

Physiological bases of organization and development in the root*.

By

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With 9 figures.

I. Introduction. Terminology.

The organization and development of roots have been well described in morphological and anatomical terms by many authors (v. GUTTENBERG 1940, 1941, ESAU 1943b, 1953a, 1960, FOSTER 1949) and recent concepts and terminologies with respect to root development have been reviewed and consolidated by ESAU (1953b, 1954) and in vol. III of this encyclopedia¹. It is not the purpose of the present chapter to repeat such information. Rather, its purpose is to bring together physiological and biochemical information which bears upon the broad subject of root development and to analyze root organization and development in terms of the physiological and biochemical processes upon which they depend. Our present state of knowledge of the intimate machinery of the cell which, through its intricate and complex workings, controls and directs the course of cellular development, is entirely inadequate to give any clear or definitive picture of the physiological basis of cell organization, let alone the further complexities of such an organized tissue system as the elongating root. However, at our present stage, sufficient information has accumulated to warrant an examination of the subject and even to outline the broad areas in which we may expect to make progress in the immediate future.

Of the various organs of the plant, the developing root has been selected most frequently for experimental studies of development. The reasons for this are many.

(1) The root is an axial structure, uncomplicated in its terminal development by the formation of lateral appendages which, unlike the shoot, are usually formed along the maturing region of the root axis as incidental branches of an axial system.

(2) The elongating root is frequently considered as a tissue system in which progressive stages of differentiation are aligned in linear sequence from tip to base. Such a sequence is readily subject to analysis and easily manipulated.

(3) The root is a heterotrophic structure under usual conditions. Lacking the photosynthetic pigments and growing in the dark, the root depends for its energy supply upon carbohydrates supplied either from the shoot system or — under experimental conditions — from some external source. Thus, complications of photosynthesis may be excluded from studies of the developmental processes.

* The following abbreviations have been used in this article: DNA, deoxyribosenucleic acid; IAA, 3-indoleacetic acid; NAA, 1-naphthaleneacetic acid; RNA, ribosenucleic acid; TIBA, 2,3,5-triiodobenzoic acid; 2,4-D, 2,4-dichlorophenoxyacetic acid.

¹ Chapter "Histology and development of the root" by C. R. STOCKING, pp. 173—187.

(4) The primary radicles of germinating seeds or the primary root tips of seedlings are readily available in quantity for chemical analyses. Reproducible data from parallel analyses of a number of roots can be secured with some assurance of reliability.

(5) Usually, excised root tips or root segments can be readily grown in sterile nutrient culture in a known synthetic medium such that one can state the minimum nutrient requirements of the root system *in vitro* and relate it to the normal picture of development in the whole plant.

(6) Within the differentiating root is formed a wide variety of cell types including most of the typical primary tissues found in the shoot, and elsewhere in the plant. The sequence of differentiation of individual elements of a tissue is essentially the same in the root as in the shoot. Thus, to some extent at least, information gained from differentiation in roots may be applicable to other tissue systems of the plant.

Although it is not the primary purpose at this time to discuss the anatomy of the root *per se*, it is appropriate that a statement be made at the outset of the terminology to be used in our discussion. Since there exists no complete agreement among plant anatomists as to the terms which should be applied to various structures in the root, it is necessary to choose a series of terms which seems generally understood and useful, admitting that the choice is an arbitrary one.

Table 1. *Anatomical terminology of roots.* The anatomical terminology used in this article in discussing root organization and development is presented in columns 2 and 3. The apical meristem gives rise to the root cap and the primary meristematic tissues; the latter tissues in turn differentiate into the primary tissues of the root. Approximately equivalent terms are also given: in column 1 the histogens equivalent to the primary meristematic tissues, and in column 4 other terms equivalent to those given for the primary tissues. The table is not all-inclusive.

Histogens ²	Terminology used in this article ¹		Other terms in use
	<i>Primary meristematic tissues</i>	<i>Primary tissue</i>	
1	2	3	4
<i>Dermatogen</i> ² . . .	<i>Protoderm</i> —→	<i>Epidermis</i>	<i>Rhizodermis</i> ⁵
<i>Periblem</i> ²	<i>Ground meristem</i> →	<i>Cortex</i> , consisting of: a) <i>exodermis</i> , <i>hypodermis</i> (if present) b) <i>cortical parenchyma</i> c) <i>endodermis</i>	<i>Plerome sheath</i>
<i>Plerome</i> ² , <i>Pericambium</i> ³ . .	<i>Procambium</i> —→	<i>Vascular cylinder</i> , consisting of: a) <i>pericycle</i> b) <i>primary xylem</i> c) <i>primary phloem</i>	<i>Stele</i>
<i>Calyptragen</i> ⁴ . . .	↑ <i>Apical meristem</i> (Root cap initials if present) ↓ <i>Root cap</i>		<i>Promeristem or Generative meristem</i> <i>Calyptra</i> or <i>Columella</i> ⁶ and root cap cells

¹ ESAU (1943b, 1953a) gives a detailed account of the origin and use of these terms;

² HANSTEIN (1868); ³ DE BARY (1877), V. GUTTENBERG (1940); ⁴ JANCZEWSKI (1874); ⁵ V. GUTTENBERG (1940); ⁶ NĚMEC (1901), V. GUTTENBERG (1940).

To help clarify my own usage of terms and to assist those who are not themselves deeply involved in anatomical terminology, I have brought together what seem to be more or less equivalent terms which may be found in the literature and

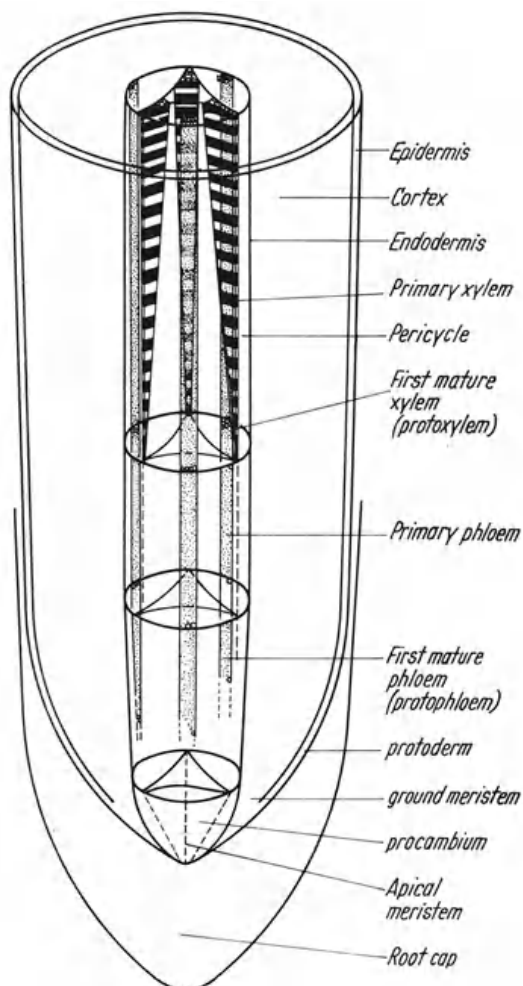


Fig. 1. Diagram of longitudinal view of root tip showing the root cap, the region of the apical meristem, the primary meristematic tissues and the primary tissues into which they differentiate. Although the relative positions of the differentiating tissues occur as illustrated, the actual distances have been modified for convenience in illustration. For details, see text. (From TORREY 1953.)

equated them in tabular arrangement to the terms which I shall use in this review.

In Table 1 are presented the terms which will be used, together with equivalent or approximately equivalent terms used in the literature. The present terminology is essentially that used by ESAU (1953a), FOSTER (1949; 1956, table 123), STOCKING¹ and others. A rather precise statement of the essential characteristics of the main tissue and cell types has been made by FOSTER (1956). Fig. 1 is a diagrammatic representation, in longitudinal section, of the terminal portion of a root, showing the root cap, the apical meristem, the primary meristematic tissues which originate from the apical meristem, and the primary tissues which differentiate from the respective primary meristematic tissues. Although the relative sequence of tissue differentiation in the acropetal direction is illustrated here, the actual distances between the different stages of tissue differentiation have been much compressed for convenience in illustration. Thus, while mature protophloem elements may first differentiate between 200—800 μ from the apical meristem region, the first mature protoxylem elements usually occur much further behind the apical meristem, varying widely with the species of plant and the rate of root elongation. First mature xylem elements are reported to occur within half a millimeter (500 μ) of the apical meristem in some roots or as far

away from the apical meristem as 10 millimeters in others (HEIMSCH 1951; STOCKING in vol. III of this encyclopedia, see above).

II. General features of root organization and development.

The root originates early in embryogeny with the differentiation at the suspensor end of the embryo axis of a root apical meristem, usually covered by root cap cells. With elongation of the embryo axis during seed development, the root apical meristem remains in a subterminal position below the root cap. In the mature embryo, the embryonic radicle-hypocotyl axis is terminated by a more or less complete root cap and the central procambial cylinder of the hypocotyl is continuous with that of the radicle, extending into the region of the apical initials.

¹ Vol. III of this encyclopedia, see above.

The apical initial region of the embryo includes a group of cells which is, itself, relatively inactive in cell division (STERLING 1955) but at whose boundaries cell divisions occur relatively frequently, producing radiating rows of cells which differentiate into the primary meristematic tissues of the root. In the embryonic root the future vascular tissue arrangement is usually already blocked out in the procambial cylinder (MILLER and WETMORE 1945).

Seed germination is first dependent upon imbibition of water by the dry seed. Then follows an activation phase during which the various metabolic activities of the embryo are triggered, preparatory to the start of seedling growth. Many changes in the embryo radicle must be presumed to have occurred during the stages of germination immediately following the imbibition of water, such as the activation of enzyme complements within the cells, changes in nucleic acids in the nuclei of the radicle cells, the mobilization within the root of energy-supplying materials, the synthesis of new materials—all preparing the radicle for subsequent elongation and differentiation. Although scattered observations have been recorded concerning these early changes, a precise picture of such changes with respect to the developing radicle itself has not been achieved. This area of knowledge remains for future investigation.

With the elongation of the radicle, seed germination passes over into the normal processes of root growth and development. The transition involves many anatomical as well as physiological changes. REEVE (1948), for example, has described the contrasting structures of the apical meristem of the embryonic pea radicle and the subsequently developed meristem of the pea seedling root. There is good evidence that apical meristem organization may change during the ontogeny of the plant and that no fixed pattern is universally applicable within a given species.

Development of the root system typically proceeds through three distinct phases (Fig. 2): (1) The elongation of the main root axis, which results from the interaction and integration of the processes of cell division and cell enlargement and is accompanied by cell maturation; (2) the initiation of branch roots from

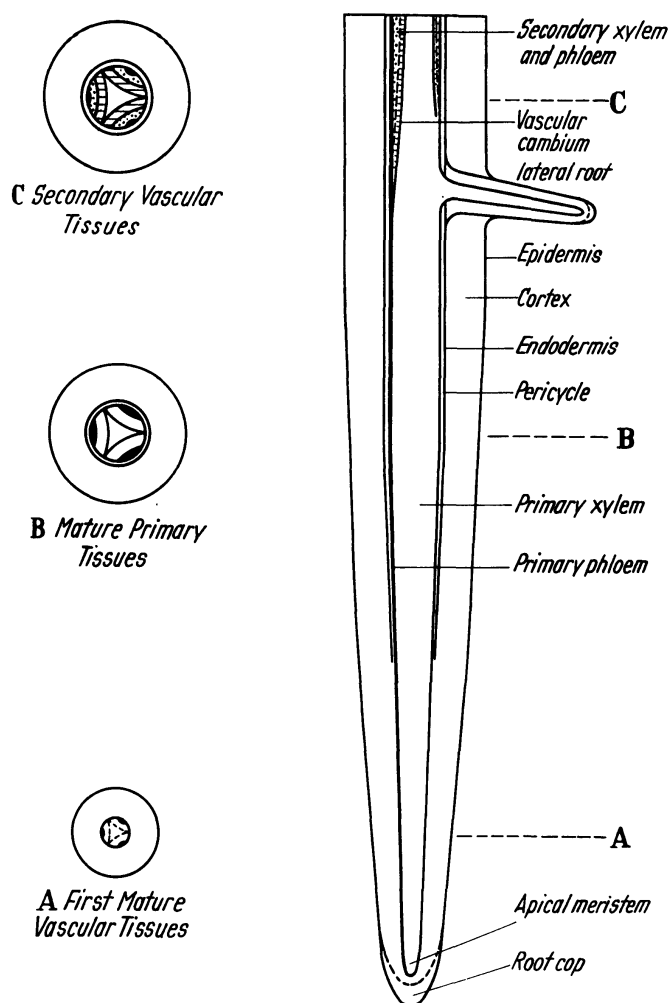


Fig. 2. A diagrammatic representation of the organization and development of a root as seen in longitudinal and transverse sections at different levels. The major features of root organization and development are shown: the region of the apical meristem, the differentiation of primary tissues, the formation of lateral roots from the pericycle and the production of secondary vascular tissues from a vascular cambium. (From TORREY 1959a.)

the primary root axis, each branch root in turn being capable of elongation and further branching; (3) the increase in thickness of the root axis through the formation of secondary tissues from a vascular cambium. Of these three main features of root development, the greatest interest centers about the first which we can refer to as the development of the primary tissues of the root. It is about this phase that we have the greatest information. No less interesting is the process whereby roots initiate roots, centering first around the process of cell division in the pericycle, then the subsequent organization of a new meristem within the mother root tissues, and finally the emergence of the branch root from the main axis. Here our facts are less abundant. Also of great interest and importance, both theoretical and practical, is the problem of vascular cambial activity in roots and its physiological control.

III. The organization and development of the primary tissues of the root.

1. The organization of the root apex.

a) Developmental histology of the apical meristem of the root.

Descriptive studies.

The concept of the apical meristem. The apical meristem of the root usually designates the actively dividing region at the root tip which includes and immediately surrounds the apical initials, a group of poorly defined cells which are conceived to give rise more or less directly to the primary meristematic tissues of the root. The meristem in its broad sense thus includes the initiating cells, which perpetuate themselves and remain active in cell division, and their derivatives, which undergo specialization through cellular differentiation. The concept of the apical meristem (or root promeristem) arises from consideration of the more or less sharply defined tissue organization to be observed in histological section of the developing root tip. Numerous attempts to achieve precise description and definition of initial regions or zones based on the interpretation of histogenic relationships observable in fixed and prepared materials have given rise to a variety of conflicting terminologies and interpretations. Many investigators refer back to JANCZEWSKI (1874) who described some five or more types of organization of the apical region of roots which he believed could be found in the roots of the vascular plants. No common agreement seems to have been reached even among investigators who study the root apices of the same species of plant (see POPHAM 1955a). Evidence to be discussed later suggests that the organization of the apical region changes during the ontogeny of the plant and during changing environmental conditions surrounding the root during development. Comments by POPHAM (1960) with respect to variability in shoot apices probably apply about equally to root apices. The basic assumption in descriptive analyses of root meristem organization is that one should be able, by careful and detailed study of roots sectioned at various times during development, to discover the ultimate sites of origin of the various primary tissues of the root comprised of distinct cell types. Some of the difficulties and restrictions of this concept with respect to the root have been interestingly discussed by HEJNOWICZ (1955).

Theories on the organization of the apical meristem of roots. The ultimate sites of origin of the primary root tissues, lineal descendants of embryonic cells, must perpetuate themselves in the developing root in order for the continued formation of the characteristic root structures observed during root development. Analyses have been based on the observed incidence and orientation of mitotic figures, the

arrangement and relationships of cell walls, the sequences and tiering of cell rows, divergences in cell lineages and patterns of cellular differentiation and maturation. From such observations have evolved the rather formalized concepts of root meristem organization implicit in such studies as those by JANCZEWSKI (1874). Two general theories concerning apical meristem organization of the root have developed from such studies. According to one view (see especially v. GUTTENBERG 1947), the apical initials comprise a very small number or, frequently, only one cell from which can be traced all the tissues of the primary root body. Unquestioned examples of this type of development are to be seen in the activity of the apical cells of certain pteridophytes. Other investigators conceive of the apical initials as a group of many cells with different arrangements and orientations in different species. The observation that different primary meristematic tissues of the root seemed to originate from separate initials led HANSTEIN (1868) to formulate the concept of histogens, *i.e.*, that different sets of initials give rise to distinct tissue systems of the root. Thus the dermatogen consisted of a single layer of cells which gave rise to the epidermis, the periblem was a generative layer within the dermatogen-producing cells of the cortex and the plerome was designated as the innermost layer giving rise to the tissues of the central cylinder. Distinct histogens are most clearly evident in the grasses. Upon this concept was based the rather elaborate classification of root apical meristems proposed by JANCZEWSKI (1874).

The number of initial cells and the quiescent zone.

Number of initial cells. In recent years, certain of the limitations of this approach to the organization of the root apex have been pointed out and alternate approaches to an understanding have been suggested, and in some cases, tested.

In an attempt to determine the nature of the apical meristem in *Crepis capillaris* and *Vicia faba* BRUMFIELD (1943) analyzed the distribution during root development of cells with distinctive chromosomal rearrangements following X-ray treatment. From the sectorial chimeras which were observed, BRUMFIELD concluded that the whole root developed from perhaps as few as three cells and that the histogen concept of HANSTEIN could not apply.

CLOWES (1953, 1954) has criticized the conclusion reached by BRUMFIELD. His criticisms are based upon studies of cell lineages in regenerating root tips after simple surgical operations had been performed on the tips of seedling roots of *Vicia faba* and *Fagus sylvatica* (1953) and *Zea* and *Triticum* (1954). Small sectors of tissue were removed at various levels from the apices of elongating roots. After a period of further root development, the roots were fixed and sectioned. Arrangement of cell complexes in the regenerated regions of the meristem and tissues derived therefrom led CLOWES to conclude that in all these plants the cytogenenerative center from which all future tissues of the root are derived is multicellular in nature with the initials grouped in such a way as to be interpretable in terms of a modified histogen arrangement. According to this view, then, many cells are involved in the origin of root tissues at the meristem and not just one or a few.

The use of surgical operations in experiments designed to determine the dimensions of the cytogenenerative center of the root apex—such as were used by CLOWES—are subject to criticism, since it is a well-known fact that differentiated, even fully mature, cells which normally will not undergo further division can be induced to divide by wounding of the tissue (NĚMEC 1905, D'AMATO 1948b). Thus in these experiments by CLOWES, the delimitation of the apical initials is

made particularly difficult—with inherent errors which would lead to the conclusions favored by CLOWES.

Occurrence of the quiescent zone. From anatomical considerations of normal roots, CLOWES (1954) postulated the existence in the apices of grass roots of a quiescent zone in which cells seldom or never divide. On the periphery of this inactive zone lie the actively dividing cells which represent the apical initials of the root. By feeding C^{14} -labeled adenine to intact elongating roots and observing the pattern of its incorporation in DNA, using autoradiographs of the sectioned roots, CLOWES (1956a, b) has been able to demonstrate clearly in *Zea*, *Vicia* and *Allium* the existence of a zone in the root apex where cells synthesize no DNA and

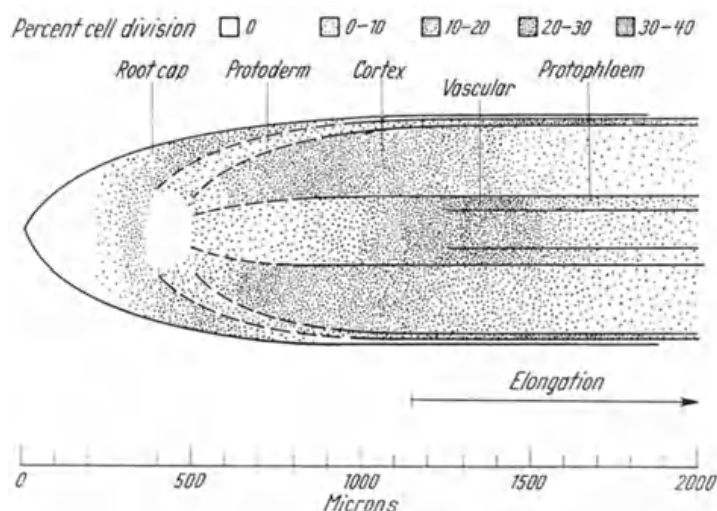


Fig. 3. Diagrammatical representation of the distribution of cell divisions in the onion root tip at 12 noon, showing the location of the quiescent zone. (From JENSEN and KAVAJIAN 1958.)

therefore may be presumed to be unable to undergo cell division (HOWARD and PELC 1951) or contribute to the construction of the root. CLOWES (1956b) visualizes the apical initials then as “cells over the whole surface of the quiescent centre (which) divide so that the inner daughter cells remain meristematic and the outer daughter cells differentiate after further divisions”. The presence of a group of cells of very low division rate has been detected also by JENSEN (1958a) and

JENSEN and KAVAJIAN (1958) in root apices of *Vicia* and *Allium* from measurements of mitotic frequency and DNA distribution (Fig. 3).

Significance of the quiescent zone. The existence of a quiescent zone in the apical region of actively growing roots is of great interest. This quiescent region may well be traced back in ontogeny to embryonic stages, such as the inactive region described in lima bean embryos by STERLING (1955), although CLOWES (1958a) has evidence from autoradiographs suggesting that the quiescent center is in fact absent in the embryo. Its presence clearly affects the nature of the apical initial region and the minimal constructional area within the root, and will influence and/or determine the cellular patterns formed at the root apex. How general is the occurrence of this quiescent zone in roots of the angiosperms remains to be seen. CLOWES (1958a) has also studied the development of the quiescent center during the initiation of lateral root meristems in *Pistia* and *Eichornia*, as well as its origin in the primary root meristem during seed germination. It will be of considerable interest to see if roots of the different histogenic types proposed by JANCZEWSKI (1874) show differences in the occurrence or shape of this inactive region. Further studies concerning changes in this quiescent region during ontogenic development and its persistence beyond seedling development will also be of great interest.

Recently, further re-examination of the problem of the number of initials in the root meristem has been carried on using irradiated roots. DAVIDSON (1959, 1960) traced cells with changed chromosome complements into lateral

roots and concluded that a much greater number of initial cells are present than suggested by BRUMFIELD (1943). Apparently the quiescent zone of non-dividing cells is less vulnerable to X irradiation and can contribute cells to a newly regenerated root meristem (see also CLOWES 1959a). The whole matter of root apical meristem organization has been reviewed by CLOWES (1959b).

Cell division activity in the root meristem.

Distribution and rates. It must not be inferred from the above discussion, however, that cell divisions at the root apex are restricted to the periphery of the quiescent zone, or to any limited apical initial region in the apical meristem. Although it is true that the terminal region of the root apex is the site of active cellular divisions, the "meristematic" region may be of considerable length along the root axis—in terms, not of just microns, but of millimeters—and cell divisions may occur in root tissues which no longer fit the classical concept (see, *e.g.*, SINNOTT 1938) of an isodiametric, densely protoplasmic, meristematic cell. In fact, most evidence shows that cell divisions are most rapid some distance basal to the base of the root cap. In *Zea*, ERICKSON and GODDARD (1951) have shown that the relative elemental rate of cell formation (cell divisions/unit time/unit of root length) is greatest about $1,000\ \mu$ (1 mm) proximal to the root cap base. In the same plant, measurements of CLOWES' illustrations (1956a) place the apical initial region about $150\ \mu$ proximal to the root cap base. Similarly in roots of *Allium*, it has been shown that cell division is infrequent in the region immediately beneath the root cap base (the region of the apical initials) and is most frequent some distance from the root cap base, depending upon the particular tissue referred to (JENSEN and KAVAJIAN 1958). Thus, it would appear that the rate of cell division in the region of the apical initials is relatively low and increases for a distance equal to several times the length of the apical initial region before it begins to decrease in rate as cell maturation begins.

HEJNOWICZ (1959) has made a study of photographic records of the growing root epidermis, combined with counts of mitoses in fixed sections of all tissues of the root. He states that the rate of cell formation per cell, exclusive of the quiescent center, is fairly constant throughout the meristem and that the duration of mitotic stages of dividing cells is also constant in the meristem.

Cell division rates in different tissues of the meristem. Rate of cell division *per se* is hardly a useful index for designating the apical meristem; on the contrary, in the apical initial region cell divisions will occur only to the extent necessary to maintain the fundamental cellular pattern of the root. Second and third generation divisions occur more frequently, proximal to the initials giving rise to the cells which produce the permanent tissues of the root in their final patterns and dimensions. Certain tissues continue to divide more rapidly than adjacent tissues, *e.g.*, the primary phloem region maintains cell divisions at a more rapid rate than primary xylem so that the complete primary xylem pattern is blocked out and fixed at a point closer to the root apex than the phloem (TORREY 1955). Similarly, in *Allium* (JENSEN and KAVAJIAN 1958) the maximum number of cell divisions occurs in the cortex before cortical cell elongation begins, whereas in the central cylinder region the maximum occurs after cell elongation has begun. HEIMSCH (1960) pointed out in cultured tomato roots the role of periclinal divisions in the inner cortex in determining root diameter. Here, more divisions occurred opposite phloem strands to give an unequal number of cortical cell layers. Thus, these differences in rates of cell division combined in a complex way with differential rates of radial enlargements and elongation give rise to

morphological regions behind the apical meristem which can be characterized histologically as the primary meristematic tissues: the protoderm, ground meristem and procambium.

b) The heterotrophic nature of the root apex.

In understanding the activity of the apical meristem and the distribution of cell divisions in time and space during root development, it is the ultimate goal to be able to understand these processes in terms of the physiological and biochemical factors which limit and determine particular patterns. As PRIESTLEY (1928) has pointed out, all cellular processes at the apical meristem are determined by the available materials, provided to it from the rest of the plant, interacting with the materials that the cells themselves are capable of synthesizing from these external supplies.

In roots, we have a limited knowledge of the chemical activities of the root tip. It is a heterotrophic structure, dependent on older portions of the root and the shoot for a variety of materials. Since it is usually subterranean and non-photosynthetic, the root requires from the above-ground, green portion of the plant an energy supply, which is usually translocated into the root in the form of sucrose or other simple sugar via the phloem. RICHARDSON (1953, 1956) has shown in seedlings of *Acer saccharinum* that root elongation is ultimately dependent upon photosynthesis of the shoot system although the root may draw for its immediate carbohydrate supply upon reserve carbohydrates stored in the root itself¹. Mineral elements, both macro- and micronutrients, enter the root either *via* root hairs in the maturing regions of the root, moving into the region of the root tip in aqueous solution probably in the xylem tissue (KRAMER and WIEBE 1952), or are absorbed directly into the tissue near the tip region itself (OVERSTREET and JACOBSON (1946). In so far as it has been studied (see reviews by WHITE 1943, 1951, STREET 1957), isolated root tips grown *in vitro* require all the known mineral elements essential to the plant as a whole.

Vitamin requirements.

Requirements of different root species. In addition to these requirements, the root tip is usually unable to synthesize certain vitamins or other growth factors which are essential to the continued normal activity of the root. Evidence on this point in organ culture is extensive and conclusive. One of the most striking requirements is the almost universal requirement for vitamin B₁, thiamin (WHITE 1943). In most cases where careful studies have been made, isolated roots grown *in vitro* have been found to require an external supply of thiamin. In the absence of this vitamin, cell divisions in the apical region of the root soon cease (ADDICOTT 1939, 1941). In the intact plant, the vitamin is synthesized in the green leaves and shoot and moves downward into the root through the phloem (BONNER 1944). Thus, there must be a continuous supply of the vitamin, thiamin, to the region of the meristem. If this supply decreases or is cut off, apical meristem activity ceases. In certain plants, *e.g.*, corn, the root tips may be capable of synthesizing thiamin (McCLARY 1940, STREET 1957) and, in such cases, other growth factors may be limiting meristematic activity.

In roots, the vitamins which are not synthesized by the root but must be provided to it from the shoot are in every sense true plant hormones and are usually considered as such (THIMANN 1952, BONNER and BONNER 1948, *etc.*). Yet

¹ See also chapter "The significance for development of water supply, osmotic relations, and nutrition" by A. ALLSOPP, pp. 504—552 of this volume, specifically p. 525.

in the root cells, they function in their usual role as vitamins. In animals, vitamin deficiencies usually exhibit themselves by distinctive symptoms in a particular tissue system in which the cells are especially susceptible to lowered concentrations of a given substance or to the metabolic changes which ensue following a decrease in the level of the essential factor. Vitamin deficiency symptoms in root systems become evident when root tips are grown *in vitro* in the absence of an essential vitamin. The most striking symptom seems to be cessation of cell division and disappearance or diminution of the apical meristem.

A similar picture exists for a number of roots which require another B vitamin, nicotinic acid or niacin (ADDICOTT and BONNER 1938). Here again, the substance is synthesized in the shoot system and translocated to the root probably via the phloem. Still other organic substances which the root is unable to synthesize, such as pyridoxin, *etc.*, may similarly be provided by the shoot system.

Significance for the behavior of the root meristem during development. The use of organ cultures in the study of the heterotrophic nature of isolated roots has contributed a considerable amount of information concerning the materials which normally reach the root from the shoot. Yet, surprisingly, the possible significance of these facts to the behavior of the root meristem during development has been largely overlooked. Soluble carbohydrate and organic growth factors (vitamins, *etc.*) are continuously moving *via* the phloem into the region of the meristem where they are essential to continued meristematic activity—the carbohydrate as energy source and the vitamins as co-factors for the respiratory systems which make the energy available as high energy phosphate bonds in the form of adenosinetriphosphate. The movement of these factors from the shoot into the root establishes a longitudinal concentration gradient along the length of the root with the lowest levels in the extremities of the root tip, the meristem itself and the root cap. When cultured *in vitro*, the apical region of pea roots (0.5 mm including root cap) develops best when provided with vitamins at concentrations 10 times higher than that required by the usual 5.0 mm explant (TORREY 1954). To all intents and purposes, in roots which require a particular vitamin, for example, thiamin in tomato roots, nicotinic acid in pea roots, *etc.*, that vitamin is a “cell-division factor” and to the degree that it is in limited supply, will limit cell processes in the meristem, including the process of cell division.

Since these substances move through specific elements of the phloem tissue into the region of the root meristem, it is tempting to suggest that radial gradients from the sieve tube outward into the center of the vascular cylinder exist which are gradients of a substance or substances which limit cell division. In the proximity of functional sieve tube elements, cell divisions continue at a rapid rate, but toward the center of the root, in the region where large late metaxylem elements are found, these factors are in limiting supply and cells continue to enlarge without dividing.

Few attempts to explore such possible explanations of regions of high rates of cell division which would be bases for tissue patterns in the root tips have been made. BÜNNING (1951, 1952) has described the importance of “induction effects” in the blocking out of vascular patterns in the root meristem of *Sinapis* (see p. 1277). His interpretation, however, centers around the influence of the early enlarged central metaxylem elements in the meristem region on the plasma density of adjacent cells in the vascular cylinder which become xylem elements. He has not devised an experimental approach to this problem.

WIGHTMAN and BROWN (1953) in studying the effect of thiamin and nicotinic acid on meristematic activity in pea roots, showed that these vitamins had marked

effects on cell division in the meristem. They found that the period during which a cell remains in the non-vacuolated condition after cell division in the meristem is influenced by the availability of these vitamins. They believed that whereas thiamin had a direct effect on cell division, nicotinic acid acted in some way to prolong the period between the formation of a new cell in the meristem and its subsequent vacuolation during cell differentiation. Here again, an internal gradient of the vitamin might well affect the cellular activities which establish tissue patterns.

Micronutrient elements.

(*Boron, iron and others.*)

In a manner, similar to the vitamins, the supply of certain mineral elements, especially the micronutrient elements, to the region of the apical meristem may limit cellular activities, leading to abnormalities or complete cessation of root development. A case of particular interest is that of boron. Boron-deficiency is manifest most rapidly in the apical meristems of shoot and root with the latter usually showing symptoms first. The typical deficiency symptoms are cessation of cell division, necrosis of the meristem and death of the root tip. It has been suggested by GAUCH and DUGGAR (1953) that boron forms a complex with sugars in the plant and that the sugar-borate complex which is ionizable, moves readily through cell membranes and represents the translocated form of carbohydrates in plants. Boron deficiency in root tips of intact plants represents in fact a sugar deficiency which leads to the death of the cells and tissue. Movement *via* the phloem of the sugar-borate complex establishes still another organic material supplied to the root in a distinct gradient pattern. NEALES (1959) has clearly shown a boron requirement in excised roots of flax grown *in vitro* which is satisfied by 0.05 mg/l boron. Although NEALES found no evidence to support the idea that boron was essential for movement of sugars in roots, he did state that processes of cell division and cell maturation are primarily affected by lack of boron.

That micronutrient elements are essential to the activity of the root meristem, and that they may be supplied to the apex from older tissue of the root was apparent in the experiments of TORREY (1954) with excised root tips of different initial length grown in organ culture. A requirement for molybdenum, iron, manganese and zinc could be demonstrated with 0.5 mm-tips which was not manifest if larger root tips were used.

Recently, BROWN and POSSINGHAM (1957) have studied the role of iron in the development of pea roots. In pea root tips, 1 cm long, excised from germinating seeds and grown in a nutrient medium lacking iron, they found that cell divisions in the tip continued normally up until the 7th day of culture and then abruptly ceased. Thereafter, cells at the meristem enlarged radially and became vacuolated so that the entire tip region was characteristically swollen and enlarged. During this period, protein content per cell continued to increase, paralleling that in the control roots. Thus, lack of iron in the meristem stopped cell division but not protein synthesis. By feeding Fe^{59} to seedling roots, then sectioning and using autoradiographic procedures, BROWN and POSSINGHAM demonstrated that the largest amounts of iron in the root tip were localized, not in the mitochondria of the cells where it might be expected to function in the cytochrome system, but in the nucleus itself. Such localization suggests that the iron plays an important role in mitosis and a deficiency affects this process directly¹.

¹ Note added in proof: BRANTON and JACOBSON (1962) have shown such nuclear localization to be an artefact of fixation and that iron occurs uniformly throughout the cytoplasm.

Conclusions.

The preceding discussion has emphasized the necessity for continued meristematic activity in the root tip of a number of different types of substances—carbohydrates, vitamins, and mineral elements—all of which may be supplied to the meristem acropetally from the basal or shoot end of the root along specific pathways—either via the phloem tissues or the xylem—or, upon reaching the undifferentiated regions of these tissues near the root tip, by diffusion through differentiating primary meristematic tissues into the apical initial region. The inevitable acropetal movement of these materials suggests that they may influence or in some way determine the acropetal differentiation of specific tissues which is such a striking feature of primary tissue differentiation in the root. Only limited evidence of this base to apex influence has in fact, been found.

It is suggested that such influences, if they exist, will be difficult to demonstrate. In the above speculations concerning the possible role of the vitamins in determining tissue patterns by establishing cell division gradients, proof would be difficult. Yet it seems to the author that experimental approaches are in fact possible, especially the use of radioactive tracers to follow the movement of substances and the use of autoradiographic techniques to localize effects to specific tissue systems. It is clear that any evidence, either positive or negative, will be of interest and value.

c) The autonomous nature of the root apex.

The problem. The apical meristem of the root is an active region of the root in many ways. If the quiescent zone is itself relatively inactive metabolically, the immediate proximal region of the root meristem is highly active and the root tip itself—the first 1—2 mm or so taken as a whole including root cap, is a region of considerable metabolic action. A comprehensive discussion of the gradients of physiological and biochemical activity along the root axis will be presented later (pp. 1281 *et seq*). The meristem region is of peculiar interest, however, in terms of its activity as the organizing center for root development. It has been postulated by WARDLAW (1944), that “whenever the apical meristem . . . is in a state of active growth of such a nature that the distinctive character of the meristematic cells is maintained, the initial differentiation of vascular tissue will be observable immediately below the apex and in a path of substances diffusing from it, one or more of these substances being causally involved in that process”. Such a view which was conceived specifically in reference to the shoot meristem bears examination equally with reference to the root meristem. Here again, one is concerned with the ultimate regions of the root—the quiescent zone, the apical initial region, and the primary meristematic tissues which give rise by cellular differentiation to the primary tissues, including the primary vascular tissues of the root. This is a region of a millimeter or so in length, representing diverse tissues and cell types. Although it is feasible to give precise anatomical and histological descriptions of the region, definitive chemical and physiological analyses are difficult.

One of the implications of WARDLAW's hypothesis is that, to a certain extent, the apical meristem is autonomous and acts independent of the mature tissues to which it has given rise in certain respects at least. Such a view does not deny the established facts already summarized that the root apex is dependent upon the mature root structure and the shoot system of the plant of which it is a part. But it suggests that in certain respects at least the meristem imposes itself and its effects upon the differentiating cellular system which it has itself produced. By isolating the root tip from the plant and providing it in sterile nutrient culture

with the materials which it requires and which normally are provided from the shoot, it is possible to begin to examine the autonomous control of the meristem during root development.

In the first place, it is a striking fact that roots in organ culture do grow as normal roots. In general, the rate of elongation is somewhat less than that of a seedling root. Further, distinctive patterns of root morphology are retained. Thus, BONNER and DEVIRIAN (1939) have pointed out that pea roots do not branch very readily in culture but show the apical dominance of the root seen in seedling growth, whereas tomato roots show much branching and a fibrous type of growth. This maintenance of a genetic pattern of development *in vitro* indicates that isolation per se does not completely upset the developmental pattern. To a certain extent this pattern can be influenced *in vitro* and some information gained concerning the mechanisms which control the fixed pattern (see also Section VI on root branching; p. 1310 *et seq.*). It is also of interest and importance that isolated root tips grown as organ cultures, show essentially normal patterns of tissue differentiation, similar to those in seedling structures. Even root tips of very tiny dimensions, which lack any mature vascular tissues, such as 0.5 mm tips of pea roots (TORREY 1954, 1955) can develop into normal roots showing a normal sequence of tissue differentiation.

Experimental evidence. Further information concerning the activities of the apical region in tissue differentiation can be derived from studies in which very small tips or segments of root are cultured. Thus REINHARD (1954), using pea roots, isolated tissue fragments 0.2 mm³ to 0.5 mm³ from different anatomical regions of the root tip and cultured them in sterile nutrient agar medium containing macronutrient salts, sugar, thiamin, the amino acid cysteine and IAA. Over culture periods of 4 to 6 weeks, he found that all individual tissue fragments (his explants *I—VI*; see Fig. 4) produced irregular callus tissue masses. A tissue piece derived from the apical initial zone and adjacent tissues (explant *II*), 0.2 mm³ in volume, first formed a callus mass which after several weeks produced new roots which were characteristically like lateral roots in origin and appearance. Isolation of larger segments including root cap, apical initial zone and the adjacent tissues of the central cylinder (explants *I, II* and *III* together), 0.7 mm in length, led to development of normal differentiated roots in 8—10 days with no intermediate stage of callus formation. In such cases, the patterns of tissue differentiation appeared more or less unrelated to pre-existing patterns of the root from which the tips were derived. REINHARD concluded that an isolated apical meristem region of a certain minimum degree of organization can proceed in subsequent development to produce root structure independent of the other tissues to which it is usually attached. A related conclusion was reached by TORREY (1955) that the tissue pattern in isolated 0.5 mm pea root tips grown in culture arises, not as the result of inductive influences of older mature tissues, but as a product of the activities of the apical meristem region itself.

Two interesting facts should be considered in reference to these studies. In the first place, it should be noted that with a sufficiently large tissue fragment taken from the apical initial region, organized roots develop rapidly, in a matter of a week. In REINHARD's work (1954) explant *I, II* and *III* taken together developed as a normal root during the first 8—10 days. Similarly, TORREY (1954) used 0.5 mm tips and grew normal roots readily in the first week after isolation. If there exists an organized meristem in a small tissue piece, root development is rapid under optimum cultural conditions. If, however, smaller tissue pieces are used, *e.g.*, 0.2 mm³ in volume, even if the tissue piece includes the apical initial region and its immediate cellular derivatives, an organized root does not develop imme-

diately. Rather, an intermediate stage of callus tissue formation occurs and thereafter, a root meristem is organized and develops therefrom which elongates subsequently after several weeks.

Origin of organized meristems in analogous systems. It has been found likewise true from studies of root callus tissue (*e.g.*, TORREY and SHIGEMURA 1957)

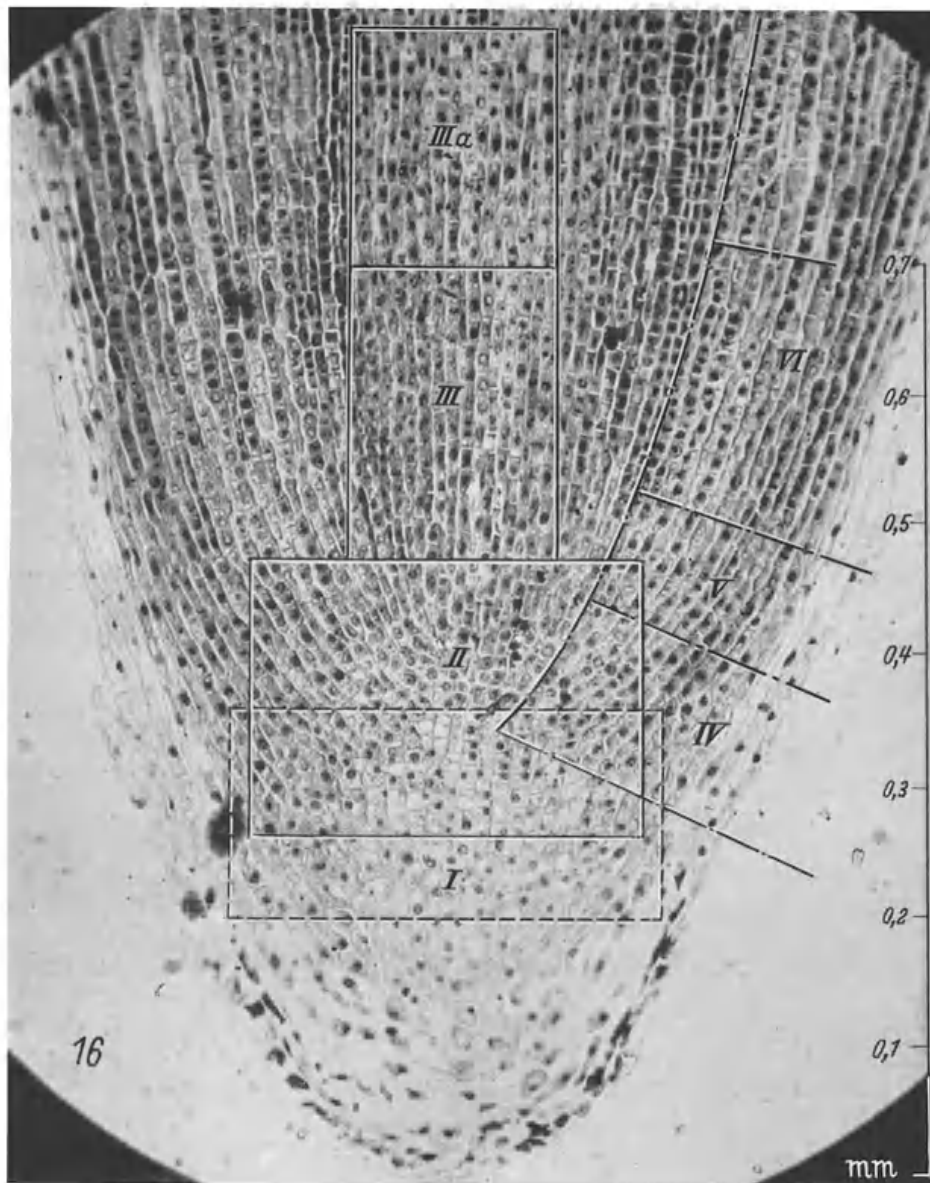


Fig. 4. Median longitudinal section of the root tip of *Pisum sativum*, showing the numbered explants cultivated by REINHARD (1954). [From REINHARD (*l.c.*).]

that diffuse unorganized callus tissue masses, under appropriate cultural conditions, are capable of giving rise to organized root meristems with normal tissue organization and development. This capacity to undergo tissue organization depends upon the environmental conditions in which the tissue resides and can be affected by the balance of the chemical constituents in a medium which influences and, in some cases, determines that development (SKOOG and MILLER 1957, *etc.*).

That there is a time lag in the organization of a meristem structure from a diffuse tissue mass is understandable since oriented cellular divisions must occur

in a tissue mass of sufficient volume and cell number to allow for the necessary organization. STEWARD *et al.* (1958) and STEWARD (1958) have discussed in interesting fashion the problems of organized development in these early stages from single-cell origins.

It would seem, further, that once organized, the apical meristem of a root is capable of perpetuating itself so long as the cellular orientation is not disrupted by direct traumatic damage to cells or by inavailability of cellular components which are essential to continued cell division and differentiation.

A close analogy is found in the development of the embryo from the zygote *in vivo*. From a single cell in the embryo sac, surrounded by the most favorable nutrient medium of the endosperm, the developing embryo passes through the globular stage, as a diffuse mass of callus-like tissue showing little cellular differentiation, thence to the progressively more organized heart-shaped and torpedo-shaped embryo where for the first time, the organization of the embryonic radicle becomes apparent. *In vivo* under optimum conditions this requires some time (8 days for *Phlox drummondii*, MILLER and WETMORE 1945; 12 days for *Phaseolus lunatus*, STERLING 1955). It is not unexpected that the process of organization is prolonged or impossible under the suboptimal conditions of culture *in vitro*. Thus, to date no success has, in fact, been achieved in approximating *in vitro* the complex chemical and physical environment which normally surrounds the globular embryo and which is essential to its normal subsequent development¹. Similarly, the conditions essential for the division of single isolated cells grown *in vitro* are poorly understood and the time periods involved in the formation of a multicellular callus tissue, not to say an organized meristem, from single cells is very prolonged (MUIR 1955, MUIR *et al.* 1959, TORREY 1957a). REINERT (1959) reported the induction of true, bipolar embryos in agar-grown callus tissues of carrot by sequential passages of callus tissues on different nutrient media for rather long cultural periods. He believed each different nutrient condition contributed to the organizing and development of the young plants². The forces which give rise to organization in a diffuse tissue mass are likewise little understood although these are the forces which must be understood if we are to understand the processes of cell differentiation and organ formation.

General conclusions. There is good reason to believe that the progression from one cell to a multicellular callus tissue mass to an organized structure such as the root meristem or in the case of embryogeny, to the young embryonic plant as a whole, involves a progressive transition from marked heterotrophism to increasing autotrophism. Evidence for this view comes from the established fact that progressively smaller embryos show progressively greater requirements with respect to nutritional requirements. Thus mature whole embryos can be grown on simple nutrient media and torpedo- and heart-shaped embryos require in addition a relatively complex mixture of vitamins, amino acids, and other constituents. The complex requirements of the globular embryo have yet to be worked out. Similarly, callus tissue systems reflect a level of heterotrophism intermediate between the simple requirements of isolated root organs grown *in vitro* and the elaborate, as yet unspecified, requirements of an isolated single cell grown *in vitro*. Thus, it would seem that the organized root meristems, incident to or as an integral part of their becoming organized, develop certain synthetic capacities which give them greater autonomy in their activities as a

¹ See RIJVEN (1952) and the chapter "Physiology of embryonic development in cormophytes" by C. W. WARDLAW, pp. 844—965 of this volume, specifically p. 909 *et seq.*

² See chapter on embryonic development in cormophytes by C. W. WARDLAW, specifically p. 947 and Figs. 56—59.

meristem. It may well be that the lag period which always occurs during the organization of the active apical meristem from a diffuse tissue mass involves not only the formation of new cell lineages in a given pattern, but also the formation at the cellular level of new enzymatic capacities which prepare the meristem for its role as an active center of cell initiation.

There are, in fact, some lines of evidence suggesting that the organized apical meristem of a root is distinctive in terms of its activities, *i.e.*, enzymatic activities, synthetic capacities, chemical constituents, *etc.* These distinctive properties are evident to a limited extent in histological preparations through differences in cell appearance, staining properties, nuclear size, *etc.* Which of these distinctive properties are responsible for the peculiar capacities and nature of the embryonic region has yet to be determined. Yet it is interesting and informative to examine for what it is worth, the evidence available at present. Since such activities must be related to the level of activity of the same processes in other regions of the root it is appropriate to consider the gradients which exist along the developing root axis and then in what way the meristem region is particularly distinctive.

2. Differentiation of the primary vascular tissues.

In her review of primary vascular differentiation in roots, ESAU (1954) summarized the few major facts which are established concerning our knowledge of the observed process: "first, all vascular elements differentiate continuously and acropetally; secondly, . . . the first phloem elements mature closer to the apical meristem than the first xylem elements; and, thirdly, . . . the immature metaxylem elements are recognizable closer to the apical meristem than the other vascular elements because of their rapid enlargement and conspicuous vacuolation." She pointed out further that in slow-growing roots the first mature vascular elements are closer to the apical meristem than in fast-growing roots. These observations concern in particular the longitudinal course of vascular tissue differentiation and represent one aspect of the problem.

The second major problem which has been discussed by TORREY (1955) concerns the physiological basis for the determination of the radial and alternate arrangement of the primary vascular tissues which is so characteristic of root organization. Although these two aspects are intimately interrelated, it is helpful to distinguish between them in our thinking and discussion.

a) Anatomical considerations.

It is useful to summarize briefly the sequence of events which occurs in the primary meristematic tissues of the primary root of a typical dicotyledonous plant. In general, a very similar sequence which differs only in detail is observed also in roots of monocotyledonous plants.

Differentiation of the procambium. Immediately behind the region of the apical initials the first pattern of cellular differentiation which becomes evident is the pattern of concentric cylinders in which the solid central procambial cylinder is set off from the rest of the root tissues by radial enlargement of cells of the ground meristem in particular. The innermost layer of cells of the future cortex (ground meristem) shows periclinal divisions giving rise to radial rows of cells in the ground meristem, thereby further emphasizing the distinction between procambium and the rest of the root tissues as seen in transverse section (*e.g.* ESAU 1940, 1943a, 1953a, HEIMSCH 1951, POPHAM 1955b, TORREY 1955). The endodermis is early distinguishable as the innermost complete concentric

logically-striking features in many roots. These develop quite far behind the meristematic tip in regions of the root which one usually can characterize as composed of mature primary tissues. In pea seedling roots, thickened secondary walls of phloem fibers are reported to occur at 20—40 mm from the root apex (POPHAM 1955b). Still further from the root tip in older roots the origin of the vascular cambium marks the beginning of secondary growth.

b) Factors involved in tissue differentiation.

(Environmental, intercellular and intracellular forces.)

At least three types of forces acting on newly formed cells in the root apex may be visualized. First, there is the collective influence of the *external environment* which includes a wide variety of factors, such as p_H , light, nutrients, supply of gases, *etc.*, which influence rates of root elongation and internal differentiation. Despite the very significant effects which these natural environmental factors produce on root development, it is a remarkable fact that the sequence of events associated with cellular differentiation in the root, while influenced in a relative way, seems to be little affected in its basic pattern. It is true that there must exist within an elongating root gradients, both radial and longitudinal, which are associated with the pure geometry of the tissue system in relation to its external environment. These gradients are not to be neglected and have been the subject for much speculation and some investigation by such workers as PRIESTLEY (1928), VAN FLEET (1942) and others. It is probable that the influence of the external environment on tissue differentiation is always secondary in its effect, acting in turn upon primary forces.

The second type of force acting on the differentiating root system is that attributable to *physical and chemical phenomena within the tissue system* which arise as a result of the multicellularity of the system and the interaction of constituent cells. In this category may be placed chemical factors such as hormones which possess peculiar and characteristic distribution patterns within the organ and the organism. Also of importance here are energy sources, substrates, and metabolites whose source and pathway of supply may impose limitations important in directing or determining cell differentiation. Internal physical strains and stresses which arise from the differential behavior of different cells in a multicellular tissue system might also be considered here. These internal intercellular forces which arise and act in any multicellular system are in turn affected by the external environment and may be modified in many ways by such external effects.

The third type of force of importance in considering cellular differentiation may be characterized as *intracellular* and resides in the inherent capacities of the cell as determined by the immediate history which gave rise to its cytoplasmic and genetic constitution. The potentialities of a cell as a differentiating entity in large part reside within it and its ultimate fate depends upon the expression of these potentialities under the stimulation and within the limitations imposed upon it by its position within time and space.

Suffice it to say that we have very little intimate knowledge of the action of these major forces in cell differentiation in the root. In fact, we do not know at present which of these forces is of greatest significance in determining the course of cell differentiation; we only know that it is probable that all may act during the course of differentiation of a mature cell type from a particular meristematic cell in the apical initial region of the root.

c) The longitudinal course of vascular tissue differentiation.

Levels of tissue maturation.

ESAU (1953b) reviewed much of the pertinent earlier work on the relationships which have been observed between rates of elongation and rates of differentiation and maturation of primary vascular tissues in roots under various conditions of root elongation. In general, it can be stated that under conditions which cause relatively slow root elongation, the first mature primary vascular elements occur closer to the region of the apical initials than when roots show normal elongation. The relative positions of first maturation of xylem and phloem remain constant, however. HEIMSCH (1951) found that in different morphological types of barley roots grown in the same environmental conditions the level at which the maturation of vascular elements occurred varied widely, while the initial blocking-out process was essentially similar. Thus, in unbranched seminal roots the first mature protoxylem occurred 9—10 mm from the root apex, while in seminal roots with lateral branches at 1—2 cm from the tip, protoxylem maturation occurred within 0.4 mm of the root apex. In roots formed later which showed no root branching, protoxylem first matured at about 7—8 mm from the root apex. Lateral roots showed first protoxylem maturation at 2—3 mm behind the root apex.

POPHAM (1955b) reported that seedling roots of peas growing in aerated solution elongated consistently faster than non-aerated roots. The sequence of vascular tissue differentiation was essentially the same in all roots but the levels at which tissue maturation occurred varied widely. Roots in aerated solutions which elongated most rapidly showed tissue maturation at greatest distances from the root apex. POPHAM pointed out that, because of genetic differences among seedlings, one could not make the generalization that tissue maturation occurs closer to the root apex in short roots than in long roots or in young roots as compared to old roots. Rather, rate of elongation seems to be the primary factor. Very little physiological or biochemical information is available concerning the basis of these differentiation processes and their change with different rates of root elongation. It is clear that the different cell types are affected differently by the internal physiological conditions to which they are exposed and that these differences in response must be determined in the cells at an early stage after their formation by cell division in the apical initial region.

Experimental studies.

Use of metabolic inhibitors. TORREY (1953) reviewed a number of studies in which vascular tissue maturation was observed under experimental conditions which resulted in inhibition of root elongation. As would be expected from the above discussion, root inhibition is usually accompanied by vascular tissue maturation closer to the tip. There is not always a direct relationship between these two however. In his experiments on the effects of several specific metabolic inhibitors, all of which reduced elongation by excised pea roots in culture by 90 %, vascular tissue maturation was affected differently by each inhibitor. Thus, 2,4-dinitrophenol, known to uncouple phosphorylation mechanisms from aerobic respiratory processes in cells, completely stops tissue maturation. Iodoacetic acid, which inhibits aerobic respiration by specifically affecting certain sulfhydryl enzymes, has relatively little effect on the level of vascular tissue maturation even when it inhibits root elongation almost completely. However, secondary wall formation of primary xylem elements was apparently upset by iodoacetate inhibition. Indoleacetic acid, at a concentration which inhibits root elongation almost completely, actually accelerates the maturation of primary xylem tissues

while having little effect on primary phloem. TORREY pointed out that the action of all three inhibitors centers around cellular processes associated with carbohydrate metabolism and that the key to maturation processes in primary xylem lies somewhere in this area of metabolism. An attempt was made to relate these specific inhibitory effects to particular ontogenetic steps in the course of differentiation of a primary xylem element in the root (Fig. 5). Although the determination of a specific site of a metabolic block during the differentiation of a cell is exceedingly difficult to achieve with any certainty, the attempt made in this work is illustrative of possible approaches to the problem which perhaps deserve further exploration.

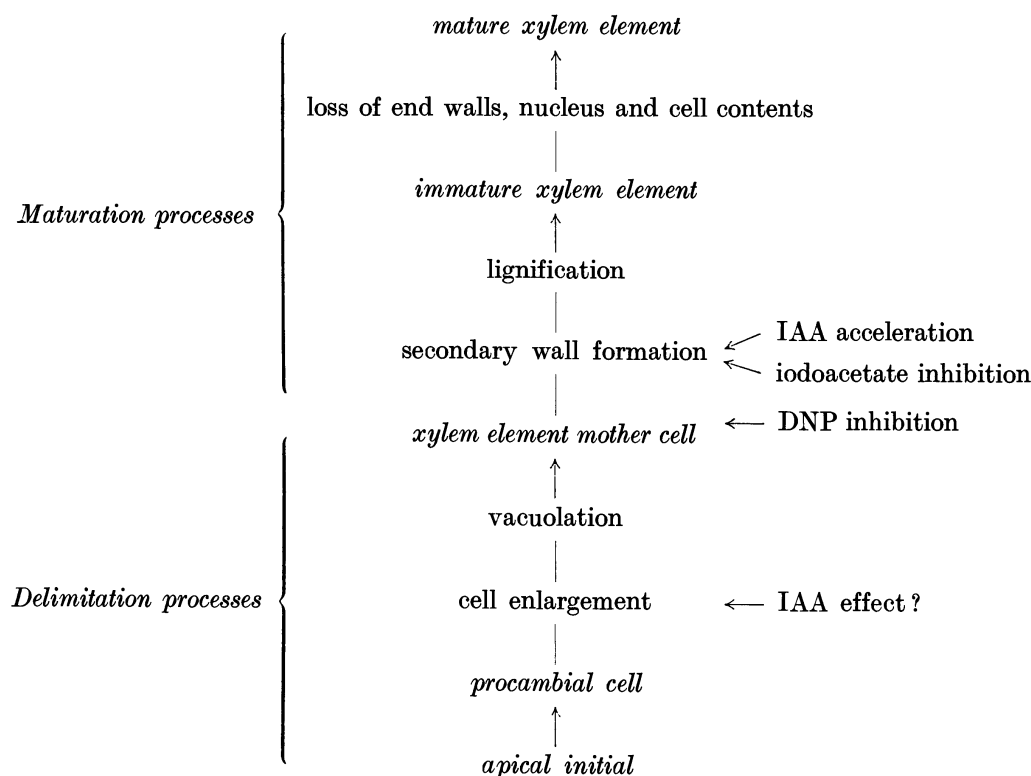


Fig. 5. Schematic representation of the ontogenetic steps in the differentiation of a mature xylem element in the primary xylem of a root. (From TORREY 1953.)

Histochemical approaches. JENSEN (1955b) used two different histochemical procedures to arrive at a quantitative measure of peroxidase activity in different tissues of the developing seedling root tip of *Vicia faba*. This enzyme is of particular interest in the differentiation of primary vascular tissues. JENSEN found that the procambium showed greater peroxidase activity than other primary meristematic tissues in the root apex and that treatment of the root with indole-acetic acid made possible the identification by histochemical means of protoxylem and protophloem elements much closer to the tip than in untreated roots. He interpreted this effect as the result of an auxin-induced enhancement of peroxidase activity in the early differentiation of the primary vascular tissues. The increase in peroxidase activity was in turn related to an increased lignin synthesis in these cells which depends on a peroxidase-hydrogen-peroxide reaction with substrates such as eugenol. JENSEN pointed out that the effect of IAA in accelerating primary xylem tissue differentiation described by TORREY (1953) in pea roots could be interpreted as the result of auxin-induced peroxidase activity in procambial tissues leading to increased lignin synthesis

and thus earlier protoxylem maturation. JENSEN's observations would suggest that the IAA acceleration effect in the scheme shown in Fig. 5 is on the lignification step as well as on secondary wall formation. It is apparent, of course, that all other steps must also be accelerated if maturation is to proceed to completion.

The concept of acropetal induction.

In the problem of the longitudinal course of tissue differentiation in the root one fact has been repeatedly emphasized in the anatomical literature—the fact of the continuous and acropetal differentiation of the vascular tissues. This observation itself seems to have given rise to the idea that such continuity could best be explained in terms of some sort of induction process whereby a maturing element, by its very maturation in linear association with newly formed cells from the root apex, induces the differentiation of the same type of cell, thereby assuring a continuous and acropetal pattern of differentiation. The concept of longitudinal induction goes back to PFEFFER (1904) and HABERLANDT (1913) and was accepted by JOST (1932). The concept implies that vascular tissue patterns, once established in the embryo radicle, are perpetuated in the primary root axis throughout the life of the root via inductive forces. The evidence against this view has been discussed by TORREY (1955). It seems reasonably certain that induction as visualized by JOST (*l.c.*) cannot in fact account for the continuity in longitudinal maturation processes nor for determination of vascular patterns in the root. Rather, the influence of mature tissues on the newly formed meristematic tissues at the root apex must be thought of in more general terms such as those concerning the supply of nutrients to the differentiating system (see discussion on pp. 1299 *et seq.* of this review).

d) The determination of radial patterns in vascular tissue differentiation.

Theoretical concepts.

The radial and alternate arrangement of the primary vascular tissues in the root of dicotyledonous plants is one of the characteristic and diagnostic features of root structure. Its determination during tissue differentiation has been a problem of great interest to anatomists and physiologists for many years. JOST (1932) stated the problem clearly and sought an explanation in terms of inductive forces originating in mature cells, which would act upon the undifferentiated cells behind the apical initial region of the root, imposing upon them the already established vascular pattern.

In recent years this view has been seriously questioned because of experimental evidence from studies of excised root tips and parts of excised roots grown in nutrient media under controlled environmental conditions. The view, first stated in a speculative way by THODAY (1939), has developed that the determination of the vascular pattern in roots is controlled in and by the apical meristem. Although the determining forces have yet to be identified in concrete and specific chemical and physical terms, the evidence is good that they have their origin in the apical meristem region of the root.

The radial vascular tissue arrangement in the primary seedling root of many species of dicotyledonous plants may be quite constant, even characteristic, of the species. Thus, for example, in roots of *Pisum sativum* three radial strands of primary xylem alternate with three groups of phloem elements for the entire length of the triarch primary root. The number of strands may, in fact, be quite characteristic of a genus or even family. The number of vascular strands in the primary root may vary from one individual to another in certain plant groups

such as the ferns (WARDLAW 1928) and in certain cases may vary within the root of the same individual [e.g., in *Vicia faba* (G. BOND 1932) and in *Zea* (HEIMSCH 1949)]. Lateral branches of the primary root axis usually show a reduced number of vascular strands compared to the main axis.

Descriptive studies.

BÜNNING (1951) described in careful cellular detail the first blocking-out by radial enlargement of the primary vascular tissues in the diarch root of *Sinapis alba*. It is his view that the early enlargement of future-metaxylem elements in the center of the procambial cylinder determines the pattern which subsequently develops. Further primary xylem elements are induced in radial rows outward from the first enlarged element. Primary phloem differentiation is inhibited, according to BÜNNING'S view, in proximity to differentiating xylem tissue and thus develops at radial points in the procambium most distant from the xylem strands. This view invokes two forces in the determination of pattern: first, inductive forces acting from one xylem element to the next in radial (and presumably also longitudinal) direction and second, inhibitory forces arising also in the xylem tissues which suppress phloem differentiation. Once differentiated, phloem tissues also cause inhibition of further phloem tissue differentiation in the immediate vicinity. As a result of these inhibitory and inductive forces, a radial and alternate pattern of vascular tissue differentiation is produced. BÜNNING'S theory (1951, 1952) rests largely upon observations of differential staining in sectioned root material, measurements of progressive changes in nucleolar volume as determined from fixed sections, studies of tissue differentiation in wounded roots and an imaginative description of the presumed sequence of events. His theory is provocative in that it allows one to think of the processes involved in pattern determination in terms of the action of diffusible or movable materials whose transmission from cell to cell determines the course of cell differentiation and hence of pattern formation. No specific materials acting in this system are known.

THIMANN (1957) has indicated the unlikelihood that simple water-soluble and readily diffusible materials such as auxins of the IAA type or vitamins such as thiamin could be responsible for induction or inhibition phenomena like those suggested by BÜNNING since sharp concentration gradients, which would be required to determine differentiation in one cell and not its immediate neighbor, could not exist in a tissue where free diffusion and thus equal distribution of these materials would undoubtedly occur. THIMANN has pointed to high molecular weight compounds as the type of material most likely to be concerned in inductive phenomena. Movement of such materials probably would not involve free diffusion through cytoplasmic membranes and cell walls but would require transmission *via* cytoplasmic connections or plasmodesmata. BÜNNING (1952) observed that those cells of the pericycle which border on the outer cells of the developing primary xylem strands contain denser cytoplasm, suggesting a stimulation of protoplasm synthesis. This increase in cytoplasm density, however, was much more pronounced in pericycle cells whose entire inner tangential wall

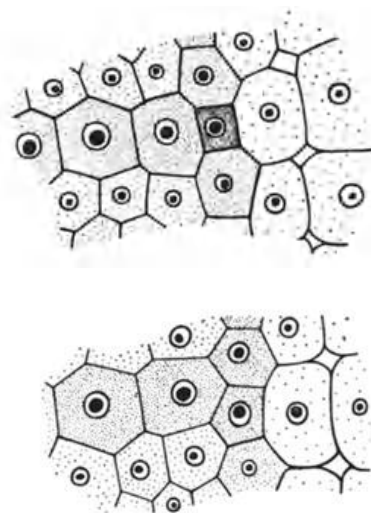


Fig. 6. Induction of formation of new protoplasm in the pericycle as dependent on the mode of contact between primary xylem ray and pericycle. (From BÜNNING 1953.)

bordered the outer tangential wall of the outermost xylem cell, than in cells which bordered the outermost xylem cells with radial walls only (see Fig. 6). This observation would fit the idea that the inductive forces pass from cell to cell along interfaces which could be expected to have direct cytoplasmic continuity, but not through perpendicularly-oriented cell walls. If externally supplied substances such as soluble auxins or vitamins do produce effects on differentiating tissue systems, one must look for less direct effects than through gradients induced from cell to cell.

Experimental studies.

Culture of short tips. According to BÜNNING (1952), inductive and inhibitory forces controlling tissue pattern formation must originate in the root apex. In

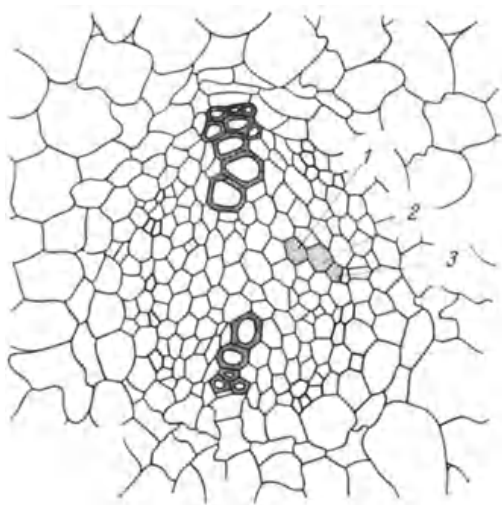


Fig. 7. Photographic tracing of transverse section of a diarch pea root grown *in vitro* from an isolated 0.5-mm. root tip. Section was cut in a region of the root where the symmetrical diarch pattern was about to undergo transition to the triarch arrangement. Future xylem elements of the new strand are stippled and numbered in the sequence of their maturation. (From TORREY 1955.)

experiments with roots of *Vicia faba* he showed that a 2 mm excised root tip, replaced on the shortened stump of the same root, would grow but that the vascular tissues of the tip did not develop in continuity with those in the root base. He concluded that the pattern had already been established in the tip. TORREY (1954, 1955) developed a technique for cultivating 0.5 mm excised root tips of *Pisum sativum* in a sterile nutrient medium. Roots of normal appearance which increased their initial length 20 fold in a week were grown routinely from excised tips. These tips included root cap ($300\ \mu$) and about $200\ \mu$ of the apical initial region and its immediate derivatives, but no mature vascular elements and only a portion of the initially blocked-out xylem pattern in the procambial core. In roots formed from such tips normal vascularization occurred.

About 80% of these roots formed the normal triarch pattern for their entire length. In about 20%, abnormalities in vascular differentiation were found; these roots were either diarch or monarch in their vascular pattern. The reduced vascular pattern was found at the root bases and for various lengths of the roots. It was believed that the reduced vascular patterns, which were symmetrical and normal diarch or monarch arrangements (Fig. 7), were the result of the destruction of the preexisting triarch pattern in the tissues at the level of the excision. New vascular patterns developed in the roots of smaller tip diameter as a result of the excision.

In diarch and monarch roots which were allowed to continue elongation in culture there occurred a transition back to the typical triarch arrangement found in seedling pea roots. The transition, which involved the appearance of a new additional xylem strand by centrifugal maturation (Fig. 7), usually occurred over a millimeter or less of root length and resulted in a new symmetrical vascular arrangement. Transitions from monarch to diarch and diarch to triarch arrangements were studied. In the limited observations made on these roots, no correlation was found between vascular complexity and either total root diameter or the diameter of the mature vascular cylinder. However, a direct correlation was noted between the number of strands formed and the diameter of the procambium

Table 2. *Relation between the dimensions and the vascular tissue patterns of excised roots of Pisum sativum grown in vitro.* All measurements are given in microns. Figures in parentheses show the number of samples. (Data from TORREY 1955, 1957c and unpublished work.)

Vascular pattern	Root at level of mature primary tissues		Procambial cylinder of root at level of pattern inception	
	Total root diameter	Diameter of central cylinder	Diameter of procambium	Number of cells across diameter
Monarch	507—780 (3)	57—103 (3)	109 (1)	9 (1)
Diarch	187—840 (6)	63—125 (6)	47—94 (3)	9—14 (3)
Triarch	304—525 (8)	62—195 (8)	94—146 (8)	14—17 (8)
Tetrarch	390—430 (6) ¹	140—175 (6) ¹	135—170 (4) ²	18—20 (4) ¹
Hexarch	430—795 (8) ¹	175—254 (8) ¹	210—310 (5) ²	18—28 (5) ¹

¹ TORREY (unpubl.); ² TORREY (1957c).

at the level of pattern inception (Table 2), suggesting that factors controlling the latter dimension were important in determining vascular arrangements in these roots.

Culture of excised pieces; surgical experiments. REINHARD (1954) also reached the conclusion that the arrangement of the tissues of the root is under the control of the apical meristem. He cultured excised pieces taken from the apical region of pea roots (Fig. 4, p. 1269) to determine the size and site of origin of pieces which were capable of developing into normal roots. Tissues of zones *I*, *IV*, *V* and *VI* each separately produced tissues characteristic of the region to which they normally give rise. Thus, zone *I* produced a diffuse mass of root cap-like cells; zone *IV* or *V* produced mature cortical tissues. Zone *II* produced first an irregular mound of tissue which then formed a central part and a cortical part. This tissue mass finally gave rise to new roots bearing the characteristics of lateral roots. Zone *IIIa* or *b* formed a callus mass with unoriented xylem tissue in its center. Only zones *I* + *II* + *III* taken together gave rise in normal fashion to an organized root. REINHARD concluded there was no evidence of determining influences of the older tissues on the younger tissues; rather, that the determination of root structure originated in the root apex.

Further evidence for control by the apical region of the vascular pattern in roots of dicotyledonous plants comes from experiments involving surgical operations on the tip itself. CLOWES (1953) reported that in a certain number of experimental roots of *Vicia faba* which had had wedges of tissue removed from the region of the apical meristem there was a reduction in the number of protoxylem arms formed in the root. He pointed out that excisions in the root basal to the apical region had no acropetal influence on the differentiation pattern. He believed that excisions in the meristem which produced a reduction in vascular strand number probably produced a decrease in the size of the meristem itself.

REINHARD (1956) also observed modified development in vascular patterns in roots of *Sinapis alba* following a longitudinal incision through the root apex. Two new roots developed from and in line with the original diarch axis. The result was the same whether the incision was oriented in the axis of the xylem strand or perpendicular to it: usually one root was triarch and one diarch or both were triarch. Clearly, the triarch arrangements could not be related to any pre-existing pattern but resulted from changed relationships in the newly regenerated meristems. REINHARD made the further interesting observation that such triarch roots, if allowed to continue elongation, reverted to the diarch arrangement. The course of such reversions in vascular pattern were described by REINHARD (1960).

The role of auxin.

TORREY (1957c) studied the regeneration of new root meristems by cultured roots of *Pisum sativum* grown in sterile synthetic nutrient media following the excision of 0.5 mm tips. He found that vascular pattern formation in the newly regenerated roots was markedly affected by certain constituents of the medium. In a cultural medium satisfactory for growing excised pea roots, regeneration of new tips was essentially unorganized and unpredictable, with little or no continuity between old and new root vascular tissues. The original triarch vascular pattern might or might not persist. In certain cases, regeneration involved lateral root initiation with reorientation of the laterals in the direction of the long axis of the main root. When regeneration was allowed to take place in a medium

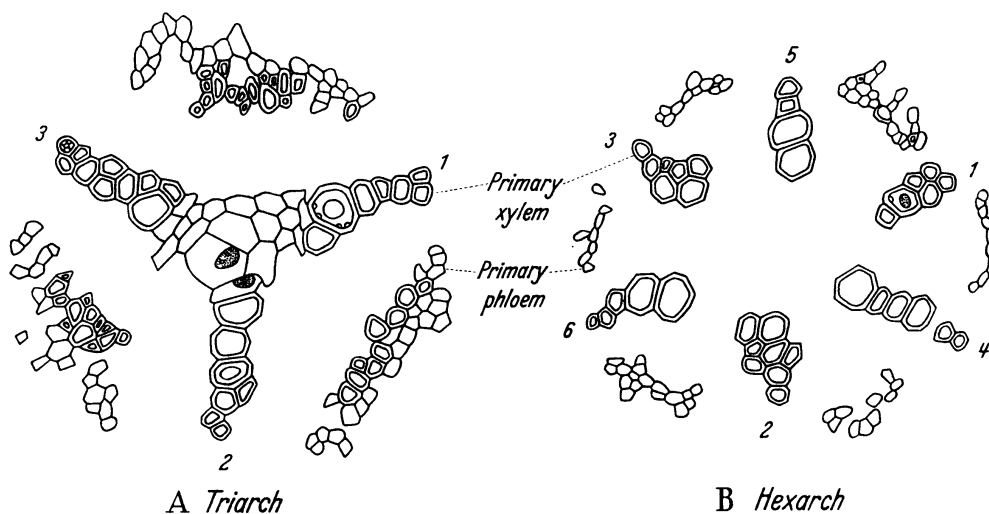


Fig. 8 A and B. Photographic tracings of transverse sections from two levels of a pea root which had been allowed to regenerate a new root tip after decapitation of the apical half-millimeter. The nutrient medium contained 10^{-5} M IAA. On the left the triarch vascular arrangement of the original root base is shown; on the right the symmetrical hexarch arrangement of the primary vascular tissues of the regenerated root is evident. The orientation and magnification of the two sections are identical. (From TORREY 1959a.)

containing relatively high concentration (5×10^{-6} M) of auxin (IAA), the new root meristem developed in continuity with the old root axis and the new roots possessed a symmetrical hexarch vascular arrangement, double the original strand number (Fig. 8). So long as root elongation continued in auxin medium, the hexarch pattern was formed. When hexarch roots were transferred to auxin-free medium, the rate of root elongation increased and the root reverted slowly back to a reduced vascular strand number, passing through symmetrical pentarch and tetrarch vascular arrangements. One or more of the strands which disappeared during the course of reduction was an original strand of the triarch root base. TORREY suggested that the auxin in the medium influenced the radial dimensions of the new meristem during the course of tip regeneration in such a way as to cause the formation of a larger procambial cylinder at the level where the vascular tissue pattern is first blocked out. Measurements of the vascular cylinder at this level (Table 2) indicate that this dimension is markedly increased by auxin treatment and the direct correlation between vascular complexity and the diameter of the central cylinder at the level of pattern inception appears to hold. TORREY suggested further that endogenous auxin levels in the root, insofar as they might control the orientation of cell divisions in the root apex, could be important in controlling patterns of vascular tissue formation in normal root development.

Further evidence for the possible importance of auxins in vascular pattern formation comes from experiments of SAMANTARAI and SINHA (1957). They found that induced adventitious roots on isolated leaves of *Ipomoea* and other plant species showed an increased number of vascular strands following auxin treatment. Their data also show that strand number is directly correlated with increasing auxin concentration. Carbohydrate supply and nitrogen provided the regenerating leaf cuttings also had an effect on complexity of the vascular pattern. Here, also, exogenous supplies of soluble materials modify fundamental patterns of vascular tissue differentiation in newly developing root structures.

The role of metabolites.

The evidence indicating that vascular patterns in the root are subject to modification in response to reduced supplies of metabolites is not clear cut. FLASKÄMPER (1910) reported that removal of the cotyledons from seedlings of *Phaseolus* and *Vicia faba*, which resulted in reduced root elongation, caused the disappearance of the pith and a reduction in the number of vascular strands formed by the root. G. BOND (1932) was unable to reach the same conclusions in similar studies with *Vicia faba* and species of *Phaseolus*. He observed reduction in vascular strand number in roots of intact control plants as well as in plants with cotyledons removed and concluded that such reduction was unrelated to removal of the cotyledons. Experiments of this sort might well be repeated under controlled conditions of nutrient culture to determine whether intact seedling root structure can be modified by nutrient supply. The anatomical observations of FRIES (1954) on decotylized pea seedlings suggest that some experimental manipulation may be possible. Clearly, since changes in the vascular pattern of primary roots may occur during normal ontogeny, physiological changes must take place in the root, presumably affecting the meristem where pattern formation occurs. Further evidence as to the nature of factors, internal or external, which influence those changes, will be of importance in formulating concepts as to the fundamental nature of vascular pattern inception.

3. Physiological and biochemical gradients within the developing root.

The growing root represents a linear sequence of cellular differentiation from the root apical meristem where cells originate by cell division, through a region where large numbers of cells enlarge in the longitudinal direction, to a region where cells show processes of cellular maturation.

Because of this evident gradient of cellular activities, many investigators have examined numerous cellular features along the length of the root with a view to discovering direct relationships between the process studied and the state of differentiation of the cell. Various experimental methods have been used ranging from the direct examination of certain activities at points along the intact root, to cutting the root into sections of various lengths—from centimeter lengths to 50 μ thick segments—and examining the activities of these isolated segments, or to dealing with individual cells isolated from a given region in the root and testing their activities in the living or fixed state. All of these approaches offer certain advantages and certain concomitant disadvantages or limitations. Some of these difficulties have been discussed with respect to the problem of studying root elongation (TORREY 1956 b). The method used will determine the presentation and interpretation of data. Thus in many studies, results may be expressed on a whole-root basis; other methods allow a discussion of physiological activity in terms of root segment length and volume; still others allow interpretation on a cell

basis. The method of expressing the data influences very much the interpretation with respect to the organization and development of the root structures concerned. It will be necessary to limit discussion to the most clear-cut data available. Frequently, because of differences in methods and of tissue systems, *i.e.*, species differences, it is impossible to arrive at any valid generalizations concerning many observed gradients within roots.

a) Gradients in general metabolic activities, dry weight and nitrogen content.

Cells of the apical initial region of the developing root are small, isodiametric, appear densely protoplasmic when stained, show little vacuolation and stain heavily with both cytoplasmic and nuclear stains. Progressively back from the region of the apex, root cells show radial enlargement, especially in the cortical region and then rapid longitudinal enlargement, which is especially noticeable in the central cylinder. Differences between tissue regions are marked, such that three distinct primary meristematic tissues are usually discernible, *i.e.*, the protoderm, ground meristem and procambium. Still further back from the tip the primary tissues of the root are differentiated at different levels.

As has been pointed out repeatedly (GOODWIN and STEPKA 1945, ESAU 1953b, TORREY 1956b), cell division, cell enlargement, both radial and longitudinal, and cell maturation are inextricably associated and overlap along the length of the developing root axis during root elongation. These processes occur in different regions of the root and in different tissues at different rates and different times.

On a root segment basis.

Measurements of particular changes in cell components or of metabolic activities along the length of the root when based on determinations of root section lengths are some measure of the over-all gradients to be observed in the root. All such determinations tend to show that the terminal centimeter, half-centimeter or even few millimeters of the root tip manifest very high values relative to the basal regions of the root. Thus, for example, data from BALDOVINOS (1953) on 1 mm corn root segments show the second millimeter of the root below the apical initial region to be the highest region, of the first 5 mm, in dry weight, alcohol-insoluble matter, total nitrogen, and ash. This region is one of beginning rapid cell elongation. Data of R. BROWN and BROADBENT (1950) for dry weight and protein nitrogen, plotted on a section basis, show peaks in the region of the apical initials and then a decrease toward the base of the root.

Similarly, data on metabolic activity, expressed on a root-section basis, indicated the tip region to be one of high total metabolic activity. Thus, many authors have reported the terminal region of the root to show highest O₂ uptake (MACHLIS 1944, WANNER 1944, BERRY and BROCK 1946). From such data, and from histological observation, the idea was established that the apical meristem, region of cell initiation, was the region of cells possessing greatest metabolic activity. This idea suggested superficially that the actively dividing cells of the meristem had a high respiratory rate associated with their mitotic activity.

In recent years, increasing amounts of data and analyses at a progressively finer level, have changed this point of view and clearer ideas of cellular changes during root growth are emerging. Evidence concerning the respiratory activity of root cells during root development where the most information is available is of particular interest.

KOPP (1948) has pointed out that calculation of WANNER's data (1944) on wheat roots, in which oxygen uptake is based on protein nitrogen rather than root

segment values, shows oxygen uptake 3 times as great in the region of maximum cell elongation as in the dividing meristem. Similar observations have been reported for roots of *Allium cepa* (WANNER 1950), *Zea mays* (BALDOVINOS 1953), *Triticum aestivum* (ELIASSON 1955, KARLSSON and ELIASSON 1955), *Pisum sativum* (R. BROWN and BROADBENT 1950), and *Vicia faba* (JENSEN 1955a). Thus, simply expressing metabolic activity on a protein-nitrogen basis rather than on an arbitrary morphological unit changes significantly the interpretation of the information with respect to interpreting cell development.

On a cell basis.

Comparison of different species. Still more informative are data based on the "average cell" of the root at any given level. Although intimate knowledge of chemical changes in each cell of the root is the ultimate desideratum in discovering the nature of root development, such information is not available with our present methods of analysis. Recent improvements in techniques, however, have made analysis at the cellular level more feasible. Thus, R. BROWN *et al.* (1950, 1952, 1953, 1955) have analyzed root segments 0.2, 0.4, 0.8, 1.0 mm thick of pea and bean for cell constituents and metabolic activities and have been able through their techniques of root maceration and cell counting to express results on a per cell basis. Using a somewhat different procedure, ERICKSON and GODDARD (1951), and ERICKSON and SAX (1956a, b) have studied 1.0 and 3.0 mm corn root segments and calculated data on a per cell basis. JENSEN (1955a) and JENSEN and KAVAJIAN (1958), studying 50–200 μ thick sections of broad bean and onion roots using Cartesian diver techniques, and distinguishing different cell types histologically, have been able to discuss changes in cell constituents at a considerable level of refinement.

It is of interest to analyze with some care the data of these workers on three different species of roots to see whether any generalizations emerge which help in understanding root organization and development at the cellular level. In Table 3 are summarized pertinent data from these studies. In the first place it is important to recognize the differences in the lengths of the meristematic region at the tip—the region where cell divisions occur. In corn, this region extends for 2 mm behind the root cap base, in pea and broad bean roots just over 1 mm. The region of cell elongation is more variable, extending in corn roots to about 7 mm from the tip, in pea roots only to 5 mm. In *Vicia faba*, JENSEN's data include only a very small portion of the elongating region (1.7–3.0 mm). According to the work of R. BROWN and ROBINSON (1955) on the same species, cell elongation continues to about 10 mm from the tip and the increase in cell volume really only enters the linear phase at about 3 mm behind the tip, after which there is a 10-fold increase in average cell volume. Thus, the three sets of data are not directly comparable except in the terminal 3-mm region and here the refinement of analytical techniques differs considerably. In certain aspects, it is possible to view JENSEN's data as a detailed analysis of the rougher data of the other two in the tip region.

Gradients of nitrogen and other constituents, of dry weight, and of respiration.

The root cap is characterized by a rather high dry weight per cell value, associated with large amounts of total carbohydrate, soluble nitrogen, but relatively low protein nitrogen on a cell basis (JENSEN 1955a). The meristem region itself is however most interesting—as the site of origin of new cells. The meristem is characterized by the lowest dry weight per cell of the entire root, a very low level of total nitrogen per cell as well as a low protein nitrogen content on a cell basis.

Table 3. *A comparison of quantitative data from roots of three different plants made by three different groups of investigators. Taking into account the differences in tissue segments studied and the portions of the roots studied, consistent trends are apparent in all physiological properties studied. The data are discussed in detail in the text.*

	<i>Zea mays</i> ¹ (corn)	<i>Pisum sativum</i> ² (garden pea)	<i>Vicia faba</i> ³ (broad bean)
Total root length analyzed	15 mm	9 mm	3 mm
Length of sections analyzed	1.0, 3.0 mm	0.2, 0.4, 0.8 mm	0.2 mm
Length of root cap	0—0.5 mm	0—0.3 mm	0—0.5 mm
Region of maximum number of cell divisions	0.5—2.5 mm	0.3—1.5 mm	0.5—1.7 mm
Region of cell elongation predominantly	2.5 to ca. 7.0 mm	1.5—5.2 mm	1.7 to ca. 10.0 mm ⁴
Dry weight per cell	Root cap high; 8× increase to 7.0 mm	Root cap high; 10× increase to 4.8 mm	Root cap high; little change
Total N per cell	Low at meristem; 2.5× increase to 8.0 mm, then constant	No data	Low at meristem; 4× increase to 1.5 mm, then decrease
Protein N per cell	Low at meristem; 1.7× increase to 5.0 mm, then constant	Low at meristem; 5× increase to 5.2 mm, then decrease	Low at meristem; increase to 1.7 mm, then decrease (9× increase to 8.0 mm, then decrease) ⁴
O ₂ uptake per cell	No data	Low at meristem; 3.5× increase to 4.4 mm, then decrease to 9 mm	High at root cap; low at meristem, 6× increase to 3.0 mm
O ₂ uptake, μ l/cell/hr.	No data	Meristem: 11.9×10^{-6} . At 2.8 mm (beginning elongation): 27.8×10^{-6} Maximum elongation: 59.2×10^{-6}	Meristem: 6.3×10^{-6} . At 2.8 mm: 32.4×10^{-6}

¹ ERICKSON and GODDARD (1951); ² R. BROWN and BROADBENT (1950); ³ JENSEN (1955a);

⁴ R. BROWN and ROBINSON (1955).

These data are confirmed in all three species. Further, in *Vicia faba*, the meristem is also a region of low total carbohydrate per cell as well as low total glucose per cell. The respiratory activity in terms of oxygen uptake is also the lowest along the root axis on a per cell basis. Although ERICKSON and GODDARD (1951) give no data on this point for corn, GODDARD and MEEUSE (1950) refer to data on corn which show that the lowest rate of respiration on a total nitrogen or protein basis is in the 1—2 mm region, which is the site of maximum mitotic activity. Thus, the meristem region of active cell divisions is dramatically characterized by its relative lack of metabolic activity.

As the cells of the root elongate, the average cell activity as reflected in chemical constituents of the cell, in respiratory activity, and in specific enzyme activity, increases remarkably—to a peak in the region of maximum cell elongation. In

corn and pea roots, dry weight per cell increases 8- and 10-fold from the meristem to the region of maximum cell elongation. In corn, protein nitrogen increases about 2-fold to the region where cell elongation is rapid, then decreases during late stages of cell elongation; in pea, protein nitrogen increases about 5-fold throughout cell elongation, then shows a decrease.

In *Vicia faba* roots, R. BROWN and ROBINSON (1955) show a corresponding increase (9-fold) in protein nitrogen per cell up to about 8 mm behind the tip just before cell elongation ceases, and thereafter a decrease is apparent. JENSEN's data on protein nitrogen in *Vicia faba* roots complicate the picture. He finds an increase from the meristem through the region of radial enlargement and then a decrease when cell elongation begins at 1.7 mm from the tip. BROWN and ROBINSON (*l.c.*) also show an initial decrease in protein nitrogen over the first 2.8 mm and then the marked increase noted above occurs during subsequent cell elongation in a region not measured by JENSEN. It would appear from combining the data that they agree quite well quantitatively where they overlap [*e.g.*, protein N per cell at 2.9 mm from root tip: JENSEN (1955a) = 0.9×10^{-10} gm; BROWN and ROBINSON (1955) = 0.5×10^{-10} gm]. The initially low protein nitrogen per cell in the meristem increases in the first millimeter during blocking out of the primary meristematic tissues at the stage of radial enlargement of the root cells, decreases in early cell elongation and then shows a much more marked increase during later stages of cell elongation. A decrease in protein nitrogen per cell in the later stages of cell elongation or as elongation ceases is then followed by a leveling of the protein nitrogen content per cell during cell maturation.

JENSEN (1958a) has found in onion root that the early rise in protein nitrogen which accompanies radial enlargement of the cells of the root levels off at the beginning of cell elongation and thereafter protein nitrogen per cell increases rapidly with cell elongation. KOPP (1948) indicated that in wheat roots, as much as half of the protein synthesis of the cell occurred during cell elongation. WANNER (1950) using onion roots found a continuous rise in total nitrogen per cell which reached a peak in the region of maximum elongation. Thus it appears that a marked increase in protein nitrogen per cell is a characteristic feature of cell elongation.

It is interesting to note that oxygen uptake per cell in pea roots (R. BROWN and BROADBENT 1950) parallels the increase in protein nitrogen of the cell. Both reach a maximum in the region of cell elongation and then drop off together in later stages of cell maturation. JENSEN also found an increase in oxygen uptake per cell in bean roots during early cell enlargement. He did not find, however, that respiratory activity dropped off accompanying the early drop in protein nitrogen during radial enlargement. Rates of oxygen uptake per cell in pea and bean meristems show remarkable agreement [pea (BROWN and BROADBENT *l.c.*) = 11×10^{-6} μ l/hr.; bean (JENSEN 1955a) = 6.3×10^{-6} μ l/hr.].

Tracer experiments. JENSEN (1957) and CLOWES (1958b) contributed to more detailed understanding of protein synthesis by following the incorporation of radioactively-labelled precursors into protein in growing root tip cells. Data from these experiments agree in general with those already discussed above. Of particular interest is the fact that some idea of the differences in rates of protein synthesis (incorporation of label) can be gained with respect to different tissue types or even individual cell types. When fully exploited, this technique may be of considerable help in unravelling problems of cell differentiation.

Relation between metabolic activity, elongation, and enzyme activity. The data summarized and to some extent analyzed above would lead one to believe

that there exists a direct and perhaps causal relationship between root cell elongation and increasing metabolic activity as manifested by increase in dry weight, total nitrogen and protein nitrogen especially, and increased respiratory activity.

BURSTRÖM (1951) in experiments with wheat roots treated with auxins and anti-auxins, investigated the relation between epidermal cell elongation and dry weight and nitrogen changes of whole roots under different conditions of root stimulation and inhibition. He interpreted his data as showing that cell elongation was not related to or dependent upon dry weight production or protein synthesis. He also found epidermal cell elongation to proceed independently of the total amounts of nitrogen-free organic compounds in the roots which included cell wall materials. Recently, THIMANN and LOOS (1957) have reinterpreted BURSTRÖM's data and believe they show that some protein synthesis does in fact accompany increase in cell volume. ELIASSON (1955), also using wheat roots, reported that increase in cell length is accompanied by a corresponding, but much smaller, increase in protein nitrogen. These data do not, however, allow an expression of events in terms of average cell changes and cannot be interpreted critically in this way. From the more critical evidence discussed above, there is good evidence that protein nitrogen per cell does show an over-all increase during cell elongation in the root.

R. BROWN and ROBINSON (1955) and ROBINSON and BROWN (1952) have examined a number of enzyme systems in bean roots, including, in particular, phosphatase, dipeptidase, invertase, glycine oxidase and proteinase. All of these enzymes had very low activity in the meristem and showed varying degrees of increasing activity (3-fold, 5-fold, 25-fold, 4-fold and 6-fold increases on a cell basis respectively) from the meristem region to the region of maximum cell elongation. They were interested in attempting to relate enzyme activity to the process of cell growth and differentiation in the root. These ideas are discussed elsewhere in this volume¹ and detailed results are given later in this chapter (p. 1294). Here, it is of interest to note that measurements of total nitrogen and protein nitrogen and their changes along the developing root, although probably a reflection of a total metabolic activity at any level, may not and, in fact, usually do not result from parallel changes in the enzymatic systems present in the tissues, but result rather from the integration of complexly varying levels of enzyme quantities which change continuously during the elongation process. Thus, in any case where a direct relationship between root cell elongation and protein nitrogen is demonstrated, the further establishment of a causal relationship must depend upon elaboration and understanding of the detailed changes in metabolic patterns.

b) Gradients in carbohydrates and cell-wall constituents.

Of considerable interest in the problems of the physiology of root organization and development are changes which occur in the cell wall materials during the process of root elongation. Here, detailed chemical information is difficult to find, a fact which can be related directly to our ignorance of the composition and chemical nature of the cell wall materials in the root. Some measurements of carbohydrate materials, related either directly or indirectly to the cell wall constituents have been made.

¹ Chapter "Cytochemical changes in cell growth and differentiation in plants" by J. K. HEYES and R. BROWN, pp. 189—212, specifically pp. 199 *et seq.*

Gradients in cellulose and other carbohydrates.

R. BROWN and SUTCLIFFE (1950) measured changes in cellulose content of isolated segments of *Zea* roots from the extension zone under different sugar concentrations. Increasing sugar concentration which stimulated segment elongation, also resulted in greater cellulose synthesis. In the absence of sugar from the external medium, some disappearance of cellulose was reported. Although cellulose synthesis proceeds during segment elongation, it occurs at a rate somewhat slower than that of segment elongation. Complex interrelations among sugar absorption, respiration, cellulose formation and cell elongation were visualized. These changes were not expressed on an average cell basis, but rather on a segment basis.

DYAR (1950) studying starch synthesis in pea root tips found the enzyme phosphorylase had greatest activity in root cap cells, but was also abundant in cells of the meristem and in the procambium. According to DYAR, the type of polysaccharide synthesized was different in the various tissues of the root.

BALDOVINOS (1953) reported that total sugars and cellulose analyzed in 1-mm root segments of corn showed a progressive increase on a cell basis along the length of the root axis which could be correlated in a general way with cell elongation. In his analyses of 200 μ sections of elongating bean seedling roots, JENSEN (1955a) measured total carbohydrate, glucose, and fructose which he expressed on a per cell basis. The root cap cells were very high in starch and cellulose, which is usually evident in seedling roots in the form of large numbers of starch grains. The cells of the apical meristem region were found to be very low in total carbohydrate and in glucose per cell. Although total carbohydrates increase about 2-fold in the region from the meristem to early cell elongation (at 3 mm from tip), the cellulose content remains constant from 1.7—3.0 mm, the region where cell elongation has commenced. Over this region of the root, a non-glucose carbohydrate increases steadily and it is suggested this may be a saccharide, perhaps composed of pentose. In a similar study of carbohydrate changes in the root tips of onion, JENSEN (1958b) reported in this root also that, while total carbohydrates increase paralleling early cell elongation, total hexose sugars increase more slowly, reflecting the formation of non-hexose cell wall components perhaps from pentose residues.

Much more data on the carbohydrate changes during root development are needed before any clear picture will emerge. Especially needed is an understanding in detailed chemical terms of the constitution of root cell walls and changes which occur during cell enlargement.

The root cell wall.

Chemical studies. Increasing attention has centered on the role of the primary cell wall in the process of cell enlargement. This interest is leading to more careful scrutiny of the wall itself, its physical nature and molecular structure. The suggestion has been made (VAN OVERBEEK 1952) that the primary wall of the root cell is fundamentally different from that of the shoot cell and that auxin inhibition of enlargement of root cells as contrasted to stimulation in shoot cells results from the basic differences in the chemical nature of their cell walls. According to KERR (1951) the primary cell wall of shoot cells contains as the main structural framework protopectin, *i.e.*, insoluble, straight, long-chained molecules of anhydro-galacturonic acid units which upon restricted hydrolysis give rise to pectic acid (free of methyl groups) or pectinic acid (partly methylated). The cellulose microfibrils constitute the discontinuous phase of the primary cell wall.

The chemical nature of pectic substances in root cell walls is less clearly specified, although VAN OVERBEEK's suggestion is based on the idea that calcium pectate is the chief form of pectic material in primary root cell walls. There appears to be little experimental basis for this idea however. WOOD *et al.* (1952) using a bacterial extract containing a mixture of pectic enzymes showed that the primary cell wall of turnip root cells contained considerable pectic materials situated apparently between the interwoven cellulose fibrils, but they were unable to indicate the nature of these substances. In a study of the gross cell wall structure of primary cell walls in seedling root tips, WHALEY, MERICLE and HEIMSCH (1952), and MERICLE and WHALEY (1953) reported observing extensive "non-cellulose reacting areas" in the cell walls, especially in cells near the apical region. These areas progressively decreased as the cells matured. The relatively crude chemical methods used in these studies did not lead to information concerning the chemical nature of these areas. CHAYEN (1952) has confirmed in bean root tips the earlier observations that the primary cell wall contains proteinaceous materials.

JENSEN and ASHTON (1960) and JENSEN (1960) completed the most careful analysis of root cell walls due to date, using chemical methods involving differential extraction of cell wall constituents combined with cytochemical localization studies which permit observations of differences in tissues. They report that all cell walls in the 2-mm tip of the onion root are comprised of cellulose, pectic substances, hemicellulose and non-cellulosic polysaccharides (each characterized by different solubility properties in various solvents). Cell morphology appears to depend largely on the presence of cellulose combined with the noncellulosic polysaccharides. Detailed changes in relative proportions of these components as growth proceeds have been described.

Electron-microscopic studies. Electron-microscopic studies (WOOD *et al.* 1952) of the primary wall of root cells indicate that the cellulose fibrils have the interwoven arrangement typically found in shoot tissues and thus root cell walls appear to be of tubular texture, which, according to FREY-WYSSLING (1953), is characteristic of all meristematic cells. FREY-WYSSLING and STECHER (1951) in an analysis of cell wall growth in parenchyma cells of maize root tips have observed localized areas of "intensive plasm growth" in which the existing microfibrils are displaced. They attributed this effect to localized loosening of the interwoven microfibrillar framework which allows area increase by what they have termed "mosaic growth". In cells from *Avena* coleoptiles, MÜHLETHALER (1950) has found evidence suggesting that cell extension is due primarily to "bipolar tip growth" in which new cellulose microfibrils are laid down in the existing framework of the wall only at the ends of the cell. This scheme would account for his observation that the tubular texture due to microfibrillar orientation does not change materially during cell growth. FREY-WYSSLING (1953) implies that bipolar tip growth may be the usual means for primary cell wall elongation and that the intercalation of microfibrils by intussusception may occur only in special cases such as area increase by "mosaic growth."

Evidence for bipolar tip growth by the primary wall of root cells is at present lacking, although clear evidence for tip growth in root hairs produced by root epidermal cells has been shown by FREY-WYSSLING and MÜHLETHALER (1949). Recent observations by SCOTT and HAMMER (1955) and SCOTT *et al.* (1956) on cell wall growth in onion roots, while confirming the earlier observations relating to the interwoven reticulate pattern of the cellulose microfibrils of the primary wall, suggest that elongation of the wall all along the length of the longitudinal axis does occur as judged from the evident physical separation of pit-like areas

during cell growth. Similar observations concerning surface growth in *Avena* coleoptile parenchyma cells have led CASTLE (1955) and WARDROP (1955) to reject the idea of bipolar tip growth in this tissue. It is clear that further detailed information is necessary before it would be wise to make any generalizations as to the dissimilarity either in structure or function of primary cell walls of root and shoot tissues.

Root hair growth. Pectic substances have been held to be important in root hair elongation (CORMACK 1949), but information available in the extensive literature relating to the physiology of root hair elongation has not generally been brought to bear directly on the problem of the primary cell wall and its elongation. Based on the differential sensitivity of root hairs and root cells to auxin reported earlier by LUNDEGÅRDH (1946), and the presumed difference in the nature of wall extension, *i.e.* apical growth in root hairs as contrasted with intussusceptive growth of the root cell wall, EKDAHL (1953) assumes that root hair elongation and root cell elongation are probably controlled by two different mechanisms. In his studies of the effects of low temperature, of applied auxins, and of osmotic conditions on cell wall extension, he has added further evidence of differences in response of root cells and root hairs. He points out, however, that the properties and composition of the apical walls of root hairs do fit the postulated nature of the primary cell wall proposed by KERR (1951). EKDAHL emphasizes the important role of pectic substances in the root hair wall and the probable significance of pectic enzymes in controlling root hair elongation. BOYSEN JENSEN (1954, 1955), on the other hand, has emphasized the role of cellulose-building enzymes in root hair growth and believes that IAA and other active reagents influence the localization of these enzymes and thus the growth pattern which follows treatment. It is reasonable to expect that further investigation into the mechanism of root hair elongation may contribute directly to our understanding of cell wall extension, but caution must be exercised in extrapolating information obtained from root hair elongation to interpretation of root cell elongation or to cell elongation in the *Avena* coleoptile or in shoot tissues in general.

c) Gradients in nucleic acids.

Analytical studies. Several analyses of the nucleic acid content along the length of the root axis have been made¹. HOLMES *et al.* (1955) determined DNA and RNA in root segments at 0—2, 2—4, 4—8, 12—20 mm from the tip of *Vicia faba* as well as cell number per segment and expressed their results on a per cell basis. They found that the values for deoxyribose nucleic acid phosphorus which were low in the region of the meristem increased from about 3.5×10^{-12} gm/cell to about 4.5×10^{-12} gm/cell in the region approximately 6 mm behind the tip, then remained essentially constant to about 16 mm. Ribose nucleic acid phosphorus on the other hand went from a low value in the meristem of 7.2×10^{-12} gm to a peak of 9×10^{-12} gm at 6 mm from the tip and dropped off to 8.3×10^{-12} gm at 16 mm. They concluded that the 30% increase in DNA-P was attributable to the presence of an appreciable number of tetraploid cells in the older tissues.

JENSEN (1958a) determined nucleic acid content in 200 and 100 μ sections of root tips of *Vicia faba* and *Allium cepa*. His data, expressed on a per cell basis, refer only to the terminal 2—3 mm; they cannot be compared directly with the data of HOLMES *et al.* In both roots he finds a low value for DNA at the region of the apical initials and then an increase to about double the meristem value at approximately 1 mm behind the meristem which remains constant in the root

¹ See also chapter by J. K. HEYES and R. BROWN, specifically pp. 203 *et seq.* and Fig. 16—18.

length measured. DNA values in the meristem were about $20\text{--}30 \times 10^{-12}$ gm/cell and increased to about 60×10^{-12} gm/cell by 1 mm. In onion, the meristem value for DNA was about 40×10^{-12} and increased to about 80×10^{-12} gm/cell. JENSEN points out that, on the basis of his quantitative data in *Vicia faba* roots, the low DNA value in the root meristem probably represents the $2n$ or diploid DNA value; the doubled value is in turn referable to either frequent cell divisions (with concomitant doubled DNA values prior to division) or to polyploidization. The DNA value of HOLMES *et al.* (1955) for the terminal 2-mm tip then represents an average value close to the tetraploid DNA level which, further back along the root, shows a still further increase. This increase may also be attributable to polyploidy above the $4n$ values of DNA.

JENSEN (1958a) reports a large increase in RNA in both roots studied, a 5-fold increase from tip to elongating region in *Vicia faba* and approximately 3-fold in onion. It is interesting that the protein nitrogen per cell in the bean roots initially follows the RNA increase but drops off strongly when RNA levels off in the elongating zone. In onion, the RNA values parallel rather closely the changes in protein nitrogen.

Tracer experiments. The above analyses confirm the observations of CLOWES (1956a) to the effect that the meristem region of the root has a low RNA content and a low rate of synthesis of DNA, facts which CLOWES relates to the relative inactivity of the meristem region and the "quiescent zone" itself. CLOWES (1959b) fed roots of *Zea* tritiated thymidine and counted silver grains over nuclei in sections with autoradiographs. By this method, an estimate of relative incorporation into DNA could be made cell by cell. He found vessel elements had very high levels of incorporation compared to surrounding tissues at the same level. PELC and LA COUR (1959), observing similar differences in incorporation with high levels of activity in the cortical cells of maturing tissues of roots of *Vicia faba* interpreted this incorporation as due to turn-over. TSCHERMAK-WOESS (1960) has presented good evidence that this increase in DNA in differentiated cortical cells is due to endomitotic reduplication. JENSEN *et al.* (1960) confirmed these general results of CLOWES and also concluded that DNA doubling in developing root cells was associated with endomitosis. Discussion of the implications of the changing DNA values along the axis of the root will be deferred until Section V concerned with cytological observations on the root (see p. 1301 *et seq.*).

d) Gradients in specific enzyme activities.

Intimately related to the gradients in chemical constituents which have been discussed are the observed gradients in activity of a number of different enzyme systems studied in developing roots. Perhaps the greatest information is available concerning respiratory enzyme systems, especially oxidase systems. In many cases, the published data relate only to activity per segment and therefore are misleading as concerns activity per cell. In other cases, histochemical localization serves to indicate local cellular activities at least at a crude level.

Respiratory enzymes. Careful detailed analyses of the activities of the important terminal respiratory enzymes along the length of the developing root have not yet been made. It may be that cutting damage involved in the use of very small root segments, *e.g.*, $50\text{--}200\mu$ would introduce too large an error to make direct manometric studies meaningful. However, several studies of respiratory enzyme activities, using 1 cm root tips or segments, have been made. These studies point up the very interesting fact that root development during seedling growth may involve a very marked transition from one set of respiratory enzymes

to another. Thus, studies of such enzymes must be made—not only along the length of the seedling root at a given stage in root development, but at different times during development. JAMES (1953) using selected inhibitors over a range of concentrations, could show that although in whole barley embryos the major electron transfer was mediated through a cytochrome system, in root tips of seedlings, electron transfer was mediated through the terminal oxidase system, ascorbic acid oxidase. JAMES and BOULTER (1955) analyzed this transition during barley development and showed that although cytochrome oxidase is functional initially throughout the seedling root its activity largely disappears by the sixth day of germination, having been gradually replaced by ascorbic acid oxidase. Such a change involves a transition from an iron to a copper enzyme system. At intermediate stages of root development, a portion of the total electron transfer in the respiring roots is apparently carried by each system. By the sixth day, direct observation of seedling roots for absorption bands of the reduced cytochrome showed their complete absence. The disappearance of the cytochromes seems to occur first in the older parts of the root and then progressively into the tip region. Apparently, there is not a complete disappearance of cytochrome oxidase (FRITZ and BEEVERS 1955, HONDA 1955a) but a striking reduction in the cytochrome system as a whole seems to occur. NORRIS and FOHN (1959) have demonstrated in onion root tips the presence of cytochrome oxidase, although they did not follow quantitative changes in its activity. Because of its postulated role in the process of salt accumulation and ion transport, the cytochrome system and its cytochemical localization in root tissues has received considerable attention. The subject has been reviewed recently by LUNDEGÅRDH (1955).

It may well be that other enzymatic systems in roots change during development. Thus, BEEVERS and GIBBS (1954a, b) have shown that in young root tips of corn seedlings, glucose utilization proceeds entirely *via* the EMBDEN-MEYERHOF-PARNAS (E.M.P.) glycolytic sequence; in old root systems of peas, approximately half of the glucose utilized during respiration was broken down via the hexose monophosphate shunt. In roots of castor bean GIBBS and BEEVERS (1955) were able to demonstrate clearly the transition from the E.M.P. glycolytic sequence to the direct oxidation of glucose during the differentiation and aging of the tissues during root development. Here again where alternate pathways for metabolic activities are possible, it seems possible that the relative activities will change during development. It thus becomes important in all studies on root development, in terms of the biochemical processes involved, to study linear gradients for distribution of activity at many different stages of development and ages of plant life. It would be of considerable interest to study possible correlations between transitions in biochemical systems and morphological changes which occur during root development such as, for example, the first initiation of lateral roots during seedling root elongation or the initiation of vascular cambium.

Ascorbic acid oxidase. Ascorbic acid oxidase has been of considerable interest and the subject of some careful studies particularly in relation to root cell elongation. NEWCOMB (1951) noted a striking increase in ascorbic acid oxidase activity in both root and shoot tissues preceding auxin-induced cell enlargement. Enzymatic activity was localized in the cell wall fraction of tissue preparations and it was suggested that the enzyme might play a specific role in the active growth of the primary cell wall. HONDA (1955b) was able to demonstrate in the roots of 5-day-old barley seedlings an active ascorbic acid oxidase which was localized in the cell wall fraction of tissue homogenates. These observations are interesting in view of an earlier study by REID (1941), who found a direct correlation be-

tween ascorbic acid content and cell size in the developing seedling root of cowpea. She found that the relative cell surface areas at different stages of development corresponded closely to the ascorbic acid concentration, suggesting that the vitamin was functional at the cell surface. JENSEN and KAVALJIAN (1956) have shown in roots of *Vicia faba* the localization of ascorbic acid on cytoplasmic particles of submicroscopic size associated with the cell surface in elongating cells. The possible roles of ascorbic acid and the corresponding oxidase system in cell wall extension in the root, as well as a possible role in salt accumulation (see HONDA 1956) remain to be clarified.

Peroxidase. The enzyme peroxidase is widely distributed in plant tissues and, in the presence of hydrogen peroxide, oxidizes a wide variety of phenolic compounds. Its presence in horseradish (*Armoracia rusticana*) roots is well known. Recent studies of its distribution in roots have been made. PILET and GALSTON (1955) found in roots of *Lens culinaris* that peroxidase activity was low in the root cap cells, relatively high in the region of the meristem and then, expressed on a protein-nitrogen basis, showed a steady decline along the length of the root. JENSEN (1955b) carried out a detailed study of the histochemical localization of peroxidase in the roots of *Vicia faba*. He reported high peroxidase activity in the cells of the root cap. Root sections in the meristem itself showed low activity, then two peaks of peroxidase activity behind the root meristem—one in the region of first differentiation of primary meristematic tissues, *i.e.*, proto-derm and procambium, and a second peak at the level of first protophloem maturation. By histochemical localization techniques, JENSEN showed that the meristem region and cortical tissues of the root showed no peroxidase activity, but that the procambial region was especially high in activity. The intensity of this activity could be increased, especially in the future vascular tissues, by treatment of the tissues with indoleacetic acid, with maximum effect at 10^{-7} M. It was suggested that the peroxidase system may be involved in lignin formation in these tissues, and that IAA, in turn, may play some role in the process. The observations of TORREY (1953) on the acceleration of maturation of primary xylem in pea roots following IAA treatment might be explicable in such terms, although direct evidence for such a relationship is lacking.

IAA oxidase. The oxidative system which causes a destruction of exogenously supplied IAA was initially described as extremely active in root tissues (TANG and BONNER 1948) especially of pea seedlings, but also seedling roots of *Avena* and spinach. Many studies of IAA oxidase in roots have been made (*e.g.*, WAGENKNECHT and BURRIS 1950, GALSTON and DAHLBERG 1954, ÅBERG and JÖNSSON 1955, PILET and GALSTON 1955, PILET 1957). GALSTON and DAHLBERG (1954) analyzed for IAA oxidase activity in 5-mm segments of seedling pea roots. They reported that, on a protein-nitrogen basis, the meristem region (terminal 5 millimeters) showed much lower auxin-destroying activity than in the older tissues and that greatest oxidase activity was found in the region of cell maturation. Prior incubation of the root tissues in IAA or other auxins or even "anti-auxins" (2,4-D, NAA, TIBA) caused a marked enhancement of IAA oxidation, which the authors discussed in terms of induced enzyme formation. ÅBERG and JÖNSSON (1955) although finding a marked IAA oxidation in pea root sections, attributed the activity particularly to cut surfaces rather than cells of intact roots. PILET and GALSTON (1955) examined the IAA oxidase system in sections of seedling roots of *Lens*, using 6 segments of 3 mm each and determining the oxidase activity on tissue breis. They found that root cap cells were high in IAA oxidase, the cells of the meristem were of low activity, expressed on a protein-nitrogen basis, and

that progressively back from the meristem, IAA oxidase activity increased, paralleling increase in cell size. The genesis of peroxide by the same root segments was found to parallel the increase in IAA oxidation. Peroxidase activity in the same tissue, on the contrary, showed a progressive decrease. The authors concluded that IAA oxidase increases with increasing age of cells which is apparently due to an increased peroxide-generating capacity in ageing cells. Because of a stimulus to both peroxide-formation and IAA-oxidation by young cells following treatment of the tissues with 2,4-dichlorophenol (DCP), the authors speculate about the possible existence of a natural DCP-like factor which upon increasing with age during cell development controls cell maturation processes and concomitant cessation of growth. No direct evidence for such a factor was given. PILET (1957) has further shown in the same tissue that if one determines IAA oxidase activity of the root tip of progressively older roots, that this enzyme is at its lowest activity when the roots are elongating most rapidly, thus further emphasizing the idea that IAA oxidase activity seems to be inversely correlated with cell elongation.

One great difficulty in determining the physiological role of the IAA oxidase system in root development is that the enzyme system appears to be, in fact, a complex of enzymes which still remains poorly defined. It is therefore difficult to ascertain the specific roles, if any, of the complex in cell elongation phenomena or to distinguish real effects from artifacts. The idea of control of root development by an enzyme system which destroys the substance which is postulated to control cell elongation in the root is of considerable interest, but further elucidation of the enzyme system itself will be necessary before any valid conclusions concerning its role in root organization and development can be made. Reference will be made later to the intimately related problem of determining the role of auxin in root development.

Dehydrogenases; sulfhydryl groups. Analytical studies of dehydrogenase systems and their distribution in roots have not been made, although the presence of dehydrogenase enzymes in roots is known and is to be expected (LATIES 1949, see also reviews by AXELROD and BEEVERS 1956, VENNESLAND and CONN 1952). The work of ROBERTS (1950) on the histochemical localization of reducing activity in a number of different roots, based on the reduction of 2,3,5-triphenyl-tetrazolium to the insoluble formazan, presumably by dehydrogenase enzymes, suggests that there exist marked longitudinal as well as radial gradients in the capacity of the root tissues to effect this reduction. Although this work cannot be related in any way to specific enzyme systems, it did make apparent the fact that the region of the root meristem is high in reducing capacity and that the procambium and later pericycle tissue are likewise particularly active in producing the formazan. The work, which is essentially a preliminary survey, suggests that a detailed analysis of dehydrogenase systems and their distribution would be of considerable interest. Here again, however, analysis of activity expressed on a cell basis is most essential.

The presence of sulfhydryl groups, usually considered to be the active group in dehydrogenases, in the meristem region of the root was noted by HAMMETT and CHAPMAN (1938). Using the nitroprusside test for —SH groups in segments of seedling roots of *Phaseolus vulgaris*, these authors showed highest concentration in the terminal 1.5 mm of the root and progressively less activity along the length of the root to about half the activity at the region of beginning cell elongation. They concluded that —SH activity was directly related to the process of cell division in the root tip. More recently, GOAS (1955) measured —SH distribution along the length of roots of *Vicia faba*, *Pisum sativum* and others, using root

sections and determining —SH activity with mesoxalic acid. She also reported a high —SH concentration in the first 2-mm tip region with a reduction to 75 % in the region of cell elongation and then to 25 % of the tip value in the region of the mature root. PILET (1957), using the same procedure for determining —SH concentration in segments of *Lens* roots, found that sulfhydryl concentration on a section basis was high at the tip and decreased back from the tip as had been found by others. However, when expressed as activity per milligram nitrogen, the tip region was relatively low in —SH and the peak in activity was reached at about 6 mm behind the tip where cell elongation occurs, and then dropped off in the region of the older tissues of the root. Although the data are not expressed on a cell basis, it is reasonable to relate —SH concentration per milligram nitrogen directly to activity per cell, judging from the studies discussed earlier. On this basis, it becomes evident that, as with many other biochemical and physiological activities already discussed, the greatest metabolic activity is in the region where cells elongate. The earlier data cited above (HAMMETT and CHAPMAN, GOAS) by expressing results on a section basis, conceal the fact that on a cellular basis the tip is relatively less active than the region where cells elongate. Similarly, ROBERTS' work using histochemical localization may lead to an erroneous conclusion — because the density of the stain in the tip region, where cells are exceedingly small and numerous for a given tissue volume, is greater than in the elongating region where the cells are less numerous per tissue volume and the stain is "diluted" in these elongate cells. It seems most probable that careful detailed analyses will show that sulfhydryl concentration per cell is low in the meristem, reaches a peak in the region of cell elongation and then decreases thereafter.

Enzymes of protein metabolism. Enzymes concerned in protein metabolism have been examined in certain root tissues. The most extensive studies of changing enzymatic activities along the root axis are those of R. BROWN and ROBINSON (1952—1956). In these studies¹, several enzymes were assayed and found to vary widely in their relative activities along the root axis. The activity of each enzyme, therefore, did not vary directly with the change in total protein of the root. Among other enzymes studied, ROBINSON and BROWN (1952) reported that dipeptidase (hydrolyzing alanyl-glycine) in broad bean root showed low activity on a cell basis in the region of the meristem; the activity increased at a regular rate to about 8 mm behind the root tip and then dropped off gradually to about 12 mm remaining more or less constant thereafter. The increase in dipeptidase activity did not reflect the initial drop in protein nitrogen per cell evident in the 3-mm tip region, but paralleled subsequent changes in protein nitrogen. Data were reported earlier for the same enzyme by BOTTELIER *et al.* (1943) in the roots of barley. Their more detailed analysis of the terminal 3-mm region showed that the root cap cells were low in dipeptidase activity, the cells of the meristem were highest in peptidase per unit nitrogen, and thereafter the activity dropped off to 3 mm. Thus, peptidase activity declined with increasing age of the cell up to the 3-mm level. They also found that peptidase activity was less at all levels in progressively older roots. The data of ROBINSON and BROWN (1952) do not reflect the drop in peptidase activity in the terminal 3 mm, but show a continuous increase to 8 mm—which represents approximately a 5-fold increase in activity. It would seem that conclusions as to the changing activity of this type of enzyme with age of cell must await further, more detailed analysis.

ROBINSON (1956), also using broad bean, examined 1.0-mm isolated root segments for proteolytic enzymes. The activity of any proteolytic enzymes present

¹ See also chapter by J. K. HEYES and R. BROWN, specifically p. 199 *et seq.* and Figs. 9—13.

in root cells is of interest in the light of the frequently observed decline in total protein nitrogen per cell in older root tissues. ROBINSON obtained evidence for two proteolytic enzymes or groups of enzymes with different p_H maxima; these enzymes showed little or no activity in the apical meristem region, increased in activity in the region of cell elongation and reached a maximum activity 10 to 12 mm behind the tip in a region where protein in the cell has begun to decrease. It is suggested that the protein level of the cell is decreased by the relatively high activity of the proteolytic enzymes, thereby affecting cell growth since the protein level appears to be a controlling factor in root cell elongation.

ROBINSON and BROWN (1952) also examined the distribution of the enzyme glycine oxidase along the root axis in broad bean roots. The activity of this enzyme parallels closely the changes in protein nitrogen, showing a minimum activity in the meristem region and an increase in either direction—into the root cap and back along the length of the root, reaching approximately a 2-fold increase at 8 mm and then dropping off sharply. No direct significance in root development was attributed to the changes in activity of this enzyme.

CLOWES (1958b), using a completely different method of analysis, has also observed a gradient in enzymatic activity related to protein synthesis. Following comparative rates of incorporation of sulfate and L-leucine into proteins by autoradiography of cells of root apices of *Zea mays*, CLOWES found a very low level of incorporation in the quiescent center and the immediate cells proximal to it. Further from the tip the rate of incorporation rises to as much as 50-fold at 3mm from the tip. Different root tissues showed different rates of incorporation.

Acid phosphatase. Acid phosphatase which is active in hydrolyzing a variety of phosphate esters in biological systems has been studied in root tissues, especially with reference to their activity in hydrolyzing sugar phosphate esters. DYAR (1950) found greatest activity in the root cap cells of pea roots, especially associated with cell walls. In the meristem region, cytoplasmic granules appeared to contain the activity. JENSEN (1956) using roots of onion, bean and pea, was able to establish the localization of acid phosphatase in the mitochondria of the cells. The cells of the root cap nearest the meristem and the cells of the developing vascular tissue were most active. ROBINSON and BROWN (1952) studied the distribution of acid phosphatase activity along the bean root axis. They also found high activity in the region of the root cap, which decreased in the meristem region and then increased about 4-fold on a cell basis to the region 8 mm behind the tip, and thereafter declined sharply. These changes paralleled closely the changes in protein nitrogen per cell¹. The carbohydrate metabolism of root cap cells appears to be most interesting and may be associated in some way with the rapid and irreversible course followed in their cellular differentiation.

Radial gradients in enzyme activity. Although it is reasonable to expect the existence of radial gradients in specific enzyme activities in developing roots, it is unnecessary to emphasize the difficulties involved in obtaining quantitative data on these gradients, let alone even demonstrating their existence. Certain histochemical procedure have been used which support the idea that such gradients do exist. The experiments of ROBERTS (1950) using tetrazolium show clearly that radial patterns in reducing capacity certainly must exist in roots and that these patterns change during the course of development. Similarly the work of JENSEN (1955b) on peroxidase distribution suggest radial patterns of distribution which may be related to vascular tissue differentiation.

¹ For more details, see chapter by J. K. HEYES and R. BROWN, specifically their Fig. 11 on p. 200.

VAN FLEET (1942) studied the oxidation of a number of "redox indicators" in reference to the delimitation of the endodermis and was able to show that the anatomical features of the endodermal layer were associated with the presence of unsaturated fatty acid peroxides in the tissues, which upon oxidation led to deposits of suberin and other condensation products of unsaturated fatty acids. VAN FLEET (1952, 1954) has reviewed a number of other studies in which radial gradients of enzyme activity can be demonstrated and levels of activity localized topographically at least by histochemical methods. In most cases the available information is qualitative since the methods are difficult to use quantitatively. These studies do, however, point the direction for more detailed studies as critical methods become available.

Concluding remarks. With the availability of sensitive autoradiographic methods of following metabolic activities, especially syntheses as shown by incorporation, it now is possible to examine a variety of biochemical systems and their occurrence in tissues and cells. This technique should become a potent tool in the study of physiological gradients within plant tissue systems. CLOWES (1958b) stated that he could distinguish differences between tissues in rates of incorporation of amino acids or DNA-precursors labelled with tritium by this method and indicated that quantitative data of this nature could be obtained readily. Such information may well be the sort necessary for understanding the physiological bases of tissue organization.

IV. The synthetic capacities of roots in relation to root development.

1. Growth substances.

a) Auxin.

Auxin content of roots. Root elongation has been generally explained in terms of cell elongation directly comparable to shoot cell elongation, except that root cells, being more sensitive to auxin concentration, are inhibited by auxin concentrations that stimulate shoot cell elongation (WENT and THIMANN 1937). This view has been brought into question in recent years (*e.g.* AUDUS 1954) and interpretations of many phenomena of root development are not always easy in these terms. It is interesting to re-examine the ideas concerning the distribution of native auxins in roots and their site of synthesis with a view to understanding the role of auxin in root organization and development. One must be concerned not only with the role of auxin in root cell elongation, but also with the possible interaction of auxin in cell division, cell differentiation and the multiplicity of events associated with root development.

The evidence concerning the synthesis of auxins by roots, or if such synthesis occurs, the site of its formation, is far from complete. THIMANN (1934), using chloroform extraction of *Avena* seedling roots and testing the extracts for auxin activity with the *Avena* coleoptile curvature test, showed that auxin concentration was highest in the terminal 10 mm root tips and decreased progressively back from the tip. The amount of auxin in the terminal 10 mm of the root was equivalent to about one-third of that present in the 5 mm tip of the coleoptile. THIMANN suggested that the root need not produce the auxin, but that it may merely accumulate in the root from the shoot. A similar gradient in auxin distribution has been reported by many authors (BOYSEN JENSEN 1936, GORTER 1936, NAGAO 1936, PILET 1951a, b). According to BOYSEN JENSEN (1936), auxin in corn and

bean roots is confined largely to the terminal 6 mm, but recent studies indicate that almost all root tissues contain some auxin.

More recent analyses of root tissues using chromatographic methods (*e.g.*, BENNET-CLARK and KEFFORD 1953, 1954, LEXANDER 1953, BRITTON *et al.* 1956, AUDUS and THRESH 1953; see also GORDON 1954) show that auxin-type compounds are to be found generally in root tissues. The exact chemical nature of these compounds and their distribution and site of synthesis remain to be discovered.

Auxin synthesis and auxin transport. Considerable indirect evidence for auxin formation or perhaps the release of "free" auxin in the tip region came from a number of different reports on the effect of root tip decapitation on auxin synthesis (see reviews of subject by VAN RAALTE 1937, WENT and THIMANN 1937, ÅBERG 1957). In these studies, diffusible auxin and ether-extractable auxin concentrations were determined. The fact that much larger amounts of auxin could be collected from excised 5 mm root tips of bean or corn standing on glucose-agar than on plain agar has suggested that formation of freely diffusible auxin occurred. The role of glucose in stimulating release of auxin is not completely clear, but VAN RAALTE (1937) concluded that glucose served some role other than an osmotic one in stimulating auxin release.

VAN OVERBEEK (1939) followed auxin formation in isolated roots of peas cultured in a synthetic nutrient medium. Using 24-hour, cold ether extraction of auxin from initial tips at the beginning of culture and from root bases after each sub-transfer, VAN OVERBEEK showed there was approximately a 9-fold increase in auxin over a 20-week period of weekly transfers. He concluded that auxin synthesis by the excised roots growing in culture had occurred. Even taking into account a possible release from bound auxins in the original root tips, the evidence favors the idea that some auxin formation does in fact occur in cultured pea roots. The site of synthesis is not established by these experiments.

Most lines of evidence suggest that *movement* of auxin in the root proceeds chiefly from the root tip toward the root base (see ÅBERG 1957, WENT and THIMANN 1937), but further critical studies of this movement are desirable, in which surface leakage of auxin along root sections is prevented and the possibility of conversion from bound auxin is taken into account. If a basipetal polarity in auxin movement in roots does exist, the existence of a gradient in auxin distribution would suggest that auxin formation occurs in the tip region where it is continuously replenished.

Effects of auxin application. The requirement for an exogenous supply of auxin by isolated cultured roots of certain species suggests that auxin formation by the root may be suboptimal or lacking completely. STREET (1957) in his review discussed a number of cases in which excised roots in culture are stimulated by the addition of indoleacetic acid to the medium (*e.g.* roots of pine, white lupine, pea, corn, rye, wheat). On the other hand, STREET (1954) found that survival of meristems of tomato roots in continuous culture was reduced by addition of IAA to the medium. The auxin antagonist, α -(1-naphthylmethylsulfide-)propionic acid, actually stimulated root elongation and enhanced meristem survival. STREET interpreted these effects as indications that in tomato roots in culture there is an accumulation at the apex of some natural auxin which reaches a supra-optimal concentration for root development, leading to reduced elongation and ultimate death of the meristem. Analysis of excised tomato roots in culture for auxin (BRITTON *et al.* 1956) has shown that non-indole water soluble substances are extractable from the roots and possess considerable growth substance activity. Thus, the auxin picture is perhaps more complex than had been anticipated.

Most roots are, of course, very sensitive to externally supplied auxins. At extremely low auxin concentrations, roots are stimulated in their elongation and at progressively higher auxin concentrations are inhibited. The interpretation of these effects of externally applied auxins is difficult in view of our present lack of knowledge concerning the role of endogenous auxins. The subject has been extensively reviewed (see BURSTRÖM 1953, TORREY 1956b, ÅBERG 1957).

b) Gibberellins. Kinetin.

Occurrence of gibberellins in roots. Our knowledge on effects and the possible role of the gibberellins in root growth and development is still highly fragmentary. While the presence of gibberellins and gibberellin-like substances (*i.e.* substances having the physiological activity of gibberellins but not known chemically) in shoots and seeds has been demonstrated in many species of higher plants, only one such effort seems so far to have been made in roots. MURAKAMI (1960) showed that the roots of rice and maize contain gibberellin-like materials; the levels, as far as they can be judged from the semiquantitative data, are of the same magnitude in shoots.

Effects of gibberellin treatment on root growth in whole plants and seedlings. Treatment of whole plants with gibberellin usually results in a decrease of the root weight and an increase of the shoot-root ratio (*e.g.*, BRIAN, ELSON *et al.* 1954) but this may be mainly a correlative effect, caused by the marked promotion of shoot growth which is the most general gibberellin effect. BRIAN, HEMMING and RADLEY (1955) found that gibberellin A₃ (gibberellic acid, GA₃) in concentrations of 0.1–10 mg/l had no significant effect on root growth in *Lepidium* seedlings. RICHARDSON (1958), in contrast, reported a definite promotion of early root growth in seedlings of *Pseudotsuga menziesii* by GA₃ concentrations of 3–10 mg/l. *Pseudotsuga* seedling roots are markedly inhibited by light, and the promotive effect of GA₃ was much more pronounced in light than in darkness; this is reminiscent of the gibberellin responses of shoots of many light- and dark-grown seedlings (*e.g.*, LOCKHART 1959). An initial stimulation of radicle growth was also observed in *Pinus lambertiana* embryos cultured *in vitro* (C. L. BROWN and GIFFORD 1958).

Effects of gibberellin on the growth of excised roots. WHALEY and KEPHART (1957) were apparently the first to study the effect of gibberellin on the growth of excised roots. Using seedling root tips of two inbred strains of maize and their hybrid, they found promotion in one of the strains and the hybrid and no effect in the other parent strain. The effective GA₃ concentrations were 1–20 mg/l.

LEE (1959) studied the effect of GA₃ and other growth regulators on two tomato root clones, one carrying the dwarf gene *wd* and characterized by a relatively low growth rate, the other carrying the normal allele (*wd*⁺) and having a higher growth rate. GA₃ had no marked effect on the *wd* roots but *inhibited* the *wd*⁺ roots even at a concentration as low as 0.02 mg/l. Auxin (IAA) and some “anti-auxins” had no promotive action. In considerable contrast to these results, BUTCHER and STREET (1960; see also STREET 1959), also using excised tomato roots but of a different genotype, found clear promotive effects of certain GA₃ concentrations on the growth of both the main and the lateral roots; higher concentrations were inhibitory. The promotive effect on the main root was due to a stimulation of both cell division and cell elongation. It was, however, evident only on media low in sugar (less than 1% saccharose) and was accompanied by an acceleration of the loss of meristematic activity (ageing) which

is a normal feature of these roots (see p. 1316)¹. The gibberellins A₁, A₂ and A₄ had much the same effects as GA₃. 1-Naphthaleneacetic acid also had similar effects, but in a much narrower range of concentration. 1-Naphthoxyacetic acid, which antagonizes auxin inhibition of growth in excised tomato roots, thus acting as an "anti auxin", *enhanced* the growth inhibition of higher GA₃ concentrations, even in concentrations which alone are stimulatory to root growth. STREET concluded that if the endogenous auxin of the root is supraoptimal gibberellin enhances the "ageing" process and that it is similarly inhibitory if the auxin is markedly suboptimal (as when anti-auxin is applied), but that at intermediate auxin levels it may stimulate the growth of the root.

While it is clearly premature to draw definite conclusions, the work with excised roots suggests that gibberellins may, after all, intervene in root growth, that the effect may depend on the nutrient level, and that, as in shoot growth, there may be an interaction with auxins.

Kinetin effects. LEE (1958) observed that kinetin (at 0.001 mg/l) stimulated the growth of both *wd* and *wd*⁺ tomato roots (see above) *in vitro*, but in a non-differential manner. STREET (1959) noted that kinetin was stimulatory to the growth of excised tomato roots in high-saccharose media.

2. Synthesis of vitamins.

a) Thiamin.

From studies with isolated roots grown in continuous culture, information has accumulated concerning the capacities of roots to synthesize the particular vitamins and growth factors essential to their growth. Most interesting is the lack of capacity in roots to synthesize vitamin B₁ (thiamin; see p. 1264 *et seq.*). With very few exceptions isolated roots in culture require thiamin. This vitamin is apparently synthesized in the green leaves of the shoot system and provided to the root *via* the phloem (BONNER 1944). BONNER and BUCHMAN (1938) showed that pea roots were capable of synthesizing the thiamin molecule from the two components—the pyrimidine and thiazole moieties. An enzyme, thiaminase, is conceived to catalyze this reaction. Pea roots also contain the necessary enzyme complement to effect ring closure from certain acyclic precursors of thiazole. ROBBINS and BARTLEY (1937) found that certain strains of tomato roots grew well if only the thiazole portion of thiamin were provided. McCLARY (1940) reported that excised roots of corn which were grown through a series of transfers (passages) in a synthetic nutrient medium containing only salts and glucose, could be shown to have synthesized thiamin during the culture period. In general, however, roots are either completely unable to synthesize this vitamin, or only at suboptimal levels for growth.

b) Other vitamins.

The requirement of nicotinic acid (niacin), of pyridoxin and of other vitamins for normal development by isolated roots varies greatly in different species or even between strains in the same species. In tomato roots, pyridoxine apparently may be replaced by glycine or a mixture of amino acids, thus bypassing the pyridoxin-requiring step in amino acid metabolism (WHITE 1951, BOLL 1954a; see also STREET 1957). Like thiamin, pyridoxin has been shown to be synthesized in the shoot portion and to move to the root *via* the phloem in the tomato plant (BONNER 1944). Certain roots synthesize riboflavin (BONNER 1942), biotin (LOUIS 1950),

¹ See also chapter "Longevity and ageing in plants and plant organs" by E. WANGER-MANN, part 2, pp. 1026—1057 of this volume, specifically p. 1050.

pyridoxin (BOLL 1954a) and ascorbic acid (WEINTRAUB 1940; see also review by BONNER and BONNER 1948). In fact, most isolated roots show remarkable synthetic capacities since they can, in fact, continue to grow for indefinite periods, forming new plant body from essentially inorganic constituents, sugar and, in most cases, one or a few vitamins. No systematic attempt to localize these synthetic mechanisms within the developing root seems to have been made. The fact that 0.5-mm pea root tips require 10 times the concentration of vitamins needed by 5-mm tips (TORREY 1954) suggests that the mature tissues may be capable of some vitamin synthesis (see also BROWN and WIGHTMAN 1952).

FRIES (1955) made the interesting observation that decotylized pea seedlings grown in the dark require not only thiamin and perhaps nicotinic acid, but also pyridoxine. The latter vitamin is not essential for growth of excised pea roots. FRIES interpreted this result as an indication that the shoot in the dark actually depletes the root of pyridoxine which it is apparently able to synthesize in amounts sufficient for its own needs.

3. Lack of synthetic capacities *in vitro*.

a) Roots of monocotyledons and of woody plants.

There remains a large number of species of plants in which attempts have been made to culture the isolated roots which have consistently failed. Thus, for example, roots of monocotyledonous plants are notoriously difficult to maintain in culture. In such roots, there may well be limitations in development associated with the lack of a certain restricted synthetic capacity within the root about which we have yet to learn. E. H. ROBERTS and STREET (1955) have reported the first successful continuous culture of excised roots of rye, by providing Difco yeast extract. Similar success with rye roots has been reported by ALMESTRAND (1957). In the former study, it was found that autoclaved tryptophane or indoleacetic acid replaced to a large extent the yeast extract requirement, but it was believed that some factor other than IAA was essential. Only 10% of the roots of rye grains tested responded to this medium. Clearly, the complete picture of the requirements of rye roots has yet to be seen. Extensive tests on a number of roots of other monocotyledons were unsuccessful (ALMESTRAND *l. c.*).

Excised roots of relatively few species of woody plants have been grown successfully in culture. BONNER (1942) reported slow elongation of excised roots of *Acacia melanoxylon* cultured continuously for 35 weeks. These roots showed no branching and no secondary thickening *in vitro*. SLANKIS (1948, 1949, 1950) found that excised roots of *Pinus silvestris* could be grown in nutrient medium containing, in addition to the usual essential mineral elements, 7% sucrose and thiamin and choline chloride. Elongation was slow; dichotomous branching could be induced by auxins.

BARNES and NAYLOR (1959a) found that several amino acids could serve as sole nitrogen source for excised roots of *Pinus serotina* grown in nutrient culture. Citrulline and γ -aminobutyric acid were especially effective; both also stimulated the formation of short dichotomous root branches. BARNES and NAYLOR (1959b) also reported the culture of roots of five species of *Pinus*; light inhibited growth in all species and sucrose at a concentration of 8% was best for growth. Glycine, present in WHITE's medium, was inhibitory to growth in all species. The amino-acid composition of the medium was found to be important in achieving good elongation.

SEELIGER (1956, 1959a, b) achieved excellent growth of excised roots of *Robinia pseudoacacia* in a nutrient medium containing 2% sucrose and thiamin,

pyridoxin and nicotinic acid as added vitamins. Root branching was frequent and activity of a vascular cambium was noted in many roots. More attention should be paid to the culture of roots of woody plants which would prove to be both profitable and of great interest.

b) General remarks.

It is conceivable that certain roots lack the capacity to synthesize one or more metabolites essential to development, other than a growth hormone of the auxin type or a vitamin. Although most isolated roots can synthesize amino acids and proteins, as well as purines, pyrimidines and nucleic acids starting with only inorganic salts, vitamins and sugar, certain roots may depend heavily on the shoot for their amino acids or the different purines and pyrimidines and thus be unable to develop in isolation *in vitro*. Further extensive and intensive studies will be necessary before a general understanding of the synthetic capacities and inadequacies of roots is achieved.

The sites within the root of known synthetic reactions are of particular interest with respect to the organization of roots and their development. All indications suggest that the major syntheses of organic constituents occur in the terminal few millimeters of the root tip and especially in the region of rapid cell elongation. The vitamins, which must be supplied to this region in order for the synthetic mechanism to continue, reach the region by a definite restricted pathway, usually *via* the phloem, and there is thereby established a gradient limiting the synthetic system. The possible implications of these gradients on root organization have already been suggested.

BROWN and WIGHTMAN make the further point (1952) that localization of the most rapidly synthesizing root cells in the mature regions of the elongating root establishes a gradient of supply of the products of these syntheses to the root meristem region and to the region where cell divisions are most rapid.

It is clearly desirable that a closer look be given to the actual loci of syntheses along the root axis, especially with respect to the auxins and vitamins, so that their respective roles in root development may be related to their sites of endogenous supply.

V. Cytological observations relating to root organization.

1. Polyploidy and cellular differentiation.

Over the past 25 years there has been evolving an intriguing picture of cytological changes which occur during root development, especially with respect to changes in the chromosomal constitution of nuclei of root cells. These changes, which have been studied extensively by plant cytologists, have been largely neglected by plant anatomists and physiologists. The most striking changes concern the development of polyploid nuclei during cellular differentiation. The subject has been reviewed by LORZ (1947), D'AMATO (1952b) and GEITLER (1953)¹.

In the region of the root meristem, it is usually found that the nuclei of meristematic cells contain the diploid number of chromosomes. Here cell divisions occur frequently. Thus, root meristems have been used extensively by cytologists

¹ See also the following chapters in this volume: "Les bases cytologiques de la différenciation et de la dédifférenciation chez les plantes" by R. BUVAT, pp. 100—145 (specifically p. 107 *et seq.*), and "Genes and cytoplasm in development" by K. MATHER, pp. 41—67 (specifically p. 49/50).

as the material of choice to study the process of mitosis and cytokinesis in plants. For this reason, a considerable literature is available on the cytology of root meristem cells.

a) The demonstration and measurement of polyploid cells.

Terminology. For many years after the early elucidation of the nature of chromosomes and of the mechanism of cell division, it was generally believed that somatic tissue cells normally possessed the same chromosomal complement, *i.e.*, the diploid chromosome number, as the fertilized egg from which the whole plant had developed. Increased chromosome numbers in tissue cells were known to be associated with abnormal tissue formations, such as crown gall tumors (LEVINE 1931) or with the peculiar proliferations which give rise to root nodules in leguminous plants (WIPF and COOPER 1938, 1940). Although early workers had shown that polyploid cells apparently occurred normally intermingled with diploid cells in some roots, *e.g.* *Spinacia* (STOMPS 1910, LANGLET 1927; *cf.* also D'AMATO 1952a), it was not until about 1937 that general recognition of this phenomenon, called polysomaty or endopolyploidy, spread among cytologists. LORZ (1937) studied the distribution of polyploid cells along the length of the root in *Spinacia* and showed that, from the diploid cells in the region of the root meristem, there was a progressive increase in chromosome number in the direction of the more differentiated cells. Thus in linear sequence, LORZ observed diploid cells ($2n$ number of chromosomes), tetraploid cells ($4n$ with chromosomes paired and then $4n$ with chromosomes unpaired), octaploid cells ($8n$ with chromosomes paired and then $8n$ with chromosomes unpaired), and so on. The now-classical studies of GENTCHEFF and GUSTAFSSON (1939a, b) on *Spinacia* established the fact of the general occurrence of chromosome doubling during cellular differentiation in the root tips by some process involving double chromosome reproduction. Subsequently, BERGER (1941) suggested on the basis of further cytological studies that chromosome doubling occurred during the resting stage or interphase between mitotic divisions. It seems generally agreed that tetraploid nuclei entering early prophase show quadripartite structures called diplochromosomes, *i.e.*, two chromosomes each with two chromatids all joined together by a single centromere. At the metaphase plate, the usually separated chromosomes lie close together showing a characteristic paired arrangement. During the subsequent division at anaphase, the chromatids split and each daughter nucleus possesses the tetraploid chromosome complement. Such cells can undergo normal mitotic division, maintaining the tetraploid number and showing no "pairing" or such cells may proceed through further chromosome doubling to the octaploid number. Such doubling is referred to as endoreduplication (LEVAN and HAUSCHKA 1953). If chromosome doubling is repeated during interphase without mitoses, polytene chromosomes result.

GETTLER (1939) used the term endomitosis to describe a mechanism for chromosome doubling whereby the doubling occurred during interphase. The nucleus entered a type of mitosis in which chromosome contraction proceeds within the nuclear membrane through an endometaphase and back again to the interphase condition. In endomitosis in the GETTLER sense, no chromosome pairs or diplochromosomes are apparent and thus can be distinguished from endoreduplication. It may well be, however, that these processes are not rigidly distinct. Both result in a doubling of chromosome number and both have been observed in the same root tissues following auxin treatment (*e.g.* THERMAN 1951).

Auxin treatment and wounding. LEVAN (1939) observed that mature, completely differentiated cells of the root could be induced to divide by treatment

with a plant growth substance such as the auxin, α -naphthalene acetic acid. Such mature cells frequently were found to be polyploid cells, showing diplochromosomes. Although LEVAN believed that chromosome doubling was associated in some way with the cellular enlargement induced by the auxin, it has since been demonstrated by a number of workers (BERGER and WITKUS 1948, HUSKINS and STEINITZ 1948b, D'AMATO 1948a, THERMAN 1951, CHOUINARD 1955) that the applied auxin acts to induce cell division in polyploid cells which have arisen spontaneously during the course of normal root development. Wounding of tissues also will induce cell division in differentiated cells which would normally not undergo division. The method of wounding represents another way of inducing pre-existing polyploid cells to undergo division. NĚMEC (1905) observed the presence of polyploid cells in division following root decapitation. D'AMATO (1948b) demonstrated that root decapitation produced essentially the same effect in inducing cell division of polyploid cells as auxin treatment.

Using the methods of auxin treatment and wounding, many authors have reported the occurrence and distribution of polyploid nuclei in root tissues, based on actual chromosome counts of metaphase division figures. Less direct means of determining the presence of polyploid nuclei have been suggested. Thus, HUSKINS and STEINITZ (1948a) studied the relation between polyploidy and the number of heterochromatic bodies in the nuclei of cells in roots of *Rhoeo*. They showed that along the root axis, there is a progressive increase in the number of heterochromatic bodies per nucleus from meristem to mature differentiated tissues, but that no simple direct relationship could be found. BRADLEY (1954) reported that volumes of cells and of nuclei in squash preparations of pith tissue of *Nicotiana* stems at various stages of cell division and at interphase fall into fairly distinct classes which are apparently related to chromosome number. With care, it is suggested, these measurements might be used to estimate polyploidy of a given tissue. On the other hand, D'AMATO (1952a) has pointed out the difficulties in using nuclear size and shape as an index to the degree of polyploidy in a tissue. It has been shown (LAIRD 1953) in animal tissues that by certain treatments, one can increase nuclear volumes experimentally without affecting the chromosome number of the cells. Here increased protein and RNA accounted for the increase in nuclear volume.

Measurement methods. Microspectrophotometric measurements of deoxyribose nucleic acid content of dividing and non-dividing cells have been also used as an index to the occurrence of polyploidy in tissues. It is believed (see PARTANEN 1959 for literature review) that measurement of Feulgen-stained nuclei either in the resting state or during division gives a reliable measure of the relative DNA content of the nuclei. It has also been fairly well established (see review by SWIFT 1953) that the amount of DNA in each chromatid set is relatively constant. Thus, measurement of the Feulgen-stained material in the resting nucleus is a reliable index of the number of chromatid sets per nucleus and can be used to determine the ploidy of the cell. One difficulty in interpretation enters in when one uses the measurement of Feulgen-staining material of resting nuclei as the basis of determining chromosome number. It is now well established that prior to mitosis, the cell doubles the DNA content of its nucleus so that at anaphase, with the separation of the two sets of chromatids, each set has a DNA value equivalent to the resting nucleus from which it was derived. This DNA content (2C amount) is interpreted as the level in a diploid (2n) nucleus. Spectrophotometric measurements show an increase in DNA during interphase from the 2C to the 4C level which represents DNA synthesis which is believed to occur usually several hours prior to the beginning of prophase. It is not possible using this

method to distinguish a tetraploid nucleus ($4n$) with an amount of DNA at the $4C$ level from a diploid nucleus ($2n$) immediately prior to division with a $4C$ level of DNA which will subsequently be reduced at anaphase to two cells at the $2C$ level. Thus, some ambiguity exists as to the interpretation of polyploid levels using this technique. However, it does give an opportunity for estimating polyploidy in non-dividing tissues.

Physiological differences between diploid and polyploid cells. There is good evidence to indicate that, in general, polyploid cells, tetraploid and higher, show decreasing tendency to undergo mitosis under normal conditions in the tissue system of which they are a part. Thus, although diploid root meristem cells divide regularly and spontaneously, tetraploid cells in division are rarely seen in untreated roots and octaploid or higher somatic cells divide much less frequently even under artificial stimulation such as wounding or auxin treatment (THERMAN 1951, CHOUINARD 1955). Certain cells do not divide at all; for example, in onion (THERMAN 1951) no divisions were induced in the small nuclei of the epidermal cells, nor the very large nuclei of the pith cells. Thus, with respect to their capacity to undergo division, there are distinct physiological differences between the diploid cells and the polyploid cells of the root. These differences, together with the characteristic distribution of the polyploid cells among the different tissues of distinctive cell types has led to considerable discussion and speculation concerning the role of polyploidization in tissue differentiation. The most extensive information is available on root tissues.

b) Occurrence and distribution of polyploid cells in roots.

Variation within the root (different regions and tissues). D'AMATO (1952b) summarizes much of the data concerning the distribution of polyploid cells in particular tissues. The root apex region is characteristically diploid, although polyploid cells appear to occur very close to the meristem region within perhaps 1–2 mm of the extreme tip of the root. SRINIVASACHAR (1959) described nuclei at the tetraploid level or higher within the meristem of roots of *Allium*. BERGER (1941) has shown in *Spinacia* embryos the presence of polyploid cells in the region of the cortex of the seedling radicle. Certain abnormalities in this typical picture of a diploid meristem region have been reported. Thus, SNOAD (1955) has reported a remarkable case of instability of chromosome number in the root tip. SASS (1957) described a situation in root tips of one strain of maize in which a large multinucleate cavity arises within the root meristem from lysis of stelar initial cells. Polyploid nuclei develop apparently from abortive mitoses. This case is quite anomalous.

In general, "the polyploid condition is associated with age and differentiation" (D'AMATO 1952b). It is of interest that chemical analyses of roots for DNA (see earlier discussion, p. 1289) show an approximate doubling of the average DNA content per cell from the region of the meristem to the region where cell differentiation has occurred (HOLMES *et al.* 1955, JENSEN 1958a). This doubling may reflect the average increase in DNA resulting from polyploidization. DEELEY *et al.* (1957) described the progressive change in FEULGEN-stained nuclear content in developing root cells of *Vicia faba* which is doubtless related to polyploidization.

Polyploid cells, mostly tetraploid, usually occur in the cells of the cortex in most roots which have been studied. This is the situation in *Spinacia* (GENTCHEFF and GUSTAFSSON 1939) and in *Allium* (THERMAN 1951, CHOUINARD 1955), *Rhoeo* (HUSKINS and STEINTZ 1948b) and a number of other monocotyledons (D'AMATO 1952a). Occasionally, octaploid divisions are observed in cortical tissues.

One of the most consistent and striking observations is the fact that the pericycle, which can be considered a meristematic tissue which exists in the differentiated tissues of the primary root, is a diploid tissue. This fact has been reported by D'AMATO and AVANZI (1948), CHOUINARD (1955), BERGER and WITKUS (1949), and HUSKINS and STEINITZ (1948b). Occasional tetraploid divisions in pericycle cells have been observed (*e.g.* in *Lens*; see HOLZER 1952). In so far as it has been studied, the evidence suggests that the endodermis is usually tetraploid or a higher ploidy (D'AMATO 1952b, CHOUINARD 1955). The early enlarged late metaxylem elements which stand out so clearly in sections of the procambial cylinder of many roots and which, once blocked out, seldom divide again, are usually octaploid or higher (see D'AMATO 1952b, CHOUINARD 1955). Thus, on the basis of limited data, there is good reason to investigate further the possible relationship between cellular differentiation and endomitotic reduplication.

Variation between species; general questions. The list of species which have been shown to have polysomatic root tissues is impressively long (D'AMATO 1952b, HOLZER 1952). There are, however, a few cases in which roots have been examined for polyploid cells and none have been discovered. Thus, D'AMATO (1950) has reported he was unable to find polyploid cells in roots of adult plants of two species of *Crinum*. LEIVONEN (1958) found only diploid division figures in roots of *Narcissus* after treatment with gibberellin or IAA, even though many mature cells were observed to divide. HOLZER (1952) reported that treatment of roots of 49 different species of angiosperms with 20 mg/l IAA caused cell division in mature cortical tissues of 27 species. Of these 15 were found to have polyploid nuclei, 12 species showed only diploid mitotic figures. It is of interest that for some of the species in which HOLZER found no polyploid divisions, other reports have shown that polyploid cells are in fact present. Thus, for example, in *Crepis*, BRUMFIELD (1943) reports that he observed occasionally spontaneously dividing tetraploid cells in untreated roots. Similarly, in *Vicia faba*, HOLMES *et al.* (1955) have referred to the occurrence of nuclei with the $4n$ number of chromosomes. From what is known of the differential sensitivity of polyploid cells to externally supplied stimuli such as auxins, it is clear that one cannot exclude the possibility of the presence of polysomaty in roots based on counts of mitotic figures until a wide range of experimental conditions has been tried.

PARTANEN (1959) has reported that in *Helianthus tuberosus* roots he could find only diploid nuclei using spectrophotometric measurements of Feulgen-stained nuclear materials. His data show a range of DNA per nucleus ranging from the $2C$ to the $4C$ level, but none above this level. Here, a relatively few nuclei were measured and conclusive evidence of the non-occurrence of polyploid cells is lacking. It will be of great interest if it is possible to establish conclusively that certain roots, which proceed through normal tissue differentiation, possess only the diploid number of chromosomes with the limits of $2C$ to $4C$ levels of DNA. Such an observation would make difficult the interpretation in general terms of cellular differentiation in relation to polyploidy. Such observations would also be of considerable interest with respect to the frequency of polyploid species within a genus, *i.e.*, it may well be that there is a direct correlation between the tendency for polysomaty within the tissues of plants of a given genus and the tendency for polyploid species formation in that genus. So far as the author is aware, no attempt has been made to relate these two phenomena.

SHARMA and SHARMA (1959) have discussed the various ways in which endopolyploidy and the occurrence of polyploid cells in vegetative tissues could give rise by means of vegetative reproduction of new polyploid individuals which in turn might lead to new races or species.

c) The significance of endopolyploidy in relation to root development.

Polyploid plants and organs. The fact that whole plants occur which are polyploids might be used as an argument against the idea that cellular differentiation and polysomaty may be, in some way, causally related. It is almost certainly true that in plants of polyploid varieties where the basic chromosome number is a multiple of that in the species, polysomaty may still occur in which chromosome doubling is now based on the increased chromosome number. Here again, adequate studies on this aspect of the problem are lacking. LEVAN (1944) reported the very interesting situation of chromosome doubling in root tips produced by second-year storage roots of diploid and polyploid varieties of sugar beet. He examined root tips for chromosome numbers of diploid, triploid and tetraploid varieties of sugar beet. In these young first-year seedlings, he found endomitotic reduplication in all three, with a higher frequency occurring in the diploids. As he did not stimulate the seedling artificially to induce divisions, the total number of mitotic counts of polyploid cells was very low (approx. 0.2%). At the second year when these same plants had produced large storage roots, new secondary roots which formed along the "root furrows" of the storage structure, were fixed and studied. Many root tips were polyploids of the initial chromosome number, whether it was originally diploid, triploid or tetraploid. Polyploid root tips were most frequent from diploid plants (18%) and less from triploids (10%) and tetraploids (5%). In these cases, whole secondary meristems were apparently derived from cells which had earlier undergone endomitotic reduplication. It is clear also that endomitosis had occurred in the somatic tissues of the polyploid varieties, with a chromosomal basis of either $2n$, $3n$ or $4n$. One further point is of interest. In the secondary roots of the storage root under normal conditions cell divisions occurred readily in tetraploid cells and cells of higher ploidy at a frequency much greater than was apparent in original seedling roots. Clearly, the physiological condition within the polyploid cells and surrounding them must have changed radically, toward a condition favoring division of the nuclei with high chromosome number. This situation, which should be explored further, might well give a clue to the problem of the differential responsiveness of cells of polysomatic tissues to cell division stimuli.

Relation of polyploidy and tissue and cellular differentiation. A final dilemma which arises with respect to the occurrence of polysomaty is the fact that within an apparently homogeneous tissue such as the cortex, there exists a mixed population of cells with respect to chromosome number. D'AMATO (1952a), basing his studies on chromosome counts of roots stimulated to divide by auxin treatment, reported that about 9% of the divisions of cortical cells in *Nothoscordum* roots were polyploid while 95–100% of the divisions of cortical cells in the thick, fleshy roots of *Bellevallia* were polyploid. No clear morphological distinction could be made between diploid and polyploid cells.

While it is doubtless true that the available evidence suggests that diploid and polyploid cells in a uniform tissue system are physiologically dissimilar, at least with respect to their capacity to undergo mitosis, it would seem that the physiological differences had nothing to do with the course of differentiation. It could be argued, however, that in such a case as the cortex, the endomitotic reduplication occurred after the cell type was already determined and that only when the chromosome doubling occurs early in the ontogeny of the cell could it affect the fate of that cell. Clearly, much work is needed to determine whether polyploidization has anything to do with cellular differentiation.

TORREY (1959a) suggested that in the organization and development of the root the important fact with respect to nuclear condition is the stability and per-

petuation of the diploid condition in the root meristem. He found in experiments with pea root callus tissue grown in liquid culture that under certain cultural conditions organized roots formed from disorganized callus tissue masses. Although the callus tissues were composed predominantly of tetraploid cells, the root meristems were always diploid. In callus which had been grown in culture for prolonged periods, organized roots could not be induced by a variety of treatments; cytological studies failed to show any diploid cells in the actively dividing polyploid tissue. It was concluded that organized meristems in this system were derived only from diploid cells under these cultural conditions.

d) Physiological aspects of endomitotic polyploidization.

Relations to auxin. Whether or not there is a causal relationship between the two processes of cellular differentiation and chromosome doubling remains to be seen. We do know, however, that endomitosis does occur during cell differentiation and that there must be some physiological basis for its occurrence. About this aspect of the problem we have practically no information. LEVAN (1939) first suggested that the cell enlargement resulting from auxin treatment of roots produced cellular changes resulting in chromosome doubling. It is now generally conceded that auxin treatment, by inducing cell division, makes manifest the polyploid cells which occur spontaneously during root development. But the causes for the spontaneous doubling remain unknown. LORZ (1947) suggested that the process of endomitosis might be under the control of endogenous auxin produced in the root. This is an interesting idea in view of the known gradient of auxin in the seedling root, *i.e.*, high concentrations in the tip and increasingly lower concentration toward the root base. According to this view, endomitotic reduplication would be suppressed by high endogenous auxin level and would occur only as the auxin level decreases. Thus, externally supplied auxin should not be expected to affect the diploid cells (D'AMATO 1952a). In fact, NOIRFALISE (1940) has shown that added external supplies of auxin reduce cell division in the meristem in *Vicia faba* roots and with increasing concentration, can actually lead to cessation of cell division in the meristem. At this concentration, cell divisions in the mature regions of the root, usually in the pericycle, may actually be considerably stimulated to division.

The stimulation of polyploid cells to division. The stimulation of mature differentiated cells to division, revealing their polyploid nuclear condition, seems to be rather non-specific. Thus, in addition to stimulation by a variety of auxins, including IAA, 2,4-D, NAA and others, polyploid nuclei are stimulated to divide by nucleic acid solutions (SHARMA and SEN 1954), a mixture of fructose, ammonium phosphate and uracil, or fructose alone (SHARMA and MUKHERJI 1956). Some of these treatments produce abnormalities in division, including reduction divisions (HUSKINS 1949) which are apparently associated with interference in the mitotic cycle (KODANI 1948). It is not safe to conclude that any of these reagents act specifically in polyploid nuclear division, nor do we have a clear indication yet if any are related to the onset of endomitotic reduplication. TORREY (1961) showed that kinetin (6-furfurylaminopurine), which stimulates tetraploid cells to divide in pea root segments cultured *in vitro*, specifically triggers the mitotic process rather than effecting DNA synthesis itself. Almost nothing is known about the physiology or biochemical processes involved in the process of endoreduplication. It may well be that advance in knowledge in this area of cytology will also be an advance in our knowledge of the process of cell differentiation.

2. Cell structure and root organization from electron microscopy.

In the last few years a new analytical tool has been turned toward the problems of cellular organization. The electron microscope with its magnification some thousand-fold greater than the light microscope and its fine resolution allows the examination of cellular detail and submicroscopic morphology not hitherto possible. Here again, roots have proved particularly useful and convenient material for study and our outlook on the problems of cellular differentiation in roots must now encompass a whole new realm of structural detail. Simply to look at a whole meristematic root cell as visualized in the electron microscope (Fig. 9) is to be aware of new implications—in which morphological designations begin to approach the molecular level.

a) The endoplasmic reticulum.

Recent papers by SITTE (1958), WHALEY *et al.* (1960), and PORTER and MACHADO (1960) summarize the available information concerning the elemental structure of root cells, especially cells of the apical meristem region in interphase and during mitosis. New structures not previously generally recognized from studies with light microscopy have been described and some attempts made to fit them into an understanding of cellular physiology. Among these structures, the endoplasmic reticulum (*er*, see Fig. 9) is of foremost interest and importance, being an elaborate membrane-bound vesicular system embedded in the ground substance, frequently continuous with the nuclear envelope (*ne*), with elements extending to the cell surface and even penetrating the primary wall (*w*) and passing into other cells (WHALEY *et al.* 1960). Changes in the endoplasmic reticulum (ER) of differentiating onion root cells have been described in detail by PORTER and MACHADO (1960) who believe that “the system [ER] may be an instrument in differentiation rather than a product of the phenomenon”. Their speculations center around the concept that the ER is physically closely related to the chromosomes in origin and thus may act to carry information important in cellular differentiation. Direct evidence for such a role in plant cells is thus far lacking. HÖFLER (1960) discussed the detailed structure of the endoplasmic reticulum and the nature of the closely related dense particulate components (“PALADE granules” or “meiosomes”) which show high ribonucleic acid content. The ultimate significance of these cellular components in cellular differentiation cannot be doubted.

Differences in the endoplasmic reticulum in cells which are morphologically distinct have been noted (SITTE 1958, PORTER and MACHADO 1960) as, for example, epidermal cells of onion root which exhibit a much more abundant ER per unit volume of cytoplasm than onion root cortex or central cylinder cells. WHALEY *et al.* (1960) note that another distinctive feature of epidermal cells of corn roots is the occurrence of two distinctive types of vesicles. Only the smaller type is found in other cells of the root meristem.

b) Mitochondria.

Other observations have been made concerning differences in subcellular particulate components in differentiating cellular systems in roots. LUND *et al.* (1958) attempted to establish correlations between biochemical activities of segments taken from different regions of the growing root tip of corn roots (*Zea mays*) and the occurrence and distribution of cytoplasmic particles, especially mitochondria (Fig. 9, *m*) and microsomes. Their description of mitochondrial “maturation” during the course of cellular development from meristematic cell

to mature cell has been brought into question by more recent studies (WHALEY *et al.* 1960). That structural changes in mitochondria do occur in maturing cells seems likely, but whether these changes relate directly to metabolic activity or in turn to cellular differentiation remains to be demonstrated. The approach used by LUND *et al.* (1958) is an interesting and useful one and it is clear that

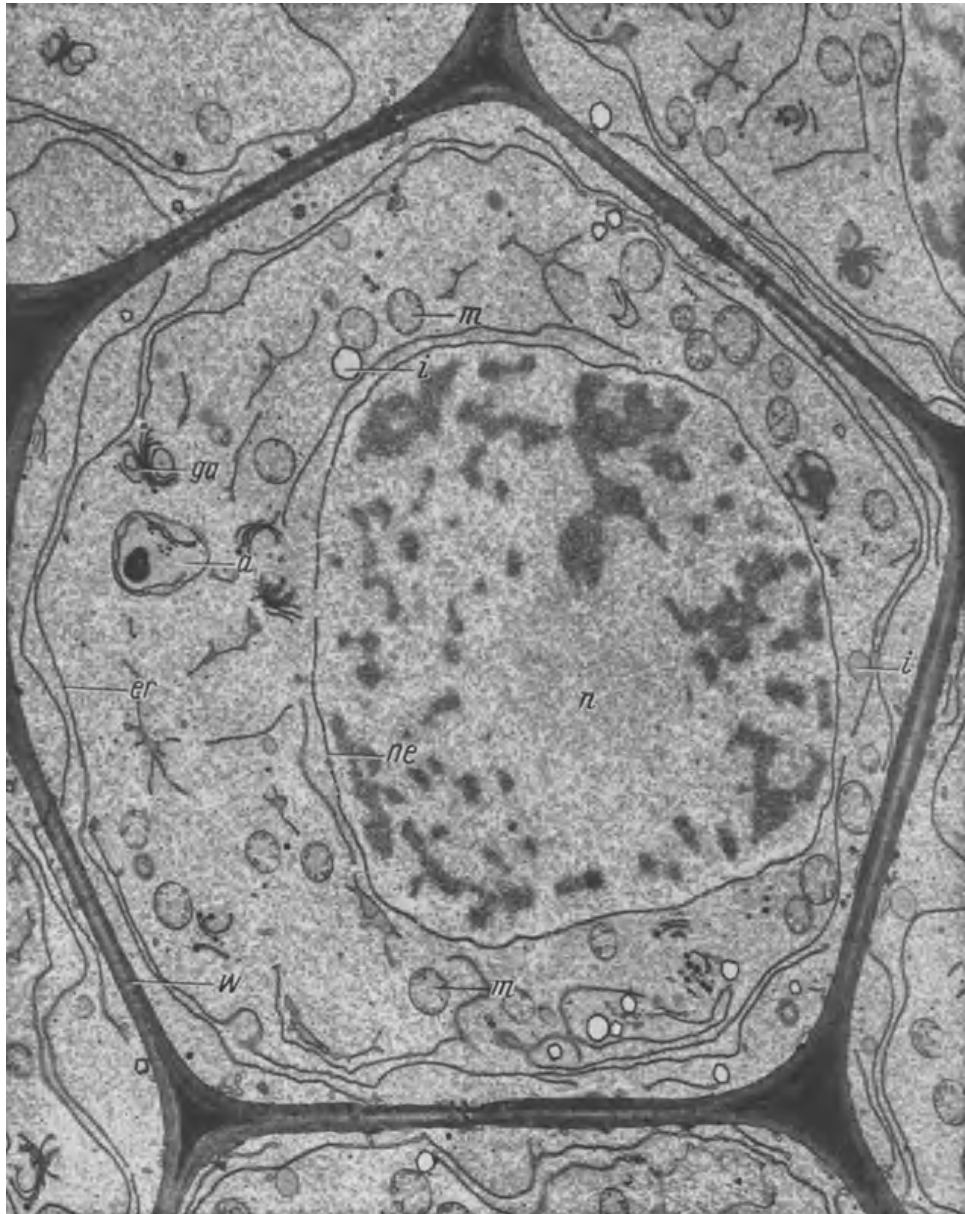


Fig. 9. Electron micrograph of a transverse section through a root cap cell in the root meristem of *Zea mays*, fixed in 2% aqueous KMnO_4 for 2 hrs. at 22° , showing the nucleus (*n*), endoplasmic reticulum (*er*), nuclear envelope (*ne*), mitochondria (*m*), Golgi-apparatus (*ga*), cell wall (*w*), amyloplast (*a*), and unidentified inclusions (*i*). Approximately $\times 5,500$. (From WHALEY *et al.* 1960.)

further studies along this line, especially as fixation and sectioning methods are improved and artifacts are minimized, can contribute much to our understanding of the detailed changes which go on during cellular differentiation and may give some clue to those changes which are indeed "instruments of differentiation" rather than products.

WHALEY *et al.* (1960) have reported that mitochondria in the apical initial region of the corn root, while structurally similar to those in the more basal,

mature cells, occurred in fewer numbers in the former region. Such an observation, which is difficult to make with any certainty because of the difficulties of localizing initial cells, does fit well with the evidence already discussed (see p. 1283 *et seq.*) concerning minimum physiological activities expressed on a cell basis in this portion of the root.

c) Other structures.

(*Golgi apparatus; cell wall; chromosomes; nucleolus; etc.*)

BUVAT (1957) and WHALEY *et al.* (1959) described the previously evasive Golgi apparatus (Fig. 9, *ga*) in plant cells as seen in differentiating root cells of corn seedlings. Remarkable changes in this structure occur during cell differentiation and the structure shows a clear orientation within cells. Some suggestion of their involvement in cell plate formation was given. Now that they can be readily visualized in permanganate-fixed plant materials, it is to be anticipated that some concept of their role in cells will be developed.

Other structures of the cell can now be re-examined in the light of new morphological knowledge developing from electron microscopy. WHALEY *et al.* (1960) discussed at length the earlier chemical literature on the root cell wall (see p. 1287) and attempted to relate the electronmicrographic information to it. The course of mitosis and changes in chromosome structure and arrangement in root tips were discussed by PORTER and MACHADO (1960) although clearly this is just the beginning of work in this direction. LAFONTAINE (1958) focused attention on the structure and formation of the nucleolus. Interesting new ideas concerning the role of vacuoles as reservoirs of metabolites which are used up during cellular differentiation have been presented (WHALEY *et al.* 1960). Other previously unrecognized inclusions referred to by PORTER and MACHADO (1960) as phragmosomes appear to play an important role in cell plate formation. Still other particulate components seen in electron micrographs of root cells remain unidentified (see *i*, Fig. 9). Clearly, much remains to be done and this is an area of research which promises to contribute significantly in the immediate future to our understanding of root cells and root organization.

VI. The formation of lateral roots during root development.

1. External factors (mineral nutrients).

Early work on plant nutrition and the effect of environment on plant growth has contributed interesting facts which should be considered. As early as 1862, NOBBE demonstrated the effects of fertilizers on the formation of root systems. By adding nutrient salts, including ammonium sulfate, calcium nitrate, and dipotassium phosphate, to certain regions of an inert clay soil contained in cylinders, NOBBE showed that the amount of branching of both primary and secondary roots was markedly increased in the fertilized zones. In similar experiments, HÄVELER (1892) found that herbaceous plants grown in containers of alternate layers of sand and fertile soil showed extensive root branching in the fertile regions and almost no branching in the sand. The effects of specific nutrient compounds in increasing root branching were shown by FRANK (1893), especially for added calcium nitrate, and by MÜLLER-THURGAU (1894) for other nitrogen compounds. More recently fertilizer experiments by WEAVER, JEAN and CRIST (1922), ROGERS (1933), SAYRE (1934) and others have clearly confirmed the fact of increased root formation and branching in areas rich in nitrogenous fertilizers.

The importance of specific ions in the growth and branching of roots has been suggested by several authors. PETTINGER (1933) found that added nitrogen had little effect in increasing root branching when the supply of potassium was limited, and that potassium added to a deficient soil had a greater effect than nitrogen in producing increased root formation in corn plants. DOWDING (1925) confirmed earlier evidence of McCALLUM (1905) that potassium was associated with the outgrowth of secondary roots and showed increased root branching of wheat roots in the presence of potassium chloride. More recent work in tissue culture studies of root growth also suggests the importance of specific metallic ions particularly potassium, which may be directly concerned in the phenomenon of root branching (WHITE 1943).

2. Internal factors.

a) Localization and pattern.

Origin and early development. In 1888 VAN TIEGHEM and DOULIOT published their exhaustive researches on the anatomical origin of lateral roots in a great number of species of plants. This work clearly established the endogenous origin of lateral roots. Lateral roots were shown to have their origin in the meristematic cells of the pericycle region of the primary root, to project by cellular division and elongation through the cortical zone of the root, and proceed by normal elongation to form lateral appendages. Variations in this general sequence have been described, but the basic facts as reported by these authors have remained unchanged since that time. In the endogenous origin of lateral roots in Angiosperms and Gymnosperms, root initiation occurs in the pericycle, just beneath the endodermis, from mature cells which have maintained their meristematic nature. It has been recognized that such points of origin are located opposite the xylem arms of the vascular bundle whenever there are three or more strands forming the vascular tissue. However, if the stele is diarch, the lateral roots arise at an angle to the central xylem band. Externally, the arrangement is clearly manifest by the arrangement of the roots in vertical rows corresponding in position to the strands of the vascular bundle within the root. The physiological basis and significance of such localization of the site of lateral root initiation is not known.

Considerable discussion arose over the mechanism whereby the young meristematic lateral root projected through the intact cells of the mother root tissue. Evidence for both mechanical pressure and for enzymatic breakdown was presented. VAN TIEGHEM and DOULIOT believed that emergence of the lateral root depended upon dissolution of the tissues. This view was also held by REINKE (1871) and VONHÖNE (1880). POND (1908) concluded from a series of experiments which tested the ability of roots to penetrate the tissues of other roots that mechanical pressures only were active, thus confirming the earlier views of PIERCE (1894) and CZAPEK (1896). It is commonly agreed that in all probability both mechanical and enzymatic breakdown occur during the emergence of lateral roots (EAMES and MACDANIELS 1947). Direct evidence, however, is lacking.

In 1900 NOLL recorded the observation that lateral roots always arise on the convex curvature of the primary root of a developing seedling. All curved roots showed the same effect, regardless of the manner in which the curvature was produced. According to NOLL, effectiveness of the curvature in determining the locus of the lateral roots depended upon its being present at the time of the "laying down" of the root primordia. No adequate explanation of the phenomenon was given.

Genetic effects and correlations. It is generally the "genetic constitution" which is held responsible for the so-called "root pattern" characteristic of each particular species. Thus tap root and fibrous root systems are considered to depend upon the respective genetic types. BONNER (1940) has noted that the particular root pattern of any species is maintained by the isolated roots grown in nutrient culture solution (see also STREET 1957). The mechanism whereby the genetic constitution impresses itself upon the root system of the developing plant is not understood, but it is suggested that the apical meristem of the root plays the important determining role in expressing this genetic constitution.

Studying the formation of side roots on the primary root of *Vicia faba*, RIPPEL (1937) noted that the normal acropetal formation of lateral roots could be prevented by early removal of the cotyledons. Lateral root formation was prevented for a period of several weeks even though the primary root elongated at the normal rate. By placing the roots of seedlings from which the cotyledons had been removed in a nutrient solution for a period of 3 weeks, lateral roots were finally produced, developing basipetally from the region of the root tip slowly toward the root base. After an additional three weeks, the primary root had formed lateral roots its full length. Removal of the cotyledons after lateral root formation had begun along the primary root resulted in the cessation of lateral root formation for a period of 2 to 3 weeks and then formation of roots at the apical end, proceeding basipetally. Removal of the shoot had no effect on lateral root formation in these experiments. RIPPEL pointed out the importance of the cotyledons in lateral root formation in the leguminous plants tested.

Using dry weight determinations as a measure of root growth, PRIESTLEY and EVERSHED (1922) and PRIESTLEY and PEARSALL (1922) established a correlation between the onset of lateral root formation and rate changes in the growth curves of the primary root. They found that the time of root branching coincided with the time of the appearance of transition points in a series of successive sigmoid growth curves drawn to describe the root growth of any particular plant. They concluded that lateral root formation occurs at the expense of growth in length of the main root. To account for the phenomenon, these authors suggested that a divergence of food materials from the main root to the lateral root meristems had occurred.

b) Significance of auxin.

With the discovery of the important morphogenetic effects of the auxins, most investigations concerning the initiation of lateral roots on roots have centered about the role of auxin in this phenomenon and its interaction with other growth factors and metabolites in the plant.

Direct treatment of intact plants with auxin solutions results in an increase in the number of lateral branches on roots in many plants (BOUILLIENNE and WENT 1933, ZIMMERMAN and HITCHCOCK 1935, THIMANN 1936, FABER 1936, NOIRFALISE 1940). Similarly, excised roots grown in nutrient solution respond to auxin treatment by initiating additional roots (DELARGE 1941, NAGAO 1942, DUHAMET 1939, 1946; L. BOND 1948, SLANKIS 1950, TORREY 1950, 1956a, GEISSBÜHLER 1953, PECKET 1957a, b). There seems little reason to doubt that externally supplied auxins specifically stimulate cell division in the pericycle leading to the initiation of lateral root branches. Whether endogenous auxin acts internally in the same manner is not conclusively established, but it seems reasonable from such studies as those of DOSTÁL (1941) on auxin distribution in seedling plants, of WARMKE and WARMKE (1950) on root initiation in root cuttings of *Taraxacum* and *Cichorium*, and of SKOOG and coworkers (1944, 1954, 1957)

on the interaction of auxins with other growth factors in initiating organs that auxin is one of the essential factors for normal lateral root initiation in many roots.

There is also considerable evidence to indicate that lateral root initiation depends upon the interaction of auxin with other factors within the plant. Two types of interaction have been suggested in the literature. The first involves the positive stimulus to root initiation from essential metabolites or growth factors which, within the root, may have a polar distribution and, through interaction with endogenous auxin, may control the sites of lateral root initiation in acropetal sequence in seedling roots. The second interaction involves an inhibition to lateral root initiation caused by naturally occurring inhibitors within the root. It is of interest to consider these two aspects of the control of lateral root initiation in the main root axis. Most data come from studies of seedling development.

c) Promotive factors.

Physiological evidence. Several authors have found evidence suggesting the importance of internal factors other than auxin controlling lateral root initiation. DELARGE (1941) found that, although an initial auxin treatment of excised roots of *Zea* and *Triticum* grown in sterile culture gave rise to lateral roots, a subsequent treatment had no effect. He postulated the depletion of a factor necessary for root initiation in addition to auxin. TORREY (1950) found a similar situation in cultured roots of *Pisum* and gave further evidence from experiments with root segments for the movement along the root axis from base toward the root tip of an internal factor which, together with externally supplied auxin, is essential to lateral root initiation. NAGAO (1942) had reported essentially the same phenomenon in relation to the inherent polarity in root segments of *Pisum* as reflected in lateral root initiation. He found that in root segments of *Helianthus* the polarity, although present, was less marked than in *Pisum* roots. NAGAO also assumed the acropetal movement of a factor or factors essential for lateral root development. After reviewing the literature concerning the movement of auxin within roots, he concluded that the evidence was inadequate to attribute the polarity of root initiation to polar auxin movement in the root. GEISSBÜHLER (1953) studied lateral root initiation in seedling and isolated roots of *Vicia sativa*. He found that in the seedling, removal of the cotyledons resulted in a displacement toward the root tip of the first initiation of lateral roots, indicating that early lateral root initiation was dependent upon factors moving from the cotyledons toward the root tip. Evidence from isolated root studies suggested further that the primary root itself later becomes independent of the cotyledons for these factors, which then are provided by the shoot, or in isolated roots in culture, from a known synthetic nutrient medium. This idea finds support in the earlier work with *V. faba* reported by RIPPEL (1937). Deficiency experiments by GEISSBÜHLER (1953) suggested that nicotinic acid was essential for lateral root initiation and that both IAA and nicotinic acid were involved in root branching. PECKET (1957b) has also presented evidence that factors other than auxin are necessary for lateral root initiation.

Effect of known substances on roots of seedlings and excised roots. FRIES (1951, 1953, 1954, 1955) studied in some detail the growth factors essential to normal root development of cotyledonless seedlings of *Pisum* grown in the dark when provided to the root from a synthetic medium. The development of lateral roots was particularly stimulated by hypoxanthine and ornithine. The position along the length of the root of the first lateral root initiation was affected by the presence or absence of the shoot. In the presence of the shoot, lateral

roots appeared first much closer to the tip than in excised isolated roots; the addition of arginine to medium in which excised roots were grown in the dark caused a shift in the position of initiation of laterals along the root axis away from the tip. Arginine also increased the number of lateral roots initiated by excised roots. These stimulations of lateral root number accompanied increased root elongation and thus cannot be interpreted as direct effects.

SKINNER and STREET (1954) have shown that isolated roots of *Senecio* grown in WHITE's medium, respond markedly in increased root elongation and lateral root formation to added lysine and arginine or ornithine or citrulline. BUTCHER and STREET (1960) report that gibberellin causes an increase in the number of lateral roots in excised tomato roots, particularly in media with low saccharose concentrations. Similar effects of added metabolites and/or growth factors on lateral root formation have been reported for other elongating excised roots but in most cases it is not certain whether this is a direct effect on lateral root initiation or a secondary effect through an influence on root elongation. BOLL (1954b) suggests that under certain conditions glycine may specifically stimulate lateral root initiation in excised tomato roots without causing a parallel stimulation in root elongation.

Effects of known substances on root segments having completed elongation. As indicated above, in studies of root development, either in seedling roots or excised roots growing in culture, it becomes difficult to distinguish effects of added substances on root initiation per se as distinct from a secondary effect due to general stimulation of root elongation. Thus, only in roots of comparable final length will the average number of laterals per unit length offer a measure of lateral root initiation response. Separation of the process of root elongation from that of root initiation is essential if any conclusions are to be reached concerning specific factors involved in root initiation. Using root segments which had completed their elongation taken from excised roots of *Pisum* grown in culture, TORREY (1956a) has shown that lateral root initiation in pea roots requires an available supply of auxin (IAA), thiamin, nicotinic acid and adenine and also one or perhaps more micronutrient elements. Deficiency of any one of these induced by cultural conditions, or by specific antagonisms with appropriate anti-metabolites, results in cessation of the cell divisions leading to lateral root initiation. It is suggested that one or more of these factors, supplied to the root from the more mature regions of the root (PECKET 1957a), from the cotyledons, or from the shoot of the maturing plant along a physiological gradient could be responsible for the observed continuing acropetal sequence of root initiation. Whether internal radial gradients of distribution of these or similar factors in limiting supply could determine the site of the initiation process opposite the protoxylem points of the main axis remains to be established. That this is a possibility is suggested by the experiments of DE HAAN and PETRICK (1935) in which they induced lateral root initiation by partially interfering with acropetal movement of materials from the cotyledons. It seems likely that in different root systems, different factors are active in the "multiple factor" control of root initiation.

Possible role of kinin-like materials. GOLDACRE (1959) suggested, from experiments with isolated flax roots in culture treated with IAA, that young root primordia themselves produce a chemical stimulus which interacts with IAA to induce new cell divisions giving rise to additional lateral roots. He believed the substance, which is kinin-like in its action, may be produced as a normal accompaniment of cell division. It is interesting that TSCHERMAK-WOESS and

DOLÉŽAL (1953) reported spontaneous divisions of polyploid (endomitotic) cells in the root cortex restricted to a region immediately adjacent to root primordia formation. From the observations of TORREY (1961), one could explain these mitoses in terms of kinin-production by the new meristem as suggested by GOLDACRE.

d) Inhibitory influences.

Physiological evidence. The inhibition of lateral root initiation by naturally occurring endogenous inhibitors may also play some role in determining the site and time of root branching. Most evidence suggests that the terminal meristem is the site of origin or at least of highest concentration of the postulated inhibitor. That root decapitation stimulates root branching has been known for some time (SIMON 1904, ZIMMERMAN and HITCHCOCK 1935, THIMANN 1936, VAN OVERBEEK 1939, TORREY 1950, GEISSBÜHLER 1953). An early interpretation of this observation centered around the idea that the terminal meristem utilized all available food materials and that, upon its removal, lateral root meristems could be initiated and develop. More recently, it has been believed that root branching is under hormonal control and some involvement of auxins has been sought. THIMANN found that in both *Pisum* and *Avena*, removal of 1 mm root tips resulted in increased root branching. Addition of auxin *via* the root base had no added effect in *Avena* roots, but produced an increase in lateral root number in *Pisum*. Since endogenous auxin levels in *Avena* roots were already high, THIMANN concluded that some other factor which originated in the tip controlled root branching in *Avena*. TORREY (1950) showed that the decapitation response was not due to substances released at the cut surface.

GEISSBÜHLER (1953) also reported stimulation to lateral root initiation by root decapitation of *Vicia sativa* and believed that this response could best be interpreted in terms of the removal of the inhibitory effect of the primary root tip itself, comparable to the removal of apical dominance by decapitation in shoots. He also interpreted the constancy of the distance between root tip and the first lateral root during root development in terms of an inhibition by the tip.

Chemical evidence. TORREY (1956a) reported decapitation experiments with isolated roots of *Pisum* which demonstrate the production by the root tip of an inhibitor of lateral root initiation. Excision of the terminal few millimeters of root segments results in a marked increase in lateral roots initiated by the segments. Preliminary experiments with ether extraction of pea root tips showed that the inhibitor was ether-extractable but no further study was made of its chemical nature. LIBBERT (1956, 1957) in an extensive series of reports has described an ether-extractable substance from roots of *Pisum* which inhibits root initiation in etiolated pea hypocotyls. He has suggested that this substance, which is not IAA, might actively control lateral root initiation in pea roots as well. The substance is heat-stable, soluble in ether, water and benzene, adsorbed by activated charcoal, is labile in H_2O_2 and alkali but stable in acid. Its chemical nature is unknown.

TORREY (1959b), using an assay for lateral root initiation which allows analysis of chromatographically-separated substances derived from ether and alcohol extracts of pea roots, demonstrated the presence of several substances in pea root extracts which are inhibitory to lateral root initiation. Although none of these was identified chemically, the evidence suggests that one of the most active inhibitors is a phenolic compound. Whether or not it plays a physiological role in the pea root has not yet been demonstrated. In this paper, reference has been made to earlier studies of root inhibitors including the work of LEXANDER

(1953), and the more recent studies by PILET (1958), and AUDUS and GUNNING (1958).

Interaction between terminal and lateral meristems. That there is a mutual interaction between the terminal meristem and the lateral root meristems has been shown by the work of STREET and ROBERTS (1952) on excised tomato roots. They believed that the cessation of meristematic activity in the terminal meristem during development in vitro was attributable to the inhibitory effects exerted by the main axis and its associated laterals. Conversely, they presented evidence that the main axis meristem exerts a depressing effect on the elongation of young lateral roots. In subsequent studies STREET (1954, 1955) analyzed the effects on root development of a number of auxins and auxin antagonists. Although the "ageing" effect leading to cessation of main axis growth in excised tomato roots was ascribed to an accumulation of a natural auxin (not IAA) within the root, there is no evidence presented to suggest that this accumulation is related in any way to lateral root meristem activities. GOLDACRE and UNT (1957) reported an interesting response to the adenine antagonist, 2,6-diaminopurine, which at a given concentration, inhibits the elongation of the main axis root of subterranean clover while stimulating the lateral root meristems.

e) Effects of light.

STREET (1953) and STREET and MCGONAGLE (1953) have shown that continuous low-intensity illumination (1—15 foot-candles, equalling about 10—150 Lux) with white incandescent light enhances main axis growth and suppresses lateral root development in excised tomato roots. Red and orange light had the same effect as white light. While stimulating main axis elongation, blue light did not prevent lateral root formation. No attempt was made to relate the interaction of terminal and lateral root meristem activity under illumination to the abovementioned auxin accumulation theory.

TORREY (1952) also observed the inhibitory action of white incandescent light on lateral root initiation in pea roots and has reviewed the relatively few studies of the effects of light on root development. He found that incandescent light with a red filter inhibited lateral root initiation markedly without affecting root elongation. Blue and green light were relatively ineffective. He suggested that red light inactivates substances other than auxin in the main root axis which are essential to lateral root initiation. It should prove profitable to examine in detail the effects of light of various wavelengths on the processes of root elongation and lateral root initiation and to compare them with the effects of light in other growth processes, such as shoot elongation, leaf growth, flower formation and seed germination.

f) Relations in the formation of lateral roots and root nodules.

NUTMAN (1948, 1949, 1952, 1953), who has made a careful study of the factors controlling nodule formation on roots of red clover, found that the rate of formation of lateral roots and of nodules was under the same hereditary control. Assuming that the two structures are physiologically homologous, NUTMAN has given evidence for the mutual inhibition of initiation of laterals and nodules. He postulates that the meristem regions of the two types of structures produce an inhibitor which prevents further initiation of lateral appendages, either nodule or lateral root. Excision of lateral root meristems and nodules led to an increase in the number of nodules subsequently formed. The suggestion was made

(NUTMAN 1953) that nodulation may be inhibited by specific substances secreted by developing roots, which also tend to reduce the production of lateral root meristems. No evidence concerning the nature of the inhibitor was given¹.

VII. The initiation of the vascular cambium in roots.

Remarkably little is known about why the vascular cambium differentiates in developing roots at a particular place and time during the life of the root. Careful studies of the differentiation of secondary tissues in roots are available in the literature (*e.g.* on *Daucus carota*, ESAU 1940; *Pirus communis*, ESAU 1943a; *Citrus sinensis*, HAYWARD and LONG 1942). Despite the economic importance of the so-called root crops, such as carrot, beet, sugar beet, turnips, parsnip, radish, sweet potato, yam and cassava in which vascular cambium activity plays the major role in producing a fleshy, food-storing organ, little experimental work has been carried out on the physiological basis for the initiation and maintenance of cambial activity in such roots.

Interesting speculations concerning the relation between the primary and secondary vascular tissues of the root have been made (*e.g.* THODAY 1939). One view is that the vascular cambium and its products are essentially shoot structures and only secondarily (ontogenetically and perhaps also phylogenetically) has the vascular cambium "extended" down into the root from the shoot system. Such a concept has interesting implications concerning the physiological control of cambial activity, which would presumably reside in the shoot system.

It is of some interest, then, that GARNER and ALLARD (1923) presented considerable evidence to indicate that secondary thickening in roots of *Phaseolus multiflorus* and the yam *Dioscorea alata* was markedly dependent on the day length to which the shoot was exposed with stimulation by short day lengths, indicating some form of photoperiodic control of cambial activity. This observation seems not to have been pursued further.

1. Role of auxin and other factors.

With the discovery of the probable role of auxins in controlling cellular divisions in the vascular cambium of shoots of woody plants, some studies were made of the effect of auxin supplied externally to roots. JOST (1935) has shown that cambial divisions do occur in roots after auxin treatment. Work with callus tissues derived from the cambial region of such storage roots as carrot indicates that auxins stimulate cell divisions in most of the living tissue cells but this stimulation is greatest in the region of the vascular cambium and the secondary phloem (see reviews by GAUTHERET 1955a, b). TORREY and SHIGEMURA (1957) have shown the direct initiation of the vascular cambium in excised pea seedling root tips within 3 days after treatment with IAA. It seems probable that auxin initiates cell divisions leading to vascular cambium formation in the root as in the shoot.

There is good evidence that other factors than auxin are essential to continued meristematic activity of the vascular cambium in roots. Thus, CAPLIN and STEWARD (1952) found it necessary to provide tissue explants from the vascular cambium region of carrot roots with coconut milk to maintain active cell proliferation. TORREY and SHIGEMURA (1957) reported that tissue from the vascular cambium region of pea roots continued cellular division only when provided

¹ For further discussion, see chapter "Origin and developmental physiology of root nodules" by P. S. NUTMAN, pp. 1355—1379 of this volume, specifically p. 1364 *et seq.* and p. 1368/1369.

powdered yeast extract in the presence of auxin (2,4-D). KÜNNING (1950) gave evidence that cambial activity in *shoot* systems may be stimulated not only by auxin, but also by other, specific factors. He found thiamin, ascorbic acid and yeast extract all increased the activity of the vascular cambium; the effect, however, was considerably inferior to that of auxin.

One difficulty in studying the factors controlling vascular cambium activation in the root is the lack of a completely satisfactory test system. Callus tissue derivatives of the vascular cambium of roots very soon lose their distinctive anatomical (and perhaps physiological) character and thus no longer serve as test systems of vascular cambium behavior. BESSET (1953a, b) has devised a test for cell-division-stimulating substances based on the response of radish hypocotyl tissue, but these results may not in fact be referable to the root as well.

2. Cambial activity in excised roots.

There are clear indications that a simple test system could be developed using root organ culture. Although it was early believed that excised roots in culture produce no secondary tissues (WHITE 1943), a number of cases of vascular cambium formation by excised roots have been reported. DORMER and STREET (1948) found vascular cambium formation in excised tomato roots grown without subtransfer for 5—6 months. SEELIGER (1956) found cambial activity in excised roots of *Robinia pseudoacacia* (see p. 1300). TORREY (1951) reported induction of a vascular cambium by decapitation of isolated pea roots growing *in vitro*. Since the vascular cambium always appeared a constant distance proximal to lateral roots which were also induced by decapitation, he suggested that auxin produced by the lateral meristems might be the controlling factor in determining the site of vascular cambium initiation. That other factors beside auxin are essential for vascular cambium formation in pea roots grown in culture was shown by TORREY and SHIGEMURA (1957) who found that although root tips initially excised from germinating seed could be induced to form a vascular cambium with auxin treatment, first-transfer root tips were unable to respond to auxin treatment, having been depleted apparently of the factors other than auxin essential for vascular cambium formation.

It is of interest that in comparing excised roots of peas with roots from pea seedlings after the cotyledons had been removed, FRIES (1954) showed that the vascular system, especially xylem tissue, of the former roots was considerably more extensive than in the latter case. Judging from his figures, it would appear that vascular cambium formation occurred in the excised roots but not in the attached roots. Here perhaps under the heterotrophic dark conditions of these experiments, the shoot of decotyledonized seedlings effectively removed and utilized the factors necessary for vascular cambium formation by the root.

The problem of control of vascular cambium activity is one of great practical and theoretical interest. It is a problem that has hardly been touched experimentally. Work with excised root tips grown *in vitro* seems a promising approach to the problem. Why shouldn't it be possible to grow fleshy roots in culture, such as the tap root of carrot or the bulbous radish root? Thus far, despite some attempts (carrot: LEVINE 1951; radish: WHITE 1938, BONNER 1940) these roots grow in culture producing only primary tissues and showing no signs of their inherent tendencies for secondary development. Perhaps the shoot normally provides factors necessary for this secondary development; if so, it should be possible to provide them *in vitro*.

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