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GROWTH AND DIFFERENTIATION IN LIVING PLANT MERISTEMS

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Most of our knowledge of the cellular changes taking place during growth has been derived from a study of killed and sectioned material. Cell division in living plant tissues has been observed in a considerable number of cases, but chiefly in hairs, protonemata and similar structures which are only one or two cells thick. It is desirable to learn what takes place, in terms of changes in cell number, size and shape, in a typical living plant meristem as growth proceeds. Events cannot readily be observed here because most meristems are so massive as to be opaque. Furthermore, the terminal growing point of a stem is buried in surrounding structures, and that of a root is usually enclosed in a relatively opaque calyptra.

These difficulties may be overcome by the use of appropriate material and technique. Seedling roots growing from very small seeds are so delicate, often only six or eight cells thick, that light passes through them readily. In most cases, however, direct observation of the actual meristem is prevented by the root cap; but in some of the small-seeded grasses the cap is reduced to a group of only a few rather loose cells and the meristem is thus nearly naked. Species of *Agrostis*, *Phleum*, *Poa*, *Sporobolus* and related genera provide particularly favorable material.

It has been found possible to grow these very delicate roots under essentially normal conditions by germinating the seeds on strips of moist lens paper on microscopic slides, placed in covered staining dishes. About a centimeter of tap water is left in the bottom of the dish, which keeps the air humid and the paper moist. After about four days at room temperature the young roots are usually more than a centimeter long and are ready for study. The slide is now placed on the stage of a microscope and the root covered with a few drops of water. The meristem may be examined directly under a water immersion lens, but more satisfactory results have been obtained by placing a cover glass over the root, sup-

ported by glass chips on either side. The root may now be studied under any immersion objective, but the author has found water more satisfactory than oil for contact, since the latter tends to spread over the edges of the cover. Care must be used to avoid any pressure on the root when the cover glass is applied or removed, and to keep the root continually moist, for otherwise growth will stop immediately. By means of a camera lucida, drawings may be made of the cell outlines of the surface layer of cells, the second layer and sometimes the third layer. The slide should be returned to the damp chamber as soon as possible.

Drawings may be made at intervals of from one to several hours. If a marker, such as a cell of unusual shape or size, is located in the first drawing, then all the cells may be numbered with reference to it, and located again in the second and later drawings. In this way, all the new cell walls which have appeared through division in the interval may be recognized. Changes in cell size, both before and after division ceases, may also be measured. The actual cell lineage for a long series of cells from the apex of the meristem to the point where elongation has ceased may thus be determined. The nuclei are to be seen only vaguely and details of mitosis are not visible.

These roots are very sensitive to gravity and will not continue to grow normally more than a few minutes on the horizontal stage. It is more satisfactory in some respects to keep them vertical, with the microscope in a horizontal position, but this makes difficulty in the maintenance of liquid contact between lens and cover, and in the use of the camera lucida. Photographic records may be made, but unless conditions are very favorable, these have been found less satisfactory than drawings. The chief difficulty thus far encountered is the tendency for the growing tip to twist spirally and thus sometimes to prevent continuous observation of a given row of cells.

The approximate rates of cell division and of cell elongation may readily be measured. The rate of elongation of the whole root may also be accurately determined by marking the location of a given point under the camera lucida at constant time intervals. The characteristic rhythmic nutation of the root tip may be observed and its period and amplitude measured.

The method here described makes possible a fresh approach to the study of a number of other problems of growth and differentiation in a typical terminal meristem. Preliminary results in three of these problems, as studied in the genera *Poa*, *Phleum*, *Agrostis*, *Chloris* and *Sporobolus*, are presented briefly here.

1. *Factors Determining the Shape and Position of New Cell Walls.*— The cells in all layers are in regular longitudinal rows, so that the new walls, with rare exceptions, are transverse or essentially so. In equa-

tional divisions the walls are straight, but in non-equational ones, as in the divisions giving rise to root-hair initials in some genera, they are commonly curved from the first, with the convex side toward the larger cell. A new wall is never laid down directly opposite a previously formed cross wall of any neighboring cell. If the natural position for the new cross wall would tend to bring it into such a relation, this will be avoided by a bending of the new wall so that it joins the side wall at a point at one side or the other of the place where the cross wall of the neighboring cell is attached. A new cross wall tends to join the side wall halfway between the points of attachment of the two nearest cross walls, either of this cell or a neighboring cell. This is the position which would be assumed if the walls were liquid films. In many cases, however, this position would result in daughter cells very unequal in size, and in such cases the new wall is pushed considerably nearer the point of attachment of one of the cross walls. The point where the new cross wall joins the side wall thus seems to be determined by an equilibrium between the tendency to divide the mother cell equally (or into two parts of definite proportions) and the tendency to bisect the free side-wall space.

2. *"Sliding Growth."*—Since the point where each new cell wall is laid down can thus be definitely located with reference to the walls of all adjacent cells, and since this relationship can be followed throughout the history of all these cells, it is possible to determine with some certainty whether the cells slide along each other during the process of growth. This may best be studied in the relationships between the cells of the surface layer. All the evidence indicates that sliding growth does not occur. The relative positions of the transverse walls of adjacent cells are maintained essentially unchanged from the time they are laid down until the cells are many times their original size and growth stops. There is a definite gradient in cell expansion, beginning just before division ceases, and gradually increasing until maximum growth is attained. In this all the cells participate almost equally, though the root hair initials and certain stunted cells grow somewhat less rapidly than the rest. The relative position of their walls thus changes a little, but this is clearly due to a greater growth in some parts of the wall than in others and not to sliding growth. No case has been observed where the end wall of one cell has passed by the end wall of an adjacent cell during growth. The relative wall positions between the surface layer of cells and the layer immediately below have also been determined and here, too, the positions established when the cell walls are laid down persist to maturity.

3. *The Differentiation of Root Hairs.*—In *Phleum*, *Poa* and *Agrostis*, the cells which are to produce root hairs (the "trichoblasts" of Leavitt[1]) are definitely set apart at the last cell division. This is usually either the third or the fourth division from the tip of the root. At this division the

apical (distal) member of the pair of daughter cells is always smaller than the basal. It has denser protoplasmic contents and its walls tend to bulge outward slightly. At some distance back from the tip, when elongation has ceased, this cell produces a root hair. The other cell, which rarely may divide again, never produces a hair. In these genera, therefore, differentiation for this character occurs very early and the potencies of the cells are sharply limited almost from the beginning. In *Chloris* and *Sporobolus*, on the other hand, trichoblasts ordinarily do not appear, all the cells being essentially equal in size from the beginning and all being capable of producing root hairs. Some of the cells stain differently from others, and a differentiation of root hair cells may occasionally be observed before the hairs are formed. These plants evidently provide good material for a study of the factors controlling the differentiation of root hairs.

Summary.—Materials and methods are described, by the use of which it is possible to observe and measure the multiplication and growth of cells in living root meristems. This technique has been applied to the problems of the location of new cell walls, of "sliding growth," and of the differentiation of cells which are to form root hairs.

[1] R. G. Leavitt, *Proc. Boston Soc. Nat. Hist.*, **31**, 273–313 (1904).

QUANTITATIVE ANALYSIS OF THE INTERACTION OF INDIVIDUALS

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Up to the present time, no quantitative studies of human interaction have been undertaken, with the exception of observations by Dorothy Thomas and her co-workers on the behavior of children.[1] These studies were designed to develop an "index of personality" based on the percentage of total time spent by a child handling objects, interacting with people or playing alone. Only percentage figures were secured for interaction, and the authors were more interested in developing criteria for determining the reliability of observers.

This paper represents a preliminary account of quantitative results secured through the use of a crude recording apparatus, now being superseded by an accurate instrument. Although the investigation was primarily exploratory, it is believed that a discussion of the results obtained by use of the old instrument will be of interest as an indication of the