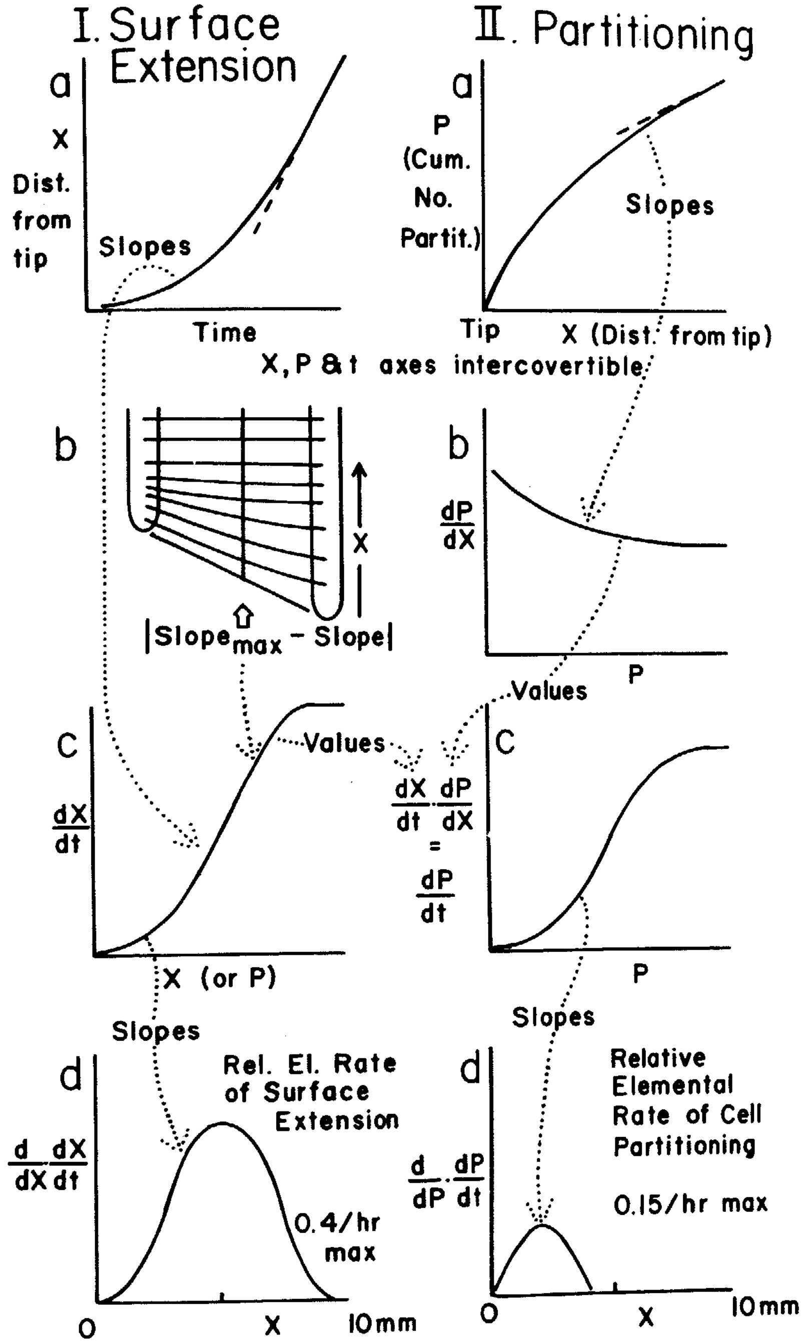

FIG. 4.—Rationale for calculation of relative elemental rates: rate determination without use of finite distances or finite times. Sufficient initial information for the two rate functions is found in the two graphs labeled *a;* the desired rate distributions are in the graphs labeled *d*. Dashed lines show extensions of straight portions of curves. *I*, Surface extension. The time course of the displacement, *X*, from the tip by a single mark, is known, *a*. Slopes are taken and plotted against distance from the tip, *X* (*c*). Or, as done by ERICKSON and SAX (1956*a*), a time exposure on moving film is made of a downward growing root (*b*). The slopes on the trajectories of successive marks, taken at a point in time given by the vertical line (broad arrow), yield the speed of departure of the point markers from the base. The absolute value of maximal velocity (that at the tip) minus the value at intervening points, yields *dX*/*dt* as a function of distance from the tip (*c*). Slopes taken on this curve and plotted versus *X* give the relative elemental rate of surface extension (*d*). The data shown approximate those for corn. *II*, Partitioning. Parallel estimation of cell partitioning rate starts with a curve relating the cumulative number of partitions down the axis (or cell file) to distance from the tip. The two curves *Ia* and *IIa* render the time, *X*, and *P* axes interconvertible. Slopes taken on curve *IIa* can therefore be plotted against *P*, *IIb*. These slopes, when multiplied by corresponding values from curve *Ic* (as plotted against *P*) introduce the time variable into column *II* in the form of *dP*/*dt* plotted against *P*, *IIc*. Slopes taken on this curve give the relative elemental rate of cell partitioning, curve *IId*. Data roughly approximate those of corn. Slope changes in the surface extension and partitioning curves appear to be independent; thus no direct competition or antagonism is evident. This outline is a simplified and modified version of the actual techniques used by ERICKSON and SAX (1956*a*, 1956*b*). It shows the ingenious feature of obtaining an otherwise difficult slope, *dP*/*dt*, by multiplying two more readily determined slopes together to yield the desired one (curve *IIc*). For actual procedures, consult the original papers.

B. Distinctness of the two processes

The processes are independently measured, as above, and are cytologically independent insofar as the completion of a cross wall by the phragmoplast is distinct from the extension of the sidewall. The parallel physical notions of stretching and demarcation are equally distinct. The processes are not alternate means to the same end, as usage of "growth by cell division" and "growth by cell elongation" implies. Nor are they found to be obviously antagonistic in growing organs, despite presumably competing for mass and energy. If they were, the sum of their rates would tend to be a constant. No such trend is seen (Goodwin and Avers 1956).

The processes are basically superimposable, capable of coexisting in any combination. At the extremes, however, biological considerations demand a kind of coupling. There cannot be indefinite partitioning without some surface (volume) extension. There cannot be indefinite extension without some partitioning. Cells do not become infinitely large or small. So, if there is an obligatory interrelation between these two processes, it occurs only at the extremes and takes the form of a coupling rather than an antagonism.

C. How the two processes dictate change in histology

In a one-dimensional system the characteristic feature of histology is the pattern of cell lengths down the axis. Mean cell length must be used as the characteristic measurement because, within the meristem, cell partitioning abruptly replaces a cell of a given length by two cells half as long. The present analysis does not resolve the details of differential cell division, this being a finer control on the partitioning process which anticipates cytodifferentiation. Differential divisions would add scatter to the data collected but would not counter their general validity.

Because each rate deals with fractional increase, the way that the two processes alter mean cell length is qualitatively obvious. It is illustrated in figure **1**, where there is cross-reference from the functions to both histology and mean cell length. If the expansion rate is zero and partitioning has a finite value, mean cell size must decrease (case 1). If extension becomes finite but at a lower value than partitioning, cell size will decrease even though surface extension is present (case 2).

When both processes occur at the same rate, mean cell length will not change (case 3) and cells will be essentially duplicated. If the values are not only equal but constant with position, and hence with time for a given group of cells, equivalent cells would be produced as an exponential function of time. It is only in this case that the many characteristics assumed for cell-colony growth obtain. This growth is undoubtedly the origin of the concept of "growth by cell division." This concept is fully valid in colonies because cell number increase and total volume (surface) increase are proportional. It is termed constant cell duplication (case 3′) to better imply the constraints that restrict its utility in characterizing organ growth. Meristems typically show cell-length decrease (case 2) or increase (case 4).

When surface extension rate exceeds that for partitioning, mean cell length increases even though partitioning occurs (case 4). When partitioning does not occur and the extension rate has finite values, cell length also increases (case 5). When both functions are zero, there is no activity and of course no change (case 6).

The quantitative relation between the processes and the histology is simply that the change in mean cell length (both sign and magnitude) is determined by the sign and magnitude of the difference of the two functions:

relative rate of change of mean cell length =
(relative extension rate) (5)
− (relative partitioning rate) .

The reasoning behind this is that the length of a short organ segment, L_s, is the product of the mean length of the cells, L_c, and the number of cells, N_c, both measured along a line down the axis:

$$L_s = L_c \cdot N_c \,. \tag{6}$$

Differentiating,

$$\frac{dL_s}{dt} = \frac{dL_c}{dt}\cdot N_c + \frac{dN_c}{dt}\cdot L_c \,. \tag{7}$$

Dividing through by L_s, or its equivalent, $L_c \cdot N_c$, yields

$$\frac{1}{L_s}\,\frac{dL_s}{dt} = \frac{1}{N_c}\cdot\frac{dN_c}{dt} + \frac{1}{L_c}\cdot\frac{dL_c}{dt}\,, \tag{8}$$

which by rearrangement yields the statement in (5). Erickson and Sax (1956*a*, 1956*b*) use dX (an element of X) for the length of the segment L_s in their more formal treatment.

An illustration of this relation is given in figure 3. An initially short zone (fig. 3 *I*, *a*) grows for 10 h. In figure 3 *I*, *b* the two relative rates are equal and constant, so both the length (volume) and the partitions double. Mean cell length is unchanged. In figure 3 *I*, *c* the partitioning rate is zero, while the extension rate is the same as in *a*. Since length has doubled and no partitions are lost, mean cell length doubles. Finally, in figure 3 *I*, *d* the partitioning rate has been made such as to quadruple the number of partitionings in the segment, so mean cell length is reduced to half.

Validity of this relationship (and the practicality of measuring it) is supported by the finding that, in general, the relative elemental rate of extension does equal the sum of the corresponding rates for change in cell length and cell number (Goodwin and Avers 1956, table 1).

D. Defining histogenetic processes and zones

The above analysis relating change in histology to the relative values of two functions allows the precise definition of processes occurring in an organ and the naming of the zones in which they occur. Cross-reference to previous terminology is difficult because common terms often have more than one meaning and these meanings are not well defined. Old terms will be given in quotes when it is felt they should be superseded.

GROWTH ZONE.—This would encompass all regions where surface expansion occurs. The growth zone in figure 1 is artificially split by having a short internal district that lacks surface expansion. This is merely to illustrate the effects of cell partitioning in isolation and is not known to occur in typical growth zones.

MERISTEM.—Derived from *meristos*, meaning to divide, this is the region within which cell partitioning (usually accompanied by expansion) occurs. Histogenetic cases 1–4 (fig. 1*A*) occur. This would presumably correspond to the old "zone of cell division." There are many narrower definitions in use (CLOWES 1961*a*).

EXTENSION ONLY (AND ZONE THEREOF).—This occurs where partitioning has zero value and extension is finite (case 5). This would be included in the old "zone of cell elongation, or expansion," but may not be synonymous with it (see below).

INCREASE IN MEAN CELL LENGTH (AND ZONE THEREOF).—This occurs wherever the extension rate exceeds the partitioning rate (cases 4 and 5) and has a broader meaning than the above. It also has been referred to by the term "cell elongation" (ERICKSON and GODDARD 1951). Note that if "zone of cell division" means meristem as defined here, and "zone of cell elongation" means increase in cell length, these zones overlap in contrast to the mutual exclusivity depicted in texts (RAVEN and CURTIS 1970). In the regions where both partitioning and extension occur, cell length is a function of both, rendering it representative of neither process.

Insofar as "cell elongation" can mean a process (surface extension) and the histological consequences of its action, in the sense of increase in mean cell length (case 5), developmental botany is in the embarrassing position of using the same term for both a process and its integral!

CELL DUPLICATION (AND ZONE THEREOF).—As already discussed, identity of partitioning and extension rates yields cells of constant mean cell length (process 3 in fig. 1). In the few systems studied (GOODWIN and AVERS 1956), the required identity is found only where the two curves cross. Hence this zone, so far, is infinitesimal. If the values are both equal and constant with position (and hence time), cells are duplicated at a constant exponential rate (process 3′). If "cell division" is taken to mean constant cell duplication, then there is no zone showing it in corn or *Phleum* roots because the rates are not constant (with position or time) as they cross. This zone may be useful only for cross-reference to cell-colony growth. Its properties, however, have been used in a model for root growth (LÓPEZ-SÁEZ et al. 1975).

Presumably the old term "growth by cell division" (or "mitosis") encompassed processes 2, 3, and 4. "During this time the leaf has been growing in height through mitotic activity of a terminal cell group" (EBERT and SUSSEX 1970, p. 293).

CELL PARTITIONING ONLY.—It is conceivable that cell partitioning could occur alone along an organ axis. This is diagrammed as case, or region, 1 in figure 1. Cell length (and volume) would halve periodically with time. Cell partitioning occurs alone in the "cutting out" of xylem cells from parenchyma in regenerating stems after wounding and in female gametophyte formation in a megaspore (SINNOTT 1960). It may occur in the striking reduction in cell volume seen in procambium formation. This reduction occurs, however, more at right angles to axis length than along it and hence may not be a feature of typical axis extension. This sense of "cell division" is implicit in the quotation, "Cell division does not of itself constitute growth" (CLOWES 1961*a*, p. 3). Case 1 was not detected in the growth zone of the corn or *Phleum* root.

GROWTH INITIATION SITE: SPECIAL REQUIREMENTS.—If the growth zone is to have steady properties with time, this must apply to both the rate gradients and the histology. For histology to remain constant at the pole or tip of the growth zone, the cell(s) must have equal partitioning and extension rates; otherwise, cell size would drift with time. This equality could be at a value of zero (the quiescent center?) or it could be at some finite value. Presumably a low value characterizes cell behavior at the pole of the stem apex. For simplicity, the present analysis is along a straight line. To apply it to real meristems, one considers a line on the surface which must bend to reach the pole of the meristem. In section, layers (tunica, etc.) pass through the pole, so there is no abrupt terminus to the pattern but rather a dome with no striking cellular discontinuity at the pole. Thus, a small group of cells (the promeristem) could all have equal rates (case 3 in fig. 1) which grade off either into case 2 or case 4. If the value for both gradients for a terminal region is zero, growth would commence at some distance down from the tip. DANIEL COSGROVE (unpublished) has shown that growth starts abruptly well down from the tip in the *Crassula multicava* root. Perhaps, in the corn root, extension starts at the lateral boundary of the columella of the root cap.

In brief, constancy of histology at the site of the start of growth excludes conditions 2 and 4 from being characteristic of that area. Condition 3 need be held for only a small region, of course. The actual condition at such a site has not been presented to our knowledge.

E. Experimental design and interpretation

A major implication of the present study for experimental design is that if finite times are involved, as is almost always the case, the region under study will undergo extensive relative migration to (often unknown) locations during the experiment. This can confound the utility of finite duration experiments, such as those used in cell-cycle analysis. A major implication for interpretation is that biochemical/cytological changes, when correlated with changes in cell dimensions in a meristem, do not correlate unambiguously with a single process.

Cell-cycle studies.—For a growing axis there are two distinct meanings for the duration of the cell cycle: (*a*) The duration characteristic for a certain distance from the tip (site-specific duration) is the time required to complete the cycle if the cells involved could remain at that position (or under those conditions) for a whole cycle. (*b*) The duration characteristic for a specific group of cells, as the cells move down the axis, is the cell-group-specific cycle time. The present kinetic analysis can measure both. Alternate cytological methods are available for both. For *Zea* roots there appear to be serious discrepancies among these methods, due presumably to artifacts or shortcomings in methodology.

Site-specific cycle duration.—Given the cell-partitioning rate curve, as a function of distance from the tip, the doubling time is simply (ln 2)/rate. As noted, Erickson and Sax (1956*b*) found continuous variation in rate in corn with a peak at 0.15 h^{-1} some 0.6 mm from the cap/root boundary (1.1 mm from tip), indicating a doubling time of about 5 h. Their data lump all cell types in the root and assume that all cells divide. There are two cytological methods that should give comparable data. The rate of entry into *S* phase (percentage of cells picking up labeled thymidine per unit time) is a comparable datum. To be characteristic of a point, the rate should be taken as the initial slope of this relation; otherwise the data would smooth out variations in the partitioning rate. Pertinent data are those of Clowes (1961*b*), which are tissue specific. Data were taken for a region, not an infinitesimally short segment of the root ("central stele just above quiescent centre"), and sizable finite times of labeling had to be used. Over the period 4 to 8 h after labeling, labeled cells increased by 22%, indicating a cycle time of about 18 h. Back extrapolation of data at 4, 8, and 16 h to zero time, by parabolic fit, gives an apparent initial slope of 9.6% h, hence a cycle time of 10.4 h. These data were taken at 18 C; Erickson and Sax's (1956*b*) were taken at 25 C. The higher temperature would be expected to reduce the duration to about 7 h, assuming the effect is comparable to that in *Allium* (Barlow 1973). This tenuous route for comparison appears to be the only one available; it indicates order of magnitude compatibility of the two methods.

A second method measures the initial rate of accumulation of metaphases in cells blocked with colchicine. Dissolution of metaphases precludes using the initial values, so data on the period 2–4 h are used. For the same region as above, the increment was only 5.3%, giving a cycle time of some 37 h by extrapolation. Using several correction factors, Clowes estimated the rate as 28 h. Both values for "fastest doubling" are so far off the kinetically calculated value that one may question the utility of the technique. This impression is strengthened by the lack of similarity in early values with the label accumulation method (above). Concurrent application of these cytological techniques with estimates of cycle time from kinetic data would be useful. These could be made tissue specific by using cell-length-pattern data in combination with mark-movement data. The freedom from chemical or radiation artifacts and the opportunity to prove that the axis is growing in a steady-state pattern (streak photograph) are special merits of the kinetic methods.

Cell-group-specific cycle duration.—The cell-partitioning curve data, plus the time/position relation, allow calculation of such cycle times (fig. 5). The cells in question must be initially identified at some transverse "plane," or thin segment. As they go through one cell cycle, the thin segment will not only expand but will be displaced further from the tip. Since cell-partitioning values vary continuously and the time/position curve is not linear, choice of the location of the initial plane of cells will have a great bearing on the cycle duration.

To find the cell doubling time for a short segment of tissue, initially at a known distance from the tip, one uses the relation (eq. [4]) that a doubling of a process of rate r is accomplished when $r \cdot t = \ln 2$. This is conceived as a constant area generated by a small r and a large t, the opposite, or any set of combinations applied over successive intervals, to give ln 2. The appropriate area must be generated on a curve of partitioning rate versus time. Thus the typical curve versus distance (X) (fig. 4 *I*, *d*) must be replotted as in figure 5*C*. One converts the initial position relative to X to initial time, t_1. Area on this plot is dimensionless, so when an area equal to 0.69 has been generated by increasing time to t_2, the partition (cell) number will have doubled; t_d is $t_2 - t_1$. The final location of the initial segment is readily deduced by finding the corresponding X_2 from t_2 on the intermediate graph (see fig. 5).

It is inappropriate to plot cell-group cycle durations as points along a distance axis, even if the distance from the tip is the initial position of the cells, because the durations obtained relate to the whole distance traversed by the cells during their cycle. Hence, a bar entry is used in figure 5*A*.

Cell-group cycle durations are, however, some-times plotted as point values (CLOWES 1975, fig. 2) on a distance axis. A sample calculation of the appropriate width of a bar-graph entry can be made using the time/distance data of ERICKSON and SAX (1956*a*, fig. 8). A group of cells initially 1.1 mm from the tip (600 μm from the cap/root line, using fig. 1 in ERICKSON and GODDARD 1951) would be displaced to a region about 3 mm from the tip during the 15–20 h indicated as the cell-group cycle duration (label, CLOWES 1975). This position is near the proximal meristem boundary in corn, making the bar span more than half of the meristem.

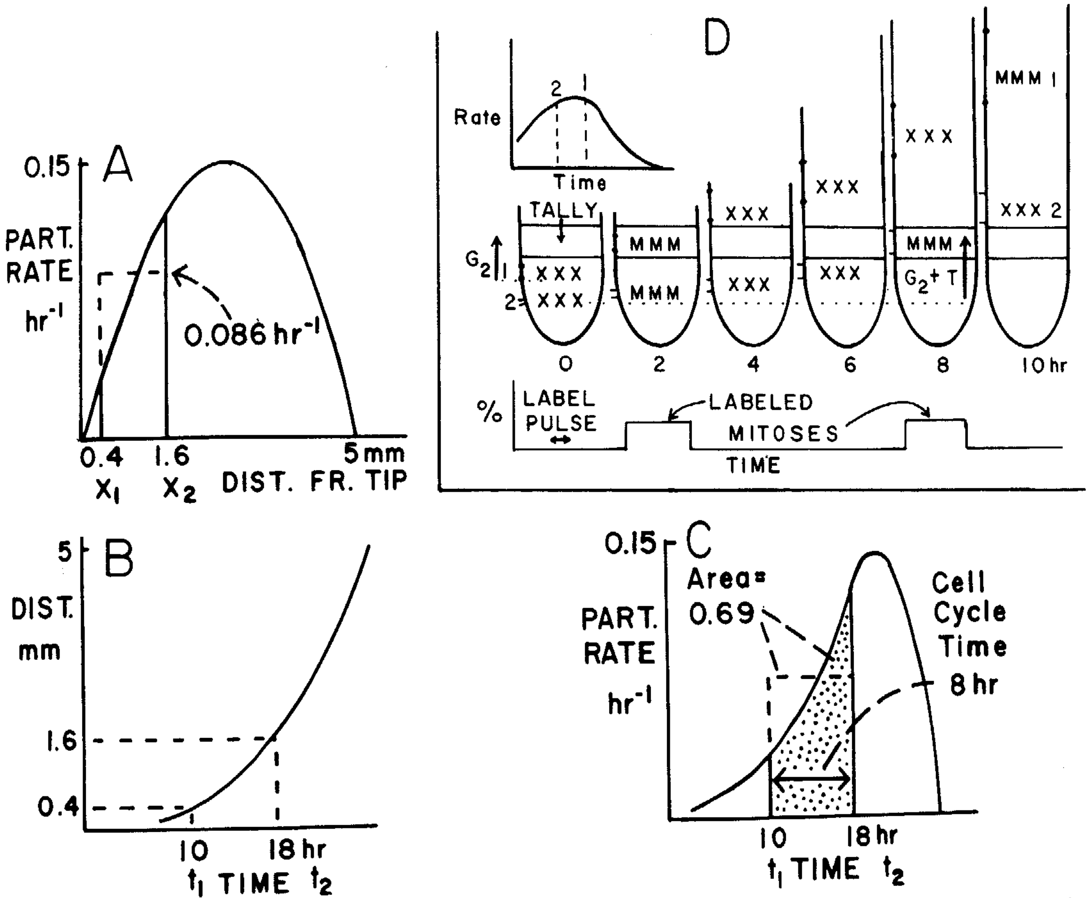

FIG. 5.—Given the curve of partition rate versus distance from the tip, *A*, and the curve of distance from the tip versus time, *B*, the time required for doubling the partition (cell) number in a thin transverse section of the axis can be determined. This time will be a measure of cell-cycle duration for the cell group in the thin section. The initial position of the section, X_1, is chosen here to be 0.4 mm. From curve *B*, the corresponding time ($t_1 = 10$ h) is determined. After plotting curve *C* (partition rate vs. time) by correlating the information contained in curves *A* and *B*, the doubling time is calculated as follows: From equation (4) the condition for doubling requires an area of 0.69 to be swept out under a curve relating partitioning to time. Starting at t_1 on curve *C* and sweeping out an area equal to 0.69 takes one to t_2 (18 h), giving a cycle duration of 8 h. Using curve *B*, it is possible to find where the cells are after they have doubled ($X_2 = 1.6$ mm from the tip). If one knew the cell-cycle time (e.g., from labeled mitosis studies) and the time course of cell movement (curve *B*), one could reconstruct the original partitioning function (curve *A*). However, even if cell movement were correctly monitored, the data from such finite-time experiments would be broad bar graphs of low resolution (dotted lines in *A* and *C*) whose height is the mean partitioning rate over the interval in question (time or distance). Cell-group specific-cycle duration obviously varies depending on the choice of initial position (starting at 1.6 mm, cycle time would be much shorter). *D*, Interpretation of labeled mitosis studies. If one marks specific populations of cells (e.g., dot pair [1] and bar pair [2]), two peaks of labeled mitoses (*M*) will appear for each group. Their temporal separation of the peaks is exactly the cell-group specific cell-cycle time, 8 and 6 h, respectively. If, on the other hand, labeled mitoses are tallied as in the graph below at a fixed distance from the tip (tally zone), two peaks of labeled mitoses are seen, one from each of the two populations. The diagram assumes mitosis is brief relative to *S*, hence the square-wave character of the percentage of labeled mitoses curve. The time before the first peak is G_2 for cells initially at position 1. The time before the second peak is total cell-cycle time for cells initially at position 2, plus G_2 for cells initially at position 1. The interval between peaks is thus the cycle time for cells initially at position 2 as they move to position 1. The time data alone, however, give no information as to the location or length of the district on the axis to which the cycle length pertains. It is certainly distal to the tally zone and separated from it by the (unknown) distance required for cells to pass through G_2 just distal to the tally zone. Inset shows that cell group 1 will be following lower partition rates, with time, than group 2; and hence its longer cycle time. Growth (division) fraction is assumed to be 1.0. If *M* is long and the duration of the wave of labeled mitoses is $S + M$, the *M* in question is that characteristic of the tallied zone. The successive *S* periods, however, are characteristic of regions 1 and 2, respectively. Thus three different positions are involved in the apparent cycle seen at a fixed tally zone. This analysis was contributed by Mr. KEITH BAUER (Stanford undergraduate).

The concept of the cell-group-specific cycle duration and the theory of the labeled-mitosis method require that a specific population of moving cells be followed during cell-cycle measurement. The second wave of labeled mitoses must be generated by the progeny of the cells of the first wave. Figure 5*D* shows the inevitable relative migration during one cell cycle (interval between two waves of labeled mitoses). Valid application of the labeled mitosis method would use many comparable marked roots and score labeled mitoses periodically within an identifiable moving district (fig. 5*D*). The results could be presented as a bar graph of time versus distance.

It appears to be the practice, however (e.g., CLOWES 1975), to tally periodically at specific fixed distances from the tip. The two waves of labeled mitoses do not have the parent/progeny relation, and the meaning of the cell-cycle duration so derived is not obvious (fig. 5*D*). The fact that cell movement is relatively slow near the tip of the axis does not mollify the situation, because cell-cycle duration (site specific) can change very rapidly with position there (in corn). Enlarging the tally area to the point where it would include some cells of both populations avails little since the method assumes a single population. There is no avoiding the cell tracking if suitable data are to be obtained (e.g., see Discussion for analysis of the growth fraction).

USE OF CELL DIMENSIONS.—Most biochemical/cytological studies on axes express data in terms of distance from the tip. This allows proper cross-reference to kinetic data, such as the partitioning or surface-extension function. Occasionally correlation is made also to changing dimensions of cells within the meristem, where both partitioning and surface extension occur. If the reference is to cell length (e.g., JENSEN 1961), this is, of course, a function of both processes and hence is not an ideal datum. By the same token, reference to a stage of radial enlargement (SCOTT et al. 1956),implying that it is a regionally dominant process, suffers from the fact that its apparent large magnitude is enhanced by the maintenance of a short longitudinal dimension due to joint action of partitioning and surface extension. Quite possibly the surface-extension rate in the longitudinal direction is just as great, if not greater, than that in the radial direction in a "region of radial enlargement."

It is the purpose of this section to point out that inferences as to processes, based solely on cell dimensions in a meristem, should recognize that cell-proportion changes have a dual basis and therefore do not offer the ideal qualities of a frame of reference.

Discussion

The elemental analysis redescribed here is both precise and flexible; it can account for any consistent periodicity (pattern of cell lengths) down a line in terms of two superimposable processes—extension and partitioning. Once these two functions are known, much additional information, such as both senses of cell-cycle duration, flow of cells or volume (length) past a given point, etc., can be derived by integrating these functions appropriately. Because it presupposes nothing biological, the elemental analysis is not a typical model but rather a neutral mode of description. As such, it can be used to portray various views about axis generation, many of which are indeed models because they employ some simplifying assumptions. The assumptions usually facilitate certain calculations, such as that for the flow of cells past a transverse plane in the axis. They do so at the expense of either averaging out a varying function over a sizable region of the axis or assuming that a certain relation, such as that between distance and cumulative cell number, is linear when it may not necessarily be linear. From the published results of GOODWIN, ERICKSON, LIST, HEJNOWICZ, and others, it appears that little simplification can be made with impunity, and the full flexibility of the method must be utilized. The sole proportionality that is safely assumed is that cumulative cell number is roughly proportional to time in the extension-only zone (this simplification is used in the Appendix).

A progression of models and descriptions for axis growth, partly taken from the literature, is given in figure 6. As the examples become more realistic, (*a*) there is a reduction in assumptions, (*b*) step functions become continuous, (*c*) the versatility of the abscissa decreases (distance from the tip does not remain proportional to cumulative cell number, etc.), and (*d*) the extension rate/partition rate ratio departs from unity in the meristem. In all models the flow of cells out of the meristem (partitioning rate integrated over cumulative cell number) and the flow of volume or length out of the whole growth zone (extension rate integrated over distance from the tip) are accounted for. In all the models, which view the axis as a string of cells, all cells save the most apical one are eventually swept out of the meristem by the progeny of the most apical cell.

MERISTEM DOUBLING MODEL (FIG. 6*a*).—This is the most direct model possible (starting assumptions for LÓPEZ-SÁEZ et al. 1975). It assumes that the meristem consists of a fixed number of cells, say 100, at the start of a growth cycle. These cells then go through one cell cycle, say of 1 day's duration, raising the meristem number to 200. Half are then converted to the nondividing state, to commence increase in cell length. This would add 100 cells/day to the mature zone of the root. An unrealistic assumption of this model is that transition from the dividing to the nondividing state is made discontinuously in time. In a meristem there is continuous flow of cells across the boundary to the nondividing zone. The distance from this boundary to the axis tip, in terms of cells, does not vary by a factor of two.

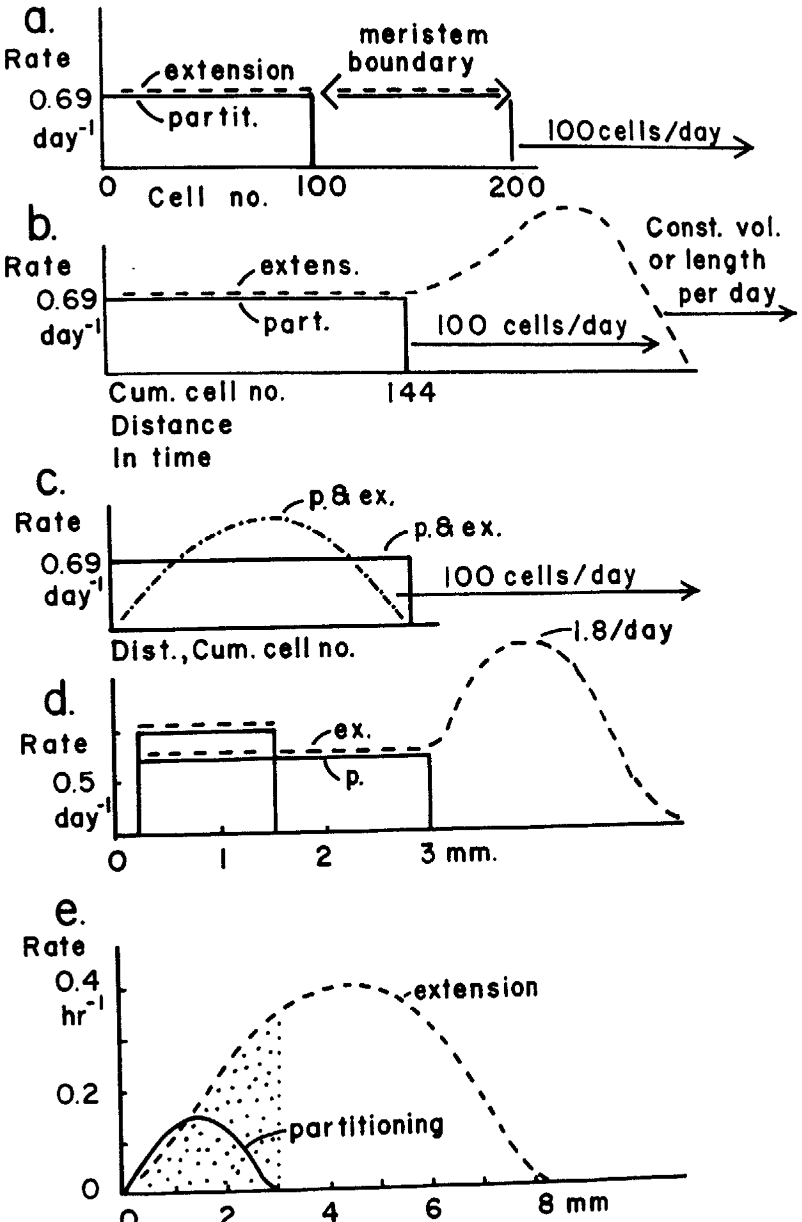

FIG. 6.—Models and descriptions of axis growth zones in common format (ERICKSON and SAX 1956*b*) with the surface extension (dashed line, *ex*), and cell partitioning (solid line, *p*) as the ordinate. Successive diagrams have fewer presuppositions as to the nature of axis development and are regarded as more realistic. *a*, Discontinuous "doubling" model. Derived directly from cell-colony growth, where meristem cell number doubles (daily) and there is a periodic (daily) loss of half the maximal cell number to the extension-only zone. This concept is presented in the model of LÓPEZ-SÁEZ et al. (1975). In terms of figure 1, the sole cell process in the meristem is constant cell duplication (case 3′). *b*, As in *a* but converted to allow an equivalent but continuous transfer of cells to the extension-only zone and to allow a fixed size and cell number (144) to the meristem. The sole process in the meristem remains constant cell duplication; a zone of extension only is added (dashed line only). The meristem boundary is fixed but abrupt. Abscissa in the meristem can be cell number, distance from tip, or ln time. *c*, Diagram to illustrate the transition from a constant cell duplication (case 3′) type meristem to a "variable" cell duplication (case 3) meristem. Since cell flow from the meristem is the area under the partitioning curve, when plotted against cumulative cell number, this flow is the same in both cases. The abscissa is no longer proportional to ln time in the meristem. Because cells are duplicated, however, distance from the tip is still proportional to cumulative cell number. *d*, Portrayal of the data of GRAY and SCHOLES (1951) showing some heterogeneity of partitioning rate in the meristem of *Vicia*. Cell-cycle times were estimated over two overlapping districts. The extension function was not measured, but cell duplication was assumed (hence the extension and partitioning functions are shown as identical in the meristem). Values for extension can be calculated for the extension-only zone (see Appendix), as position was not explicit in the data. *e*, Data of the type of ERICKSON and SAX (1956*b*) using *Zea*. There are major differences from *d*. Cell duplication is neither assumed nor found; the extension and partitioning functions are independently evaluated. Further, both functions are measured as continuous variables rather than as step functions. These advances allow mean cell length to vary in the meristem and allow the meristem boundary to be gradual. The dotted area shows that part of the axis's overall elongation rate (total area under the extension curve) which is produced in the meristematic region (about 35%).

In the present notation, this model assumes constant cell duplication and periodic harvesting to the extension-only zone. The abscissa could be distance, cumulative cell number, or ln time. Because mean cell size is constant, the extension rate (dotted line) must be equal to the partitioning rate (solid line).

CONSTANT MERISTEM BOUNDARY MODELS (FIG. 6*b*, *c*).—To convert the above model to a more realistic one with a meristem of constant size and continuous flow of cells out from it, one can arrange to have the same output (100 cells per day) from a constant population. The cell flow is described by

$$dN/dt = N \cdot r\,, \qquad (9)$$

where N is cell number and r is division rate constant. If the cells divide once daily, r is 0.69 day^{-1} and N is 144 cells. This is diagrammed in figure 6*b*. The flow out in this case is a simple product. An "elongation" (extension-only) zone is added under the dashed line. In this model the distance, cumulative cell number, and ln time axis are still proportional (and could serve as abscissa) in the meristem. If, however, the cell-cycle duration is allowed to vary with position (as in fig. 6*c*, dot-dash line) while mean cell length remains invariant, only the distance and cumulative cell number axes are proportional to each other. For the first time in this sequence, the two senses of the cell cycle must be recognized. The site-specific duration for the region at the maximum of the dot-dash curve is 1.1 day^{-1}, a tripling per day, while the effective cycle time for the whole meristem (solid line) is a doubling per day. Since the area under the two curves is the same, the flux of cells outward is the same (100 cells per day). The dot-dash line model shows a continuous gradient in cell-duplication rate. The partitioning and extension rates are equal throughout (mean cell size is invariant).

MODEL OF GRAY AND SCHOLES (1951) (FIG. 6*d*).—In this oft-quoted paper, heterogeneity of cell-cycle duration within the meristem was recognized and roughly measured. Extension rates were evaluated in the extension-only zone. Mean cell-cycle times were calculated for both the whole meristem (to a distance of 3 mm from the tip) and for its apical portion (to 1.9 mm). This means that the abscissa can be no simple (logarithmic) function of time. It was assumed that mean cell length did not vary in the meristem, so partitioning and extension curves are presented as identical there. In such a model, cumulative cell number and distance from the tip are proportional within the meristem, but not beyond it. Note that while heterogeneity of cycle duration is recognized, large regions of the meristem are averaged; resolution is quite limited. Within the extension-only zone, rates of increase in cell length were measured, taking advantage of the fact that cumulative cell number and time are indeed proportional in this region. Relative rates of surface extension, in the sense of this article, were not presented as a function of distance from the tip. This model, more realistic than those above, is described as nonconstant cell duplication followed by nonconstant surface extension. It does, however, assume constant mean cell length in the meristem.

THE DESCRIPTION OF CORN ROOT GROWTH BY ERICKSON AND SAX (1956*a*, 1956*b*) (FIG. 6*e*).—Figure 6*e* reveals that none of the simplifying assumptions employed above are valid in the corn root. Neither the partitioning nor extension function is flat (or a step function), nor are they identical except at one point. It follows that mean cell length is not constant. Hence, distance and cumulative cell number are not proportional on the abscissa in either the meristem or the rest of the growth zone. Nevertheless, as presented in the body of this paper, the major quantitative questions can be answered. Questions of volume flow, cell number flow, and cell-group-specific cycle duration can be answered when the proper function (extension or partitioning) is plotted against the proper abscissa (distance, cumulative cell number, or time, respectively) and the integration done. It is a major contribution of the elemental analysis that this necessary interconversion of the abscissa can be done by valid manipulation of data rather than by assumption, as in most models.

GROWTH IN THE MERISTEM.—Models often do not directly address the question of the rate of production of length in the meristem, the main interest being the production of cells (GRAY and SCHOLES 1951; LÓPEZ-SÁEZ et al. 1975). Since cell duplication is assumed, the extension rate is inferred to be equal to the partitioning rate (fig. 6*a*–*d*). In some treatments (e.g., GRAY and SCHOLES 1951) the production of length is discussed as if it were exclusively carried out in the "elongation zone" (zone of extension only); at least no analysis of extension in the meristem is made. The length production in the meristem itself can be readily estimated from a consideration of that part of the extension-rate curve which lies above the partitioning curve. The area under this portion of the extension curve (dotted in fig. 6*e*), compared to the total area under the curve, gives the fraction of the total growth rate that can be ascribed to the dividing region or meristem. In corn, this comes to about 35% (calculation from ERICKSON and SAX [1956*a*, 1956*b*] given in fig. 6*e*). This means that a root advancing at 1 mm h^{-1} would, if deprived of its meristem, proceed at 0.65 mm h^{-1} in the next instant.

THE GROWTH FRACTION.—This term, adopted from animal cell cultures, refers, in reality, to the "proliferation fraction," because it is not concerned with volume production but rather with the issue of what proportion of the cells are contributing to the increase in cell number. This fraction would remain constant in a system only if a constant fraction of the new cells would cease to divide. If a fixed number of cells does not divide while the remainder do divide, of course the growth fraction will rise with time. The concept is elusive in meristems because, if a cell drops out of the cycle "temporarily," this is functionally identical with having an inordinately long cycle stage (e.g., G_1, G_2) during the period when the cell was "out of the cycle."

The division fraction is estimated by comparing, in effect, the doubling time of a population (say it is 20 h) with the cell-cycle duration (say it is 16 h) of that part of the population which is dividing. The latter class is detected by the labeled mitosis method. If the doubling time is longer than the cycle time, it is concluded that one subpopulation is producing all the new cells while another one produces none. The doubling time is deduced from the rate at which the percentage of cells in mitosis accumulates in colchicine or from the rate at which the percentage of labeled cells accumulates with continuous label. These data are variable, however, and do not agree well with each other (see CLOWES 1961*b*, 1971, 1975).

The present elemental analysis methods can be applied to this issue. The doubling time for a dividing population can be calculated as in figure 5. A labeled mitosis study could be done on such a population of cells as they moved down the axis. If the cycle time were shorter than the doubling time, the growth fraction would be less than one. The kinetic methods give doubling time free from chemical or radiation artifact.

The present techniques also offer an obvious anatomical check on whether certain cells in a population drop out of the cell cycle. This has been strongly suggested for cortical cells of bean (RASCH, RASCH, and WOODARD 1967), on the basis of failure to get 100% labeling after several days of continuous label treatment. Short files, or single cells, are found to lack label in the nucleus. Clearly, these cells did not have a functional S period, while their neighbors did. But did the neighbors divide several times while the unlabeled cells did not? If so, they should be $1/2^n$ the length of the unlabeled cells, with n being the number of extra divisions (partitionings). No size difference is evident in their figures, and it can be concluded that label uptake is not a reliable measure of division. In longitudinal sections of normal meristems one would expect to see isolated cells of exceptional length (2^n longer than their neighbors) if occasional cells missed n cell cycles relative to their neighbors. This relation is independent of the amount of extension involved. It appears not to have been employed to check claims that the growth fraction is less than one.

In summary, the elemental method for description of the developmental processes offers a quantitative coupling between cell behavior and organ anatomy that is not present in other modes of study. It has limitations in that the analysis does not resolve the phases of the cell cycle, and it has not, historically, been applied often to individual tissues, although this could be done by using mean cell length data to get cumulative cell number data for a tissue. Because of the requirement for surface-extension-rate data to bring the time element fully into the analysis, the method is difficult to apply to the most apical part of the plant axis where the files are curved and where the surface is obscured by a root-cap or appendage primordia. This limitation does not apply to the label-histology approach, as used on longitudinal sections. This tactical difference does not preclude the full use of the concepts and analytical rigor of the kinetic methods which portray and evaluate the intricate, but nonetheless definable, interrelations between cell activity and organ development.

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Appendix

CALCULATION OF THE RELATIVE EXTENSION RATE IN THE EXTENSION-ONLY REGION OF AN AXIS

With the axis showing consistent growth behavior, consecutive cells down a file represent temporally equally spaced developmental stages. The time required for a cell to attain the length of its more fully grown neighbor is equal to the time taken for one cell to pass across the boundary of the growth zone as it ceases growth. This time is simply equal to the growth velocity of the axis, say 1 mm/h, divided by the mean length of the mature cells. If the latter is 40 μm, 25 cells cross the boundary per hour. It takes one cell 60/25 min, or 2.08 min, to cross. The relative extension rate for any length (L) conversion from a given cell (n) to its older neighbor ($n + 1$) is:

$$r = \frac{\ln L_{(n+1)} - \ln L_n}{(\text{growth velocity})/(\text{mature cell length})}. \quad (10)$$

Considerable variation, due to the abrupt two-fold changes in cell length brought on by the last partitionings to occur, is inevitable, but changes in mean rates should be informative. The point to be identified with this rate must be chosen somewhat arbitrarily, but distance from the tip to the cross wall between the two cells should serve. The method is not completely accurate, particularly with large cells, as it has the intrinsic drift problems shown in figure 2 (partly from ideas of BURSTRÖM 1953).

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