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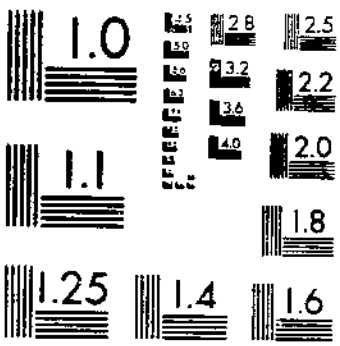

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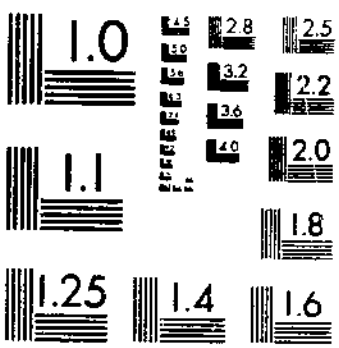

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MERISTEMS, GROWTH, AND DEVELOPMENT IN WOODY PLANTS

An Analytical Review of Anatomical, Physiological, and Morphogenic Aspects

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DEPOSITORY

A 63.9 Ag7-1 No. 1293
COP. 1

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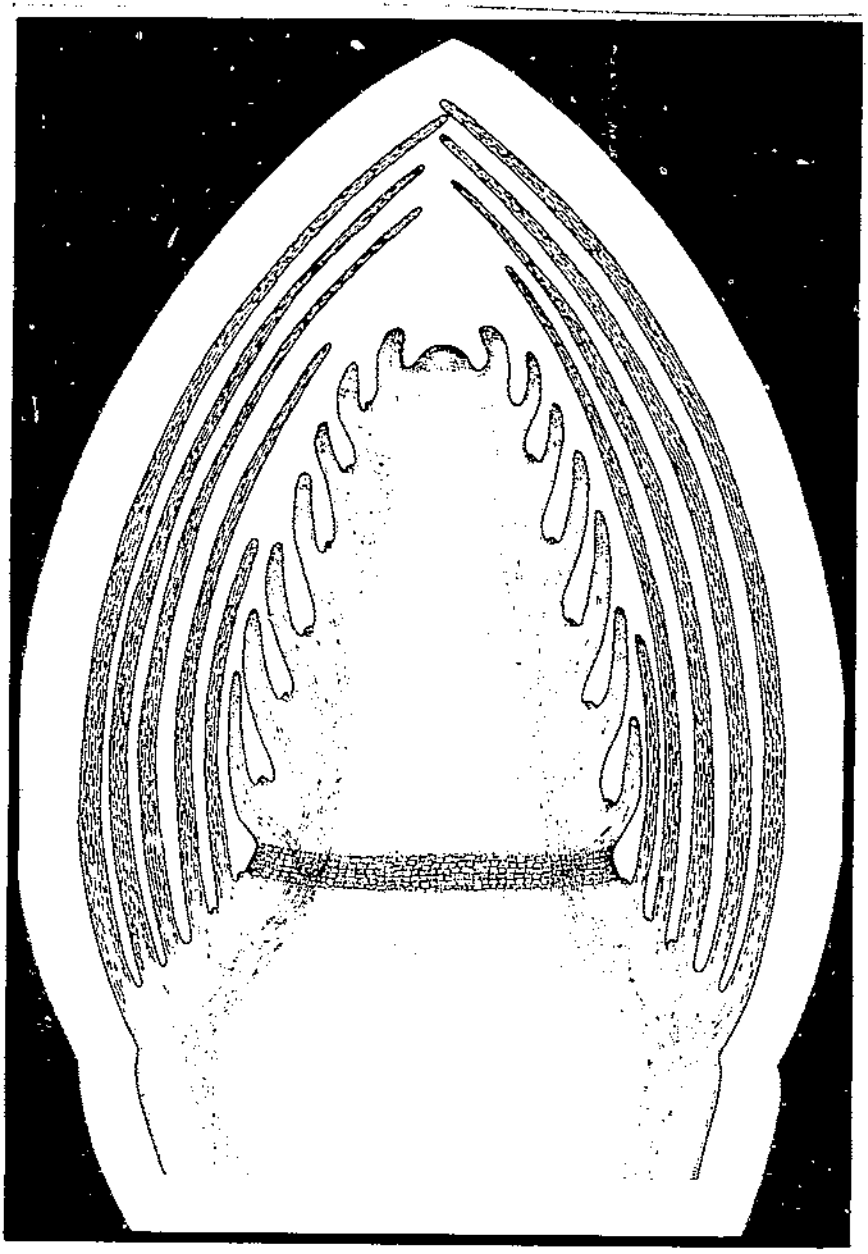
Technical Bulletin No. 1293 October 1963



PREFACE

Apical meristems are small. Lateral meristems are thin. Together they constitute a physically insignificant fraction of the total mass of a tree or shrub. Yet the whole future of the plant depends upon the activity of its meristems. Growth and morphogenesis, and the control of these processes, are largely localized in the meristems proper and in their ancillary regions of occasional cell division, continuing cell enlargement, and cell differentiation. The subject area encompassing meristems, growth, and development is basic to a wide range of research problems in forestry and horticulture.

This bulletin is intended for students and research workers, in plant physiology, horticulture, and the forest sciences, who are interested in the control of growth and development in woody plants. It is not a textbook. Illustrations duplicating those readily available in texts have not been provided. Readers are assumed to have knowledge of the basic principles of the anatomy, physiology, and biochemistry of plants, and to have access to textbooks on these subjects. I have attempted to go beyond the textbook level in analyzing complex problems, in searching for interrelations between the various islands of research information, and in providing a guide to the early as well as the more contemporary literature.

The approach is nonauthoritarian. Many questions are asked. Few are answered. Readers are encouraged to speculate and to doubt and question my interpretations as they see fit. I wish to be regarded not as an expert, or a teacher, but as a fellow student.

Although growth control in woody plants has many special aspects, it cannot be considered as a subject completely separate from that of growth control in herbaceous species. Much, or most, of the experimental work on growth regulators, photoperiodism, and photomorphogenesis was done with herbaceous species. Some of the evidence discussed and literature cited in this review is not directly concerned with trees or shrubs, but such citation and discussion is nonetheless prerequisite to intelligent consideration of the specific problems of growth control in woody plants.

Throughout the review, emphasis is put upon lines of work specifically aimed at increasing our basic knowledge of meristems and the control of their activities. The voluminous literature concerning purely empirical experimentation aimed at early application in the field is not stressed.

As a knowledge of political and social history is indispensable to social scientists, a knowledge of the history of biology is likewise indispensable to the biological theoretician and experimenter. Without the past, without an appreciation of past successes and failures, and their significance to us, our future advance would be wavering in direction and lacking in momentum. Such considerations, and the belief that discussions of sincere attempts to arrive at truth are

never obsolete, prompted use of the historical method of exposition in most sections of this review.

Plant names are generally the Latin names given in the works cited. Many original sources give no authorities for the names employed. None are given here. Some of the names used herein are not current or are in dispute. Readers who need current names and authorities must seek information in the papers cited, and elsewhere.

No review of this type can cover all related areas in addition to the central subject. The very important and closely related subjects of the control of flowering in woody plants, and the physiology of seed dormancy and the germination process, are treated only incidentally. Also outside the area of immediate concern are breaking of dormancy by deliberate wounding of plants or by applications of any of a great variety of chemicals having no known relation to any endogenous regulators.

This review is not exhaustive even within the subjects covered. The goal was to provide access to important lines of work rather than to cite all significant papers. Some references were intentionally omitted because they are included in bibliographies of other works cited. Some important papers were undoubtedly overlooked, and numerous recent ones came to my attention too late to be included. Coverage of some subject areas was modified because of the existence of relatively recent and readily available reviews by other authors. With these limitations understood, I hope that these discussions will encourage and facilitate further work on the fascinating subject of meristems and their activity or dormancy in woody plants.

A written discussion is linear. Only one aspect of a subject can be presented at a time. Words, sentences, and paragraphs follow one another. Each separate fact or idea in turn briefly commands the reader's attention. But the realm of ideas is not one dimensional. The numerous facts and ideas embodied in this review are related to each other more like various points within the volume of a sphere than like points on a straight line through space. To promote escape from linearity, numerous cross references have been provided in the text. These are indicated in italics within parentheses, either alone or separated from citations to other works by a semicolon.

I sincerely appreciate the assistance and advice received from many people during the preparation of this bulletin. Particularly helpful were Edward R. Moser, Librarian, Division of Biology, California Institute of Technology, and the staff members of the National Agricultural Library in Washington, D.C., and Beltsville, Md. Drs. Bruce M. Pollock, Harry A. Borthwick, Thomas O. Perry, and Robert M. Allen made many constructive suggestions after reading all or parts of the manuscript.

CONTENTS

	Page
Part I. Anatomy and physiological morphology	1
Organization of meristems	1
Development of the meristem concept	1
The origin of cells	1
The apical cell theory	3
The histogen theory of apical organization	4
Transition to modern concepts	5
Organization of gymnosperm shoot apices	6
Cytohistological zonation	6
Zone apicale, anneau initial, and méristème médullaire	12
Organization of angiosperm shoot apices	13
Tunica-corpus theory	13
Cytohistological zonation	16
Méristème d'attente, anneau initial, and méristème médullaire	18
Metrameristem	20
Synopsis on shoot apices	20
Organization of root apices	21
Root apex versus shoot apex	21
Apical cells and histogens	22
Körper-Kappe theory	23
Many-celled promeristems versus central cells	24
The quiescent center	27
Physiological morphology of shoot meristems and buds	30
Reactivity of shoot meristems	30
Metabolic differentiation within the meristem	30
Culture of isolated apices	31
Morphogenic regions of the apex	32
Special significance of the subapical region	34
Origin of leaves, cataphylls, and vascular tissue	35
Initiation of primordia	35
Procambium	38
Development of leaves	41
Development of cataphylls	44
Vegetative buds and the morphogenic cycle	46
The bud concept	46
A peculiar anatomical feature—the crown	47
Terminal buds	49
Axillary buds	54
Adventitious buds	58
Physiological processes in buds	59
Shoot tip abortion	62
Inability to form terminal buds	62
Physiology of apical abortion	63
Physiological anatomy and development in the cambium	65
Developmental anatomy	65
Morphogenic cycles in the vascular cambium	68
Part II. Episodic growth and dormancy of shoots	71
Concepts, nomenclature, and definitions	71
The dormancy concept and its development	71
Kinds of dormancy—definitions	73
Alternate growth and dormancy	76
Implications of episodic growth	76
Associated anatomical and cytological changes	78
Analysis of the control problem	80
Internal physiological factors	80
Why summer growth inhibition?	80
Possible root influences	81
Correlated inhibition and apical dominance	81

Part II. Episodic growth and dormancy of shoots—Continued
Analysis of the control problem—Continued Page
Experimental control of growth and dormancy in various species 84
An introduction to photoperiodism in woody plants 84
Pinus sylvestris 86
Fagus sylvatica 89
Robinia pseudoacacia 92
Catalpa bignonioides 93
Weigela florida 94
Cornus florida 95
Rhus typhina 96
Acer pseudoplatanus 96
The significance of photoperiodism 97
Are photoperiodic receptor and response mechanisms general? 97
Mechanistic implications of photoperiodic responses 99
Possible mechanisms of growth and dormancy control 100
Photomorphogenesis 100
Early work on light intensity and spectral quality 100
Duration of light 102
Etiolation 104
Phytochrome—a photomorphogenic receptor 106
Responses to light of limited spectral regions 111
A second photomorphogenic receptor? 115
Some kinetic aspects of photomorphogenesis and photoperiodism 117
Circadian rhythms in relation to photo- and thermoperiodism 119
Endogenous circadian rhythms 119
Circadian rhythms and photoperiodism 121
Circadian rhythms and thermoperiodism 124
Endogenous growth regulators 125
Introduction 125
The auxin concept 126
Auxins in buds and shoots 128
Auxins in developing long shoots versus short shoots 130
Auxins and cambial activity 133
The significance of auxins in dormancy control 138
Gibberellins 140
Kinins 146
Other possible regulators 148
Endogenous growth inhibitors 150
Interactions 154
Nonperiodic temperature effects 157
Chilling requirements 157
Unsatisfied chilling requirements and dwarfing 161
High temperature and rest induction 163
Warm baths as rest breaking agents 164
Part III. Episodic growth and dormancy of roots 166
Growth and dormancy in roots 166
Definition of the problem 166
Seasonal and episodic root growth 166
Anatomical and physiological aspects 171
Control of root growth 175
In retrospect 177
Literature cited 180

PART I. ANATOMY AND PHYSIOLOGICAL MORPHOLOGY

ORGANIZATION OF MERISTEMS

Development of the Meristem Concept

The Origin of Cells

The concept of meristems is a relatively recent one. Its formulation depended upon prior evolution of ideas concerning the cellular structure of organisms and the origin of cells. The evolution of those ideas was slow. Truths which seem obvious to us now were arrived at by the efforts of generations of dedicated men.

There were undoubtedly many brilliant minds among the botanists and microscopists of the 17th and 18th centuries. They did all they could do with the instruments available to them. But the results of their efforts advanced knowledge of cells and tissues only a little beyond the levels attained by Grew, Malpighi, and Leeuwenhoek. It was known that cork and wood are cellular in structure, but the cell was not recognized as the basic structural unit of all plant parts. Nothing was known about the origin of either cellular structure or of cells.

The great barrier to progress was chromatic aberration in lenses. Objects under the microscope shimmered with all colors of the rainbow. Details were blurred out and misinterpretation was easy. The development of achromatic lens systems was a breakthrough of great significance to biology. Achromatic microscopes became generally available to biological research institutions in about 1830. A resurgence of interest in plant anatomy and development began immediately and a great wave of progress followed shortly thereafter.

In 1830 the fact that wood is composed of mostly empty cells was generally accepted, although some question remained about the cellular origin of vessels. That other plant parts also consist of cells was, however, still not widely recognized. Modification of the cell concept to include not only the empty, thick-walled chambers of wood and cork, but also thin-walled structural units filled with liquids and gels came quickly after achromatic microscopes were in use.

On the basis of detailed studies of the structure of mosses and other plants Mirbel (1837)[1] maintained that the cell is the fundamental unit of structure in the plant kingdom. Treviranus (1835), Mirbel (1837), and Mohl (1845a, b) removed objections to the cellular structure of wood vessels by observing that vessels arise from files of cells which lose their end walls.

[1] Names and dates in parentheses refer to Literature Cited, p. 180.

A great series of further contributions was made by Mohl. Bast, bark, and other plant parts were all found to be cellular. Mohl was a very conscientious and careful observer who accurately described what he saw but did not engage in philosophical speculations. His papers, characteristically short and to the point, are still interesting and significant (see early volumes of *Botanische Zeitung*). Mohl's work overcame all objections to the cellular theory of plant structure and led to its acceptance as undisputed fact.

Solution of the problem of cell origin was also made possible by the achromatic microscope. The new knowledge that cells are the structural units of organisms did not answer the question of cellular origin. It was not at first obvious that cells are produced only by division of preexisting cells. Progress, however, was rapid during the two decades after 1830.

Brown (1831) published evidence that every living plant cell contains a nucleus, but did not realize its significance. Schleiden involved the nucleus in his explanation of cell origin, but only as a vesicle which somehow arises in a generative center and then produces the remainder of the cell. Schleiden (1842) summarized his work in a textbook which shows philosophical romanticism reminescent of Goethe's botanical writings. Nevertheless, the book had a profound effect upon botanical research because it convinced young botanists of the need for developmental studies and insisted that they use inductive methods.

Schleiden's theory of cell origin was further developed by Schwann (1839). He believed the cell to be coagulated or precipitated from sap, first the nucleolus, then the nucleus, and finally the remainder of the cell. The Schleiden-Schwann theory assigned no role to the nucleus after cell formation, and certainly did not anticipate the possibility of nuclear division. The theory enjoyed a short ascendency, but then went into decline because it could not accommodate the further findings of observers. Leadership in the field soon passed to Mohl and Nägeli.

Both Mohl and Nägeli were influenced by the Schleiden-Schwann theory, but they did not accept it as doctrine. Mohl continued his painstaking observational and descriptive work. In numerous short papers he described vacuoles, chloroplasts, and starch granules. He also described and named the protoplasm and recognized it as the essence of living matter, not merely unorganized slime. Mohl considered nuclei in the embryo sac to be derived from vesicles in the protoplasm, perhaps as envisioned by Schleiden and Schwann, but he also mentioned cell division as the normal method of cell reproduction in the vegetative parts of plants.

Other botanists became convinced that cells in growing plant parts are formed by cell division. Meanwhile Nägeli (1842, 1844) made very careful observations of cell division during pollen formation and elsewhere and described the process, including nuclear division, with great accuracy. Yet even Nägeli continued to believe in the possibility of the spontaneous generation of life and of cells throughout his entire lifetime.

Even before the concept of apical meristems was well established it was obvious that lateral zones of cell formation must be responsible for stem thickness growth. Mirbel (1837), writing at a time

when cells were thought to be coagulated from sap, used the term "cambium" in the sense of a sap or juice saturating the growing parts of plants. The term remained in use, but with new meaning, after the origin of cells by division was established. Anatomy and development in the cambium was a relatively noncontroversial subject. The outlines of our present knowledge of the cambial meristem were already evident in Nägeli's (1864) "Dickenwachsthum des Stengels." Valuable contributions were also made by Sanio (1872) and Mischke (1890), but the mechanism of girth increase in the cambial meristem itself was not well understood until later (see Bailey 1923).

The Apical Cell Theory

The large, single, apical cells of various mosses and algae were discovered and described by Nägeli (1845a, b). In apices of these plants it was obvious that all new cells were derived from preexisting cells by division. The concept of a single apical cell, dividing in a regular and predictable manner, and giving rise to all other cells of these plants, was enthusiastically accepted by the majority of botanists. The idea seemed inherently logical and at the time a working assumption that higher plant apices also possess single apical cells was a reasonable one.

Understanding of cell origin and further improvements in microscopes and in sectioning techniques had by 1850 made it possible to undertake meaningful studies of the organization of apical meristems of higher plants. The term "meristem" (from the Greek *meristos*, meaning divided) seems to have been introduced by Nägeli (1858).

Hofmeister (1852) published the first description of the organization of an apical meristem of an angiosperm. He reported a unique initial cell in *Zostera marina* (eel-grass), this cell being visible in early stages of development and dividing like the single apical cell of *Equisetum*. Later he reported *Acer* and *Fraxinus* to have cuneiform terminal cells and some other tree species to have tetrahedronal apical cells (Hofmeister 1857).

Hofmeister's apical cell theory received strong support from Pringsheim (1869), Nägeli (1878), Korschelt (1884), Dingler (1886), and Douliot (1890). The theory held that there were no fundamental differences in mode of origin of apical tissue between vascular cryptogams and phanerogams because it was supposed that, in both groups, all cells could be traced to divisions of a single apical cell. The applicability of the theory to any but embryonic apices of higher plants was soon questioned by some workers and a long controversy arose, the details of which are given by Koch (1891) and also by Schüepp (1926).

Gymnosperms received considerable attention because of their phylogenetic position between vascular cryptogams and angiosperms. Various workers reported single tetrahedral or prismatic apical cells in gymnosperm apices. A few careful observers, such as Strasburger (1872) and Groom (1885), could see no evidence for single apical cells. These dissenters were vindicated in later decades. The fact that others continued to report and describe single apical cells illus-

trates the powerful effects which preconceived ideas can have upon observers.

Reviewing the situation Douliot (1890) concluded gymnosperms, like vascular cryptogams, to have apical cells, sometimes pyramidal, sometimes prismatic, but always unique. Angiosperms he believed to have usually three, but sometimes only two, apical initial cells. While formalistically neat and satisfying, Douliot's position was not favored by time. Most of the early work was strongly characterized by formalism with little regard for the dynamic aspects of tissue development and cell function.

The Histogen Theory of Apical Organization

Meanwhile Hanstein (1868), working mostly with angiosperms, had evolved and published his histogen theory of apical organization. His ideas were based upon studies of 46 genera, including *Alnus*, *Populus*, *Platanus*, *Aesculus*, *Sambucus*, and *Robinia*. In contrast to the apical cell theory, Hanstein's histogen theory maintains that the shoot apex in angiosperms consists of a central core of irregularly arranged cells covered by a variable number of mantlelike layers. It proposes that each layer, and the core, is derived from a distinct initial cell or small group of cells (the histogens or tissue formers). Thus the origin of different parts of the apex cannot be traced to a single cell, but each part can be traced to one of a series of vertically superimposed initials or groups of initials.

Hanstein attached less importance to the behavior of individual cells than to the general distribution of growth in the apex as a whole. He did, however, attempt to assign specific destinies to various regions of the meristem, regions which in turn were derived from the series of superimposed initials. The surface layer, or "dermatogen," Hanstein believed, produced only the epidermal system; the underlying layer or layers, which he called the "periblem," produced the cortex; and the central core, or "plerome," produced the procambial and pith tissue of the axis. Hanstein originally applied his terms to zones of meristematic tissue in the early stages of development from initials, but in later literature the same terms were sometimes applied to the initials themselves.

The predestination aspect of Hanstein's theory drew a great amount of criticism which was reviewed and discussed by Schmidt (1924). A further difficulty was that in many apices periblem and plerome were not distinguishable, and in others where they were distinguishable their respective roles did not conform to Hanstein's ideas. These weaknesses were noted and discussed repeatedly (Koch 1891; Schmidt 1924; Korody 1937).

The histogen theory was applied to root as well as shoot apices. The availability of precision microtomes made it possible by 1870 to prepare good median sections of apical meristems. This led to many studies of root meristems and reports concerning their histogens. Janczewski (1874a, b) introduced a fourth histogen, the "calyptrogen," in his descriptions of roots of grasses and other plants which have a rootcap of independent origin. With regard to root apices the histogen theory attained general acceptance. In fact, Hanstein's ideas and terminology are not yet totally obsolete

and are still employed by some authors in discussing histogenesis in roots.

Hanstein's histogen theory implied complete divergence in structure between the shoot apices of vascular cryptogams with their single apical cells and those of phanerogams. This idea was, of course, strongly opposed by supporters of the apical cell theory because they believed the stem of phanerogams to be phylogenetically evolved from the stem of vascular cryptogams. Controversy about this point caused great interest in the shoot apex of gymnosperms as the most primitive surviving phanerogams. It was supposed that some lower forms of gymnosperms would be found to have distinct apical cells and that transitional forms might be discovered which would aid in the interpretation of phanerogamous apices.

Strasburger (1872) made an extensive survey of shoot apices in several groups of gymnosperms and found no evidence to support the apical cell theory. As a result he adopted a modified form of the concept and terminology of Hanstein and attempted to show that a marked intergradation of structure exists in apical meristems of various genera of gymnosperms. For example, *Araucaria brasiliana* has a discrete outer layer or dermatogen and seems closely related to angiosperms, whereas in *Cycas revoluta* and many of the Abietaceae it is not possible to draw a clear demarcation between a dermatogen and a periblem. Both Strasburger (1872) and Schüepp (1926) concluded that the gymnospermous shoot apical meristem could have been derived phylogenetically from a type having a single apical cell.

Transition to Modern Concepts

Groom (1885) had indicated that neither the apical cell theory nor the histogen theory provided a satisfactory interpretation of the structure and development of shoot apices of gymnosperms. This was also recognized by Koch (1891) who disregarded earlier formalism and gave accurate and detailed accounts of the cytohistological zonation in the shoot apical meristems in many conifers and in *Ephedra*.

Koch considered the apex to consist of two well-defined regions: (1) a peripheral mantle composed of densely cytoplasmic cells and (2) an inner core made up of larger and vacuolated dividing cells. Koch's zones did not, however, correspond to the histogens of Hanstein (1868). The central zone proposed by Koch produced only the pith, whereas epidermis, cortex, procambial tissue, and foliar organs were all derivatives of the peripheral layers. Koch (1891) also believed that the absence of a well-defined epidermis and the temporary enlargement in depth of a cell on the surface of the apex were the chief factors which had led earlier workers to report the existence of single apical cells in the terminal meristems of gymnosperm shoots.

A new interpretation of apical organization and growth was stated in a paper by Schmidt (1924). In contrast to Hanstein, Schmidt recognized only two tissue zones in the shoot apex. These were (1) the "tunica" consisting of the peripheral layers which enclose (2) the central tissue or "corpus." Hence Schmidt's theory is known as the tunica-corpus theory.

According to Schmidt, anticlinal divisions and surface growth predominate in the tunica, with the result that each tunica layer, except during initiation of leaves or buds, remains discrete and self-perpetuating. On the other hand, growth of the central corpus consists of an increase in mass and the planes of division and arrangement of cells tend to be quite irregular. Some unknown mechanism, which adjusts the balance between surface and volume growth, controls the development of leaf and bud primordia. These aspects of Schmidt's theory were discussed by Foster (1936) and by Schüepp (1938). It should be emphasized that tunica-corpus terminology suggests only a general topological zonation rather than specifically predestined cell layers or histogens (Jentsch 1957).

Whereas the apical cell theory and the histogen theory were developed with reference to both root and shoot apices of angiosperms and of gymnosperms, the tunica-corpus theory was formulated with reference to angiospermous apices (*p. 13*) and has been found to be largely inapplicable to the characterization of apical meristems of gymnosperms (*see, however, pp. 10–11*).

A discussion of modern concepts of apical organization and development is made more meaningful if the historical development of the various theories is kept in mind. It should be remembered that there may be some truth in each theory even when applied to higher plants. Some pines have single apical cells during embryonic stages (Johansen 1950). Numerous root apices and a few shoot apices, for example that of *Potamogeton crispus* (Schalscha-Ehrenfeld 1940), are apparently well interpreted by the histogen theory.

More detailed accounts of the history of both the meristem concept and of developmental morphology of vascular plants are given by Schüepp (1926) and by Sifton (1944). The most complete discussion of the early historical development of these subjects is still that given by Sachs in his "History of Botany" (English translation, 1906).

Organization of Gymnosperm Shoot Apices

Cytohistological Zonation

The shoot apices of gymnosperms are adequately described by neither the apical cell theory nor the histogen theory; furthermore, the usefulness of the tunica-corpus theory is limited because a well-defined tunica occurs in only a few taxonomic groups. It is obvious that if the apical dome is to grow and provide space for the initiation of new primordia, its surface as well as its volume must be increased.

If one or more surface layers are present in which cell divisions are exclusively anticlinal, then a tunica exists. This condition exists in many, perhaps most, angiosperms. Alternatively, if cells in the surface layers divide periclinally or obliquely as well as anticlinally, then, strictly speaking, there is no tunica. Apices of many gymnosperms have no tunica, but there are important exceptions (Johnson 1951; Griffith 1952; Fagerlind 1954). These differences in surface layers may have some phylogenetic significance. Physiologically they are interesting because they raise the question of why divisions in the outer layers of angiosperm shoot apices are almost

always anticlinal, whereas control of orientation of the plane of division is much less rigid in gymnosperms.

Korody (1937) has suggested that the gymnosperm apex be considered as a naked corpus when the tunica is absent. If not objectionable, this idea is also not particularly helpful. It should be remembered that the tunica-corpus theory was proposed as an aid in describing a type of growth, with emphasis upon orientation of planes of cell division (Schmidt 1924), and does not provide a basis for classification of tissue types or zones within the meristem. Within the framework of the tunica-corpus theory, gymnosperms may be considered as having an incipient tunica, absent in the lower forms, but in some higher forms developed to the same degree as is typical in angiosperms (Johnson 1951).

The apical cell theory, the histogen theory, and to a lesser extent the tunica-corpus theory, were concerned with the destinies and lineages of individual cells. But after 1930 new interest in physiology and developmental morphology turned emphasis toward the behavior of whole cell complexes within the meristem. The problem of understanding how the various tissues and organs of the shoot are developed from the relatively undifferentiated cells of the apex became much more important than that of locating the ultimate source of cells. This trend is evident in the work of Louis (1935), Barthelmess (1935) and Kaplan (1937).

Gradually there arose a concept of cytohistological zonation within the gymnosperm shoot apex, an idea already anticipated in the work of Koch (1891) (*p. 5*). This idea depends upon the existence, within the meristem, of zones distinguishable from one another by (1) cell size and degree of vacuolation, (2) nuclear volume, (3) staining characteristics, (4) frequency of cell division, (5) relative cell wall thickness, and (6) orientation of planes of cell division. The concept came to fruition in Foster's (1938) application of zonation in his detailed interpretation of the shoot apex of *Ginkgo biloba* (*later also in different versions, p. 12 ff. and p. 18 ff.*).

Foster recognized five zones in the *Ginkgo* apex (fig. 1). These zones are defined and described here, not because of the importance of *Ginkgo*, but because recognition of cytohistological zonation was a definite advance in understanding the organization of shoot apices.

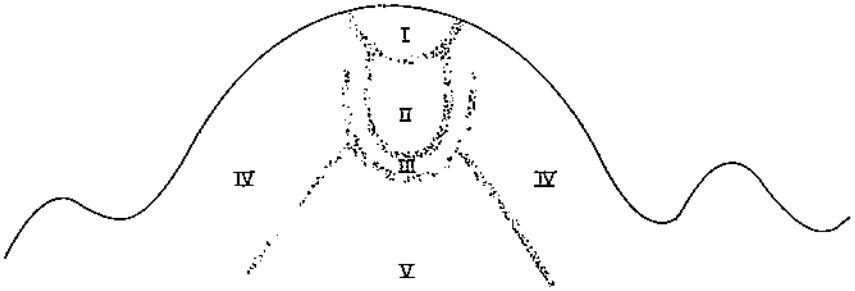


FIGURE 1.—Cytohistological zonation pattern of the shoot apex of *Gingko biloba*: *I*, Apical initial group; *II*, central mother cells; *III*, transition zone; *IV*, peripheral zone; *V*, rib meristem. The zones often have poorly defined boundaries. (After Foster 1938.)

Apical initial group (fig. 1, Zone I).—The apical initials occupy the summit of the meristem and are larger than other cells of the surface layers. The nuclei are large and the cytoplasm somewhat vacuolated. The cells are only lightly stained by safranin. There is no single or permanent apical cell, and no discernible regularity of cell division. Divisions occur with varying frequency and in various planes. The apical initials contribute directly to the peripheral zone and to the central mother cell zone. They are the ultimate, but perhaps remote, source of all cells of the shoot.

Central mother cell zone (fig. 1, Zone II).—This zone occupies a roughly spherical volume in the upper central region of the shoot apex just beneath the apical initials. Its component cells were called "central mother cells" by Foster (1938) because he believed the zone to function as a common area of propagation (but neither ultimate initiation nor rapid multiplication) of cells, which after further multiplication comprise most of the internal tissues of the apex.

The central mother cells are the largest cells of the apical meristem. Their nuclei are large and are only lightly stained with safranin. The cytoplasm is less dense and more vacuolated than in the peripheral zone. Growth of the cells is primarily in volume with no regular pattern. This results in highly irregular cell arrangement. An additional distinctive feature of central mother cells is wall thickening, presumably temporary, which sometimes resembles that of collenchyma cells. Mitoses are apparently infrequent except near the transition zone.

Transition zone (fig. 1, Zone III).—The transition zone comprises the lateral and basal margins of the central mother cell zone. It is a zone of renewed mitotic activity. When viewed in cross section the zone appears cambium-like (see Foster 1938, Plate 26). The zone contributes cells to the peripheral zone and to the rib meristem (*p. 17*). Foster did not speculate on the rate at which dividing cells of the transition zone are themselves replaced by derivatives of the central mother cells. There is no evident reason why such replacement need be frequent. Because a well-defined transition zone is lacking in many gymnosperm apices it is sometimes omitted from discussions of cytohistological zonation.

Peripheral tissue layers (fig. 1, Zone IV).—The peripheral tissue layers occupy most of the total volume of the apex and surround the central tissues with a dome-like mass carrying the apical initial zone at its apex. All cells of the peripheral layers are small and divide frequently. Their dense protoplasts are deeply stained by safranin. Although the different layers of the peripheral zone have different origins, cellular characteristics are markedly uniform throughout.

The outer layer of the zone originates from anticlinal divisions of the apical initials, but it is never discrete because periclinal divisions occur throughout its extent. This is why there is no tunica layer. The inner layers are continually augmented by daughter cells from the cambium-like transition zone. Ultimately the peripheral tissue layers give rise to the epidermis, lateral appendages, cortex, and probably also the vascular tissue of the shoot axis.

Rib meristem (fig. 1, Zone V).—The term "rib meristem" was not original with Foster (1938); but was introduced by Schüepp (1926)

to designate the primary meristem type in which cells divide at right angles to the stem, leaf, or root axis producing parallel files (*Rippen*) of cells. In *Ginkgo* the rib meristem arises from cells of the basal part of the transition zone in which there is a renewal of mitotic activity and decrease in cell size relative to the lower cells of the central mother cell zone. Some of the cell wall thickenings of the latter may be carried over into the rib meristem zone. The rib meristem consists of files of cells in which transverse divisions and extension growth predominate. Occasionally new files of cells are introduced by periclinal or oblique divisions (Foster 1938).

The rib meristems of long and short shoots of *Ginkgo* exhibit pronounced differences in behavior (*for references and discussion of physiology see pp. 130–131*). In the short shoot rib meristem activity is ephemeral. It gives rise to maturing pith cells only a short distance below the transition zone. Consequently there is little internodal elongation. The extensive internodal elongation of long shoots partly results from much more persistent rib meristem activity.

In young internodes the peripheral tissue regions may also take on the aspect of rib meristem and are included with it by some authors. In this sense, internodal tissues are very largely derived directly from the rib meristem, though the ultimate and remote source of cells lies in the more apical zones.

As in Ginkgoales, the organization of shoot apices of the various genera of Cycadales is not interpretable in terms of single apical cells, discrete histogens, or a tunica-corpus structure. Moreover, the cytohistological zonation applied by Foster (1938) to *Ginkgo* can be used with surprisingly little modification to interpret cycad apices.

An untrained observer first looking at median longitudinal sections of shoot apices of *Ginkgo biloba* and the cycad *Microcycas calocoma* would not expect an anatomist to assign similar zonal organization to both. The apex of *Microcycas* is, in fact, a good example of how misleading cell patterns can be as indicators of loci of meristematic activity if they are not analyzed with extreme care.

Rows of cells appear to radiate, fountainlike, upward and outward from a central area beneath the apical dome. Yet the initials are actually in the upper surface layers. The rows of derivatives converge downward toward a central mother cell zone (Foster 1943). This is logical if, instead of a fountain, one imagines a sector of a cross section of a woody stem. In the latter the rows of tracheids and rays also radiate from a central area, but the cells had their origin in the cambium, not in the pith region.

Zonation in cycad apices is more variable and frequently not as well defined as in *Ginkgo*. The zone of apical initials is difficult to delimit and may gradually grade off into the peripheral zone. Anticlinal and oblique divisions in the surface layers may sometimes add new vertical series of cells, deflect others, and produce a conspicuous fanning out of cell files as in *Microcycas calocoma* (Foster 1943).

Cycad apices usually have a central mother cell zone similar to that of *Ginkgo*. But in *Cycas revoluta* vertical files of cells may occur throughout the zone making it indistinguishable from the rib

meristem below (Foster 1939a). In *Zamia* the central mother cells may be very large, highly vacuolate, and have thickened walls with primary pit fields; or, if the apex is rich in starch, no central mother cell zone may be distinguishable at all (Johnson 1945). A cambium-like transition zone may be regarded as present whenever there is an easily definable central mother cell zone, but usually the transition zone is not as well defined as in *Ginkgo*. Peripheral tissue and rib meristem zones are always present, though the latter is sometimes not distinguishable from the central mother cell zone.

Popham (1951) used the presence or absence of a cambium-like zone beneath the central mother cells as a criterion to divide gymnosperm apices into two groups. On this basis the cycads, *Sequoia sempervirens*, and *Pseudotsuga taxifolia* are assigned to the *Ginkgo* type. Most other investigated gymnosperms, including members of Pinales, Taxales, Gnetales, and Ephedrales, are grouped in Popham's *Abies-Cryptomeria* type. In these there is no cambium-like zone between the central mother cells and the subjacent rib meristem, and the central mother cell zone itself may resemble rib meristem more than its counterpart in *Ginkgo*.

It is noteworthy that only leading shoot apices of *Sequoia sempervirens* have ginkgoid zonation, whereas apices of lateral branches lack the cambium-like zone (Sterling 1945a) and fall into the alternate category. Obviously more information is needed on the behavior of the cambium-like layer with regard to the various phases of shoot growth and development. The transient occurrence of a somewhat similar layer in some angiosperm apices has been correlated with specific phases between initiation of foliar primordia (*pp. 16–18*).

Apical zonation in Pinales is not as diversified as in Cycadales, but is nonetheless more variable and less well defined than in *Ginkgo* (Cross 1943a, b; Kemp 1943; Sterling 1945a, 1946). Generally, zonation patterns encountered in the shoot apices of the various taxa of Pinales can be considered as modifications of the *Ginkgo* pattern described by Foster (1938).

Sacher (1954) distinguished three types of apical zonation within the order Pinales. These are (1) the ginkgoid type (*Pinus*, *Pseudotsuga*, and other genera of Pineaceae) in which there is no discrete surface layer, (2) the taxodioid type (members of Taxiodiaceae, Cupressaceae, and Taxaceae), characterized by a discrete surface layer except for the apical initial region, and (3) the araucarioid or tunica-corpus type in which a complete tunica layer is present. The latter type is comparable to that commonly found in angiosperms. According to Sacher (1954) there are easily recognizable differences even within the genus *Pinus* in that "soft" pines (subgenus Haploxylon) exhibit a ginkgoid zonation whereas "hard" pines (subgenus Diploxylon) show a less distinct pattern.

The occurrence of apices with tunica-corpus structure within the Gymnospermae (Johnson 1950; Griffith 1952; Fagerlind 1954; Guttenberg 1955; Sterling 1958) in no way detracts from the value of the zonation concept. Cytohistological zonation and tunica-corpus structure are not mutually exclusive. The former merely indicates that cells in definable areas of the apex are morphologically and/or

physiologically different from cells in other definable areas. The latter implies that there is one or more discrete outer layer of cells which does not contribute to the inner zones or corpus. Cytohistological zonation can and does occur in apices which also have a tunica-corpus structure. In such instances the tunica layer can be considered as part of an enlarged apical initial zone with its own initials at its apex. The corpus then includes the remaining apical initials and the internal tissue zones.

The shoot apices of gymnosperms generally show seasonal changes in form, size, and activity corresponding to the periods of winter rest and dormancy (*for definitions see pp. 73–75*), bud expansion, and the period of formation of the new bud.

There is some disagreement as to whether such changes are fundamental or superficial. Kemp (1943), Sterling (1946), and Singh (1961) reported that in *Torreya*, *Pseudotsuga*, and *Cephalotaxus*, respectively, there is a decrease in the distinctness of zonation during the dormant period. On the other hand, Sacher (1954) found that in *Pinus lambertiana* no basic change or decrease in distinctness is evident in the zonation of the apex throughout the annual growth cycle (*p. 51*). Parke (1959) reported that the volumes of the various zones in the shoot apex of *Abies concolor* change markedly during the annual growth cycle, but that the basic pattern of zonation remains unaltered (*p. 50*).

An additional point is that apical organization may change during ontogeny even beyond the embryonic stage. For example, in the shoot apex of *Gnetum* in the cotyledonary stage there is no tunica and zonation is diffuse. As the plant grows, apical zonation becomes more distinct. Periclinal divisions in the outer layers become increasingly rare until the tunica-corpus condition is approached (Fagerlind 1954). Physiologically speaking, orientation of planes of division of surface cells is more closely controlled in adult than in juvenile plants.

It is agreed that changes in size and shape do occur, whether fundamental or superficial, and that comparisons of one species with another are not valid unless both are in the same physiological and morphological state with respect to ontogeny and their annual cycle of growth.

Also disturbing to attempted correlation of apical structure with phylogeny are indications that a relationship exists between apical meristem structure and shoot vigor. In *Sequoia sempervirens* (Cross 1943b; Sterling 1945a) and in *Agathis lanceolata* (Sterling 1958) those shoot apices which are smaller in size have a better defined surface layer than do larger ones. The dormant shoot apex of *A. lanceolata* can be adequately described in terms of the tunica-corpus theory if the individual apex being examined is a small one. Larger dormant apices (from strong terminal buds) have better defined histological zonation and more frequent periclinal divisions in the outermost layer.

Use of patterns of apical structure or zonation in attempts to determine phylogenetic status will be on rather doubtful ground until it is determined whether the apex of the weak lateral or the vigorous main shoots are definitive and whether apices should be dormant or active when collected.

Zone Apicale, Anneau Initial, and Méristèm Médullaire

Numerous authors have expressed the view that all tissues of the shoot are ultimately derived from the relatively superficial apical initial cells, and that the central mother cell zone, itself derived from the initials above, contributes cells to the peripheral tissue zone and to the rib meristem.[2]

There is, however, some disagreement regarding the extent to which the apical initials and the central mother cells actually participate in tissue formation during gymnosperm shoot growth. Most investigators assume the apical initials and central mother cells to be actively meristematic, whereas some workers, mostly in France, believe these areas to be the least active or even quiescent. The disagreement stems from the difficulty of determining relative *frequency* of mitosis in different zones of the meristem when there is little information on the relative *duration* of mitosis in these zones (*p. 19*).

On the basis of inferred differences in mitotic frequency, the French plant anatomist, Camefort (1950, 1951, 1956a, b), applied the concept of cytohistological zonation to interpretation of the gymnosperm shoot apex somewhat differently than did Foster and others in the United States (*p. 7 ff.*). He recognized only three zones, which are the following:

Zone apicale.—The *zone apicale* corresponds to the combined apical initial and central mother cell zones of Foster's terminology (*p. 8*). Cells of the *zone apicale* are reputed to be the least active of the entire apex. They are poor in ribonucleic acid and have very feeble powers of proliferation. For example, in the *zone apicale* of *Picea excelsa* Camefort (1956a) observed only 2 mitoses as compared with 198 in the subjacent zones.

Anneau initial.—The peripheral tissue zone or flank meristem of other authors corresponds to Camefort's *anneau initial*, a term proposed earlier by Plantefol (1947) with reference to angiosperms. The cells of the *anneau initial* are rich in ribonucleic acid and proliferate actively. This highly meristematic zone produces the foliar primordia, the cortex, and vascular tissue of the stem.

Méristèm médullaire.—Camefort's third zone, the *méristèm médullaire*, is located below the *zone apicale* and is surrounded laterally by the *anneau initial*. The *méristèm médullaire* is largely equivalent to Foster's rib meristem (*p. 9*). It produces cells which mature into pith.

Camefort (1956a) objected to the idea that the so-called apical initials and the central mother cells are meristematically active. Whereas cell arrangements and wall configurations seemed to point toward the apical cells or central mother cells as centers of cell origin, the actual function of these zones as such had, he maintained, not been demonstrated.

Would cell patterns be very different if the supposed apical initials and central mother cells divided only rarely? Again, cell patterns are indicators of ancestry and lineages. Even if it is

[2] Foster 1938a, b, 1940, 1941a, b, 1943, 1949; Cross 1939, 1941, 1942, 1943a, b; Johnson 1939, 1943, 1944, 1951; Gifford 1943; Kemp 1943; Sterling 1945a, 1946; Allen 1947a, b; Griffith 1952; Sacher 1954.

granted that apical initial cells are the remote ancestors of all cells of the shoot, is it necessary to assume that the remote ancestors continue to contribute new cells in an apex beyond the embryonic stage? Are not additional divisions of descendent cells in the peripheral and central areas sufficient to produce all the tissues of the shoot? Unequivocal answers to these questions are not yet available (*pp. 17, 34*).

The points of controversy between adherents to the French ideas and others can be viewed as matters of degree rather than conflicts at the fundamental level. Camefort (1956a, b) has not claimed that cells in the *zone apicale* never divide (see also Buvat 1955). Moreover, Foster (1938) originally described the central mother cell zone as one of relatively low mitotic activity, which has at its lower and lateral boundaries a transition zone of renewed meristematic character (*p. 8*). In essence the disagreement is partly semantic and partly revolves about the real question of the role of apical cells and central mother cells in shoot ontogeny (*p. 19*).

There is little factual information concerning the function of the apical zone in gymnosperm shoot ontogeny. Chouinard (1959a), after a detailed study of the shoot apex of *Pinus banksiana*, concluded that the cells of the apical zone simply divide passively when the wave of proliferation coming from below reaches the apex. Such divisions allow the apical zone to harmonize its growth with that of the subjacent zones. In the view of Chouinard, construction of the juvenile shoot of *P. banksiana* can be accomplished almost entirely through the histogenic activities of the subapical meristematic zones which are capable of regenerating themselves in their own upper regions. This is in agreement with Camefort's (1956a, b) ideas.

The idea of a semi-quiescent *zone apicale* within the growing shoot apex of gymnosperms, if the existence of such were confirmed by strong evidence from a variety of genera, might eventually induce formulation of new concepts of apical organization and physiology. Some of the resistance to acceptance of the inactive *zone apicale* concept may possibly be the result of lingering influences of the apical cell and histogen theories with their strong emphasis upon apical and near apical cell division. However, uncritical acceptance of new ideas is also to be avoided.

The present situation, then, is one of controversy which could bring new understanding. A somewhat similar controversy exists with regard to angiosperm shoot apices (*p. 18 ff.*).

Organization of Angiosperm Shoot Apices

Tunica-Corpus Theory

Typically, but not invariably, the domelike part of the shoot apical meristem of angiosperms has a structure suggesting that the one to several outer layers of regularly arranged cells are discrete and arise from specific groups of initials. Divisions in these layers appear to be almost exclusively anticlinal. The tissue mass beneath the superficial layers is characterized by a more random arrangement of cells. Thus the structure seems to conform to Schmidt's (1924)

tunica-corpus theory (*p. 5*). (Foster 1939b; Sifton 1944; Jentsch 1957; Clowes 1961).

In this view the angiosperm shoot apex typically consists of a central region, the corpus, in which planes of cell division may be quite randomly oriented, and a one or several layered superficial region, the tunica, in which planes of cell division are almost entirely anticlinal. Schmidt's (1924) original definition of the tunica allowed that a small fraction of the divisions therein would be periclinal. This loose definition was adopted by some authors (Reeve 1948; Gifford 1954). Others have preferred a stricter definition and designate as "tunica" only those layers in which *no* periclinal divisions may be detected at a given time (Popham 1951; Clowes 1961). The strict definition is adopted here. The term "mantle" has been used instead of "tunica" in the loose sense (Popham and Chan 1950).

Originally evidence for the existence of discrete surface layers in angiosperm shoot apices was deduced from the arrangement and aspect of cells in fixed and stained sections. Later additional evidence was provided by investigations of the development of periclinal chimeras. The remarkable stability and persistence of some of the latter seems consistent with the existence of a discrete tunica layer. But some evidence obtained from chimeras also raised doubts about the adequacy of the tunica-corpus concept in describing so dynamic a system as the growing shoot apex.

After studying colchicine-induced polyploid chimeras in the three regular outer layers of the shoot apical meristem of *Datura*, Satina et al. (1940) reported that the two outermost layers formed a tunica, whereas the third contributed cells to the corpus. Baker (1943), by means of chimeras, found a self-perpetuating tunica to be present in *Solanum tuberosum*. Likewise Dermen (1945) demonstrated the presence of distinct apical layers in *Oxycoccus* by using colchicine-induced chimeras. At first he considered these to be histogenically independent.

Later, Dermen (1947) concluded the apical layers of *Oxycoccus* to be somewhat unstable and the tissues derived from them to be variable. He did not consider his work to support the tunica-corpus theory, and implied that the latter had no real histogenic merit. Dermen may have placed more emphasis upon histogenesis and predestination than Schmidt (1924) intended (see Jentsch 1957). Nonetheless, the number of regularly arranged layers in the *Oxycoccus* apex is so variable (Dermen 1945, 1947), and any tunica-corpus boundary so transient, that the tunica-corpus concept is not very helpful in describing the apex as a dynamic system.

It was long thought that periclinal chimeras could not exist in plants lacking a true tunica layer, but Thielke (1954, 1957) has shown this to be untrue. In *Tradescantia fluminensis* there are no periclinal divisions at the very summit of the apex although they occur elsewhere in the surface layers. Thus there is no tunica, yet periclinal chimeras do persist. These conditions may not be unusual. Therefore the persistence of periclinal chimeras is not in itself unequivocal evidence for the existence of a self-perpetuating tunica. The uses of induced chimeras in studying the behavior of shoot

apices is further discussed by Guttenberg (1960), Dermen (1960), and Clowes (1961).

It is easy to determine the number of tunica layers in a median longitudinal section of a specific apex under observation, but other apices of the same species may have a different number (Popham 1960). Furthermore, observation of a layer of cells showing no evidence of periclinal divisions offers no guarantee that the cells would not have divided periclinally or randomly in the near future. One reason why this is true is that some of the subsurface layers may consist of regularly arranged cells which by synchronous periclinal division produce new layers within. These layers actually arise by periclinal division, but there is no evidence of periclinal division within any one layer.

In fixed and stained material such regular layers are not readily distinguishable from true tunica layers having no periclinal divisions and may be interpreted as part of a tunica having a variable number of layers. These difficulties and their implications have been discussed by Gifford (1954) and by Jentsch (1957). Reeve (1948) described fluctuations in the depth of tunica in *Cornus californica*, *Lithocarpus californica*, *Quercus kelloggii*, *Salix laevigata*, *Garrya elliptica*, and other woody species. The observed fluctuations were periodic and were interpreted as resulting from an organized mode of growth. Reeve also stressed the need for greater emphasis on "dynamic principles and apical evolution" in application of the tunica-corpus concept.

The number of tunica layers reported in angiosperm shoot apices has varied from none to six (Zimmermann 1928; Foster 1939b; Schalscha-Ehrenfeld 1940; Thielke 1951; Jentsch 1960). According to Thielke (1959) *Saccharum officinarum* has no discrete tunica layer at all and exhibits an apical structure more similar to that typical of gymnosperms than of angiosperms. Popham (1958) also reported that *Chrysanthemum* apices sometimes lack a tunica.

It is now recognized that the number of parallel surface layers may vary during the ontogeny of the plant and also with seasonal growth changes. Periodic changes in apparent depth of the tunica may occur in relation to the initiation of leaves. In *Dianthera americana* the number of apparent tunica layers varies regularly from one at leaf initiation to three during intervening periods (Sterling 1949). Similar changes may occur in some other species (Reeves 1948) but are not necessarily universal. They are not obvious in *Viburnum rufidulum* (Cross 1937a) or *Liriodendron tulipifera* (Millington and Gunckel 1950).

As in the case of gymnosperms, some workers consider such periodic fluctuations, where they occur, to be insignificant (Reeve 1948; Rouffa and Gunckel 1951), whereas others believe that they represent a basic change in apical structure (Kliem 1937; Schnabel 1941). The situation was reviewed by Gifford (1954), and has more recently been treated by Jentsch (1957, 1960).

Jentsch believes that the disagreement arises mostly from failure to recognize that the corpus of an apex may exhibit a stratification of its outer layers which are then difficult to distinguish from any original and persistent tunica layers. The shoot apex of *Hippuris vulgaris* may have four, five, or six apparent tunica layers (Jentsch

1960), but whether a single apex undergoes changes in the number of layers during its ontogeny is difficult to determine because direct observations cannot be made without destroying the apex.

The tunica-corpus theory aids in describing an apex on the basis of planes of division of existing cells and their ancestors. It is less helpful in studies of the developmental morphology and physiology of the apex. Although there are some indications of metabolic differences between inner and outer layers (Sunderland et al., 1956, 1957), such differences may not be correlated with the presence or absence of a discrete tunica. Furthermore, in large angiosperm shoot apices it is obvious that cytohistological zones do exist within the so-called corpus. There is, in fact, no reason why cytohistological zonation akin to that of gymnosperms (*p. 6 ff.*) cannot be used to describe angiosperm apices.

Cytohistological Zonation

The first detailed description and discussion of cytohistological zonation in angiosperms was that of the *Heracleum* shoot apex by Majumdar (1942). Later others[3] documented the widespread occurrence of a zonal structure superimposed upon a tunica-corpus organization. It should be understood that recognition of cytohistological zonation does not demand abandonment of the tunica-corpus theory by those who prefer the latter. The two approaches to description of apical organization can be complementary rather than antagonistic.

The typical cytohistological zonation pattern of gymnosperm apices (fig. 1, p. 7) can be used as a point of departure in visualizing zonation in angiosperm apices. Opinions expressed and terminologies employed in the literature are, however, quite variable. Thus far apices from only a small number of angiosperm species have been studied in detail. Although general patterns are just beginning to emerge, it is now safe to say that details of zonation vary between species, between individuals of the same species, and probably vary also during different phases of the growth cycle in the same apex (Popham 1960).

Many of the detailed differences in zonation and planes of cell division are probably too superficial and variable (Millington and Fisk 1956) to justify using them as criteria for classifying apices. A general feature in common with gymnosperms is a central apical to subapical zone of vacuolated cells. In the central axial area beneath this is a central mother cell zone. As in gymnosperms, it is surrounded by a densely cytoplasmic peripheral zone. The rib meristem is also a common feature. Gifford (1954), Popham (1960), Guttenberg (1960), and Clowes (1961) have critically discussed various aspects of zonation in angiosperm shoot apices. Jentsch (1957), however, has not found zonation useful.

Of special physiological interest is the reported occurrence in some angiosperms of a cup-shaped, cambium-like zone similar to that found in cycads and other gymnosperms (*see pp. 8, 10*). Such a zone has been described in *Opuntia cylindrica* (Boke 1941), *Bellis*

[3] Hsü 1944; Philipson 1947, 1949; Millington and Gunckel 1950; Gifford 1950; Rouffa and Gunckel 1951; Kasapligil 1951; Boke 1951; Gifford 1954.

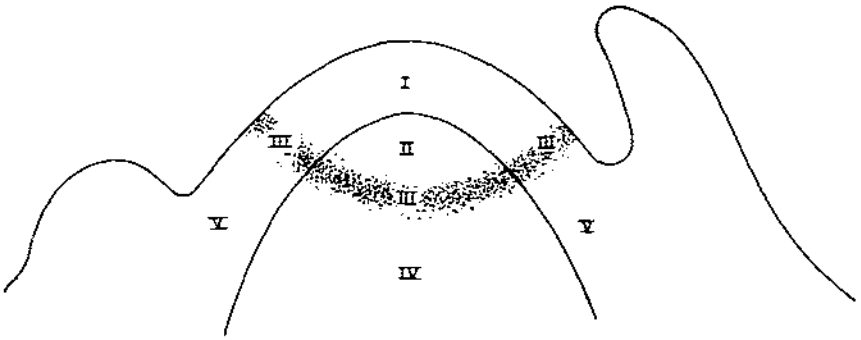


FIGURE 2.—A cytohistological zonation pattern applicable to angiosperms: *I*, Mantle layers; *II*, central mother cell zone; *III*, cambium-like zone (not always present); *IV*, rib meristem; *V*, peripheral zone. (After Popham and Chan 1950.)

perennis (Philipson 1946), and *Chrysanthemum morifolium* (Popham and Chan 1950). It is also present in some woody Ranales (Gifford 1950).

In *Ginkgo*, Foster (1938) regarded the cambium-like zone merely as a transition region between the low mitotic activity of the central mother cells and the more active peripheral and rib meristem zones (*p. 8*). Philipson (1946), however, finding the zone to be present in some *Bellis perennis* apices and absent in others, suggested that its presence is a transient state perhaps confined to the earlier part of each plastochron.[4]

The cambium-like zone is absent during the late phase of each plastochron in *Chrysanthemum morifolium*. In this species the zone becomes distinct in the central part of the apex during the early phase of the plastochron and is fully developed at mid-plastochron. Concomitant with full development of the zone is the reattainment of maximum height and diameter of the apical dome (exclusive of primordia and their basal buttresses) and enlargement of the youngest primordium (Popham and Chan 1950).

After studying the cambium-like zone in *Arabidopsis*, Vaughan (1952) suggested that the oriented divisions during mid-plastochron are a means by which the apex attains a condition favorable to initiation of another primordium. This idea is of significance in relation to the available space theory of determination of leaf primordia (*p. 37*).

Popham and Chan (1950) have included the cambium-like zone in a scheme of cytohistological zonation applicable to angiosperms. In this scheme the mantle layers (fig. 2, Zone I) include a large part of what many authors call tunica. The zone is larger than the somewhat comparable apical initial zone in gymnosperms. Divisions are entirely or largely anticlinal in the outer layers but more randomly oriented in the inner layers. The central mother cell, rib

[4] A plastochron (Gr. *plastos*: formed + *chronos*: time) is the time interval between two successive periodically repeated events such as the initiation of leaf primordia of their attainment of specific stages of development.

meristem, and peripheral zones (fig. 2, Zones II, IV, and V, respectively) have characteristics similar to their gymnosperm counterparts.

When present, the cambium-like zone (fig. 2, Zone III) is likely to be more extensive than the transition zone of *Ginkgo* (fig. 1, Zone III) described by Foster (1938). It is cup-shaped and extends through the peripheral tissue and mantle layers to the surface. Its exposed periphery forms a ring around the apex, a ring which could correspond, in a sense, to *l'anneau initial* of Plantefol (1947) and other French workers.

The cambium-like zone should not be relegated to insignificance merely because it has been found in only a few species. If the zone is distinguishable only during certain stages of each plastochron, then, it will often be missed. Furthermore, in woody angiosperms the apex produces primordia in regular and rapid sequence, and has well-defined plastochrons, during only a part of the yearly growth cycle. Careful study may reveal the presence of this zone in additional species.

Like the rib meristem, the cambium-like zone is characterized by regularly oriented cell divisions. The mechanisms controlling orientation and frequency of cell divisions in this zone may be closely related to control of leaf initiation. Such a relation seems plausible because activity of the cambium-like zone raises the apical dome, making available additional surface area for initiation of primordia. Evidence that the amount of available space between existing primordia and the apical summit may be a factor in controlling initiation of primordia is discussed later (*pp. 37–38*).

Méristèm d'Attente, Anneau Initial, and Méristèm Médullaire

Those who employ cytohistological zonation, the tunica-corpus theory, or both, in describing apical organization in angiosperms generally assume that all cells in the apical dome are meristematic, and that cells of all zones contribute to histogenesis, though not necessarily equally. The opinion among a group of French plant anatomists has, however, been at variance with this idea. As in the case of gymnosperms, they believe that the summit areas of vegetative angiosperm apices are meristematically inactive and that histogenic activity is mostly subapical.

Buvat (1952, 1953, 1955) has suggested a zonation scheme for angiosperms which is closely related to Camefort's (1956a, b) scheme for gymnosperms discussed earlier (*p. 12 ff.*). Again the most active zone is the peripheral and subterminal *anneau initial*. The supposedly semi-quiescent apical and subapical regions, comparable to the apical initial or mantle layers and central mother cells of the English language literature, are grouped into a *méristèm d'attente* (after Bersillon 1951). The rib meristem region is again called *méristèm médullaire*.

In the vegetative apex the *méristèm d'attente* (literally the waiting meristem) experiences few mitoses, but if the apex becomes re-

productive the *méristèm d'attente* becomes most active whereas the *anneau initial* and *méristèm médullaire* become quiescent instead (Buvat 1952). Some support for these ideas was provided by data indicating cell divisions in *Vicia faba* (Lance 1952, 1953a, b), *Lupinus albus*, and *Triticum vulgare* (Buvat 1952, 1953) to be concentrated in the *anneau initial*. A modified view was given by Catesson (1953) who allowed that a few divisions may occur near the apex. Buvat (1955) has also conceded that some mitoses do occur in the *méristèm d'attente*. Wardlaw (1957a) has made a very considered criticism of these ideas and points out how little is yet really known of the physiology and biochemistry of the apex.

By means of time-lapse photography of living, growing shoot apices of *Lupinus albus*, *Vicia faba*, and *Asparagus officinalis*, Ball (1960a) found that, in these species at least, there is no restriction of cell divisions to the periphery or to any region comparable to an *anneau initial*. There is likewise no central *méristèm d'attente* in which cell divisions are markedly less frequent (see also Tepper 1960). The duration of superficial cell divisions Ball observed in *Asparagus* apices was only 3 to 6 minutes.

Ball's finding lends some support to Newman's (1956) suggestion that the process of cell division in the apical dome is of shorter duration (as distinguished from frequency) than elsewhere in the shoot. If, in general, cell divisions in the apical dome are of short duration, then reports of low frequency of observation of mitoses in the *méristèm d'attente* region are open to reinterpretation.

Data obtained by Partanen and Gifford (1958) with P^{32} labeled phosphate and by Clowes (1959a) with C^{14} labeled adenine, suggest that in both angiosperms and gymnosperms cells of all zones of the apex synthesize nucleic acid, and therefore are presumably able to divide. The real and unanswered question, however, is the relative rate at which cells in the various zones actually do divide. Clowes (1961, pp. 60–69) has discussed the data on this point. He concluded, and I agree, that cells in all regions of the apex probably do divide, but that some weak evidence exists indicating a lower frequency of division in cells at the summit than in the flanks of the meristem.

It should be noted that Buvat (1955) does not ask us to believe that summit cells never divide. He admits them to be ancestral initials or mother cells, but only by virtue of their position, not because they have any special inherent qualities.

After nomenclatural differences are removed, the controversy concerns passivity of apical cells versus their active or even indispensable role in shoot morphogenesis. I believe that dividing summit cells, like other dividing cells, have an effect upon the behavior of the apex. I also believe that differences in environmental conditions (oxygen supply, diffusion gradients of metabolites, etc.) can account for the different behavior of different groups of cells. If the summit cells behave differently from other cells it is probably because environmental differences have brought to the fore different segments of the total fund of information which is encoded in the nuclear material of all the cells. Simply stated, cells behave as they

do because they are where they are. If this view is correct, discussions of relative passivity versus indispensability of certain groups of cells are of little significance.

Metrameristem

Is it possible to develop an ideal theory of apical organization which will be applicable to both angiosperms and gymnosperms? Buvat attempted to make his theory generally applicable, but it has attracted little cosmopolitan support. Recently Johnson and Tolbert (1960), after studies of *Bombax* (tropical trees) apices, advanced another unifying concept, that of the metrameristem (Gr. *metra*: womb).

The metrameristem in gymnosperms is visualized as consisting of the apical initial cells and the central mother cell zone. In angiosperms it encompasses, for example, the central part of the mantle and the mother cell zone of Popham and Chan (1950) in *Chrysanthemum morifolium*, the cuplike central zone of Millington and Gunckel (1950) in *Liriodendron tulipifera*, and generally corresponds in its geometry to the *méristème d'attente* of Buvat. The metrameristem is often strikingly obvious in sections of *Bombax* apices and is quite evident in many other groups as well (Johnson and Tolbert 1960; Tolbert 1961). This idea has yet to meet the test of time and criticism.

Synopsis on Shoot Apices

Of what significance to the physiologist or developmental morphologist are the various schemes of organization of shoot apical meristems? Each reader will undoubtedly have his own answer. In my opinion these schemes are useful as long as they promote localization and analysis of physiological and morphological problems (*for examples see pp. 11, 18*). When emphasis is put upon formalized nomenclature and upon minor differences between members of related taxonomic groups usefulness declines.

As the characteristics of a species remain unchanged through all taxonomic controversies, so also are the properties of zones or layers of the apex independent of the various names or supposed destinies which may be assigned to them on the basis of examination of fixed sections. It should be recognized that in spite of differences in detail between taxonomic groups there is a general homology of organization (*p. 32*) in all higher plant shoot apices.

The important physiological-morphological questions posed by all shoot apices are much the same. What controls the plane of orientation of cell division? Or frequency of cell division? If cells in different regions of the apex behave differently because of their location (see Schüepp 1952; *also p. 19*), what are the cellular level environmental factors which determine that behavior? What controls initiation of primordia (*p. 35 ff.*)? Techniques are now available (for example, see Jensen 1962) which allow these and other questions to be approached with some hope of making progress.

Organization of Root Apices

Root Apex Versus Shoot Apex

If we accept the premise that cells in different regions of the shoot apex behave differently because of environmental differences (*p. 19*) associated with their relative positions within the mass of meristematic tissue, then we would also expect root apical initials to behave differently from shoot apical initials because of present or past differences in cellular environment.

Many higher plant species are capable of vegetative propagation during which shoot tissues give rise to roots or vice versa (*see also p. 30*). This is compatible with the belief that large segments of the total genetic information in developing root or shoot cells are normally inoperative, but that this inoperative information is nonetheless passed on to descendant cells. It seems logical to me that the environment (including thermodynamic and kinetic factors) of each cell should determine which of the possible biochemical and biophysical processes shall prevail.

The environment of a developing cell within a tissue is the resultant not only of light, temperature, water and nutrient supply, oxygen tension, etc., but also of conditions and processes already established in neighboring cells. The same reasoning could be applied to each cell generation back to establishment of shoot-root polarity in the embryo. The persistence through many cell generations of characteristics which initially arise as responses to environmental stimuli may be regarded as a kind of somatic cell heredity (Brink 1962).

Throughout this section the reader may profit by keeping in mind the possibly predominating influence of cell environment upon cell metabolism, growth, and differentiation. He can, as well as I, speculate upon how different the environment of deep-seated root apical initials must be from that of the more superficial shoot apical initials, and how wounding, stress conditions, or chemical or radiation treatment might alter cellular environments in both apices.

Information and terminology concerning apical meristems of roots and shoots is only poorly coordinated, probably as a result of the relative lag in research on root meristems. Coordination of knowledge of root and shoot meristems of a single species was attempted by Allen (1947a, b) using *Pseudotsuga taxifolia*. His work points out the difficulties of establishing homologies between tissue regions of the shoot and root.

Allen suggested that the stele of the primary root is homologous with the whole primary shoot, and that the root cortex and rhizodermis are not counterparts of the shoot cortex and epidermis. The embryonic root initials appear in a subterminal position. They cut off new cells both inside and outside with respect to the surface of the apical dome. The outer derivatives give rise to the cortex and epidermis, the inner ones to the stele. The embryonic shoot initials are on the surface of the apex and have inward derivatives only.

Allen (1947a, b) proposed that the inside derivatives of root and shoot apices are equivalent. Thus, in his view, the stele of the root

is homologous with the whole shoot. In support of this idea he suggested that zonation in the meristematic area producing the root stele is somewhat similar to that of the shoot apex. He also suggested that the root endodermis may be homologous with the epidermis of the shoot because lateral appendages originate near the surface of the stele just beneath the endodermis, whereas in shoots they originate just beneath the epidermis.

Allen's ideas are different from those expressed by Arber (1941). The latter regarded the shoot as in some degree analogous to a periclinal chimera, with an internal component of rootlike nature. On the basis of this hypothesis Arber suggested roots and leaves to be comparable "since they are both, in different ways, partial-shoots."

Root meristem initials are typically deep-seated and are separated from the external environment by the rootcap. In the shoot, however, some of the initials may be components of the surface layer. Another striking difference is that the root cortex often appears to arise from outward derivatives of the initials whereas in the shoot the cortex necessarily arises from inward derivatives. Some hesitation is justified in regarding root and stem cortex as equivalent.

Roots have no lateral appendages comparable to leaves. Hence there are no nodes and internodes. The lack of nodes in turn makes impossible root structures homologous to the buds of shoots. This lack is also reflected in a more uniform growth and in less variation in the size and shape of the apex. It does not, however, preclude the formation of characteristic dormant structures in some roots (*p. 171 ff.*).

Apical Cells and Histogens

Members of those lower groups of vascular plants which have single apical cells (*p. 3*) in their shoot meristems may have single and totipotent apical cells in their root meristems also. Both root and shoot apical cells may be tetrahedral, but they differ in that the root apical cell lies within a mass of its progeny, whereas the shoot apical cell is truly terminal and has one face exposed to the environment.

The fern *Marsilia quadrifolia* has root apices with single, tetrahedral apical cells from which all root tissues, including the cap, are derived (Clowes 1961). Such apices are well described by Hofmeister's (1857) apical cell theory (*p. 3*). But some fern species have single root apical cells only when young and multicellular apical groups when more mature (Ogura 1938). Thus even among pteridophytes the apical cell theory is not universally applicable.

Hanstein's (1868) histogen theory substituted for the single apical cell concept three axially located, vertically superimposed groups of initial cells. These usually single-layered tissue initiators or histogens were called dermatogen, periblem, and plerome (*pp. 4–5*). The derivatives of the dermatogen were presumed to form the epidermis, where as the periblem and plerome formed the cortex and stele, respectively. The origin of the rootcap was realized to be variable. Janczewski (1874a, b) proposed the term "calyptrogen" to designate a fourth histogen which produces the cap tissue independently in monocotyledons.

With modifications this approach to understanding root apices gained wide acceptance, and histogen theory terminology is still found in contemporary literature. However, the original concept of three or four discrete and predestined histogens has been found too rigid. Much effort was expended on classification of apices according to the number of distinguishable histogens and the destinies of their derivatives (see Schüepp 1926). Aside from possible phylogenetic implications, such apical typing is no longer of great interest except that it illustrates the wide differences existing within the higher plant groups. These differences are briefly summarized in the following paragraphs.

Many of the investigated gymnosperms and angiosperms have only two groups or layers of initials, the inner one forming the stele, and the outer one the cortex and rootcap. There may be no clear division between the cap and the cortex except where mechanical rupture has occurred. A well-defined epidermis is lacking. The outer layer, called rhizodermis by some authors, is merely the surface of the cortex.

In Juglandaceae, Tiliaceae, Umbelliferae, and in some members of Rosaceae and Leguminosae there are also only two tiers of initials. The stele and the inner cortex arise from the inner set whereas the remainder of the cortex and the cap arise from the outer set. In these groups also the rootcap is not distinct and the epidermis or rhizodermis is the outer layer of the cortex. In a wide scattering of dicotyledons, all parts of the root appear to arise from one initial region (but not one apical cell) in which the cells are not susceptible to formal grouping into histogens. With respect to these the histogen theory fails.

But members of various other families of dicotyledonous plants have a very precise apical organization based upon three tiers of initials or histogens very much in keeping with Hanstein's theory. These families include Rosaceae, Solanaceae, Cruciferae, Scrophulariaceae, and Compositae. One tier of initials gives rise to the stele, the second to the cortex, and the third to the epidermis and cap. The existence of roots with such precise organization may account for the survival of the histogen theory terminology in the root literature though it is little used with respect to shoots.

It is now becoming evident that many of the earlier interpretations were too formal and too static, that recognition of the actual functioning initials is quite difficult, and that mere enumeration of apparent initial groups is not sufficient explanation of the complex zonation found in some apices (Allen 1947a, b; Clowes 1950). Furthermore, roots of some common plants, such as *Vicia faba* (Clowes 1956b) have no distinguishable initial groups or histogens at all. While it is true that roots of some species conform beautifully to the histogen theory, the latter lacks general applicability and is of little help in understanding the dynamic aspects of root growth.

Körper-Kappe Theory

The *Körper-Kappe* theory of root apical organization proposed by Schüepp (1916, 1926) is not incompatible with the histogen theory though its approach is different. It is based upon cell pat-

terns and orientation of cell divisions rather than cell destinies. In view of the great amount of attention given Schmidt's (1924) related tunica-corpus theory of shoot apical organization, it is somewhat surprising that the *Körper-Kappe* theory has remained so little known. In spite of its lack of popularity, the theory is applicable to various patterns of root organization. Examples of its use are given by Wagner (1939) and Clowes (1950, 1961).

The physical basis of the *Körper-Kappe* theory is the following: The cells of root apices as they appear in median longitudinal sections are arranged in rows which appear to originate from some cytogenic center. Examination of a segment of tissue reveals that the number of rows of cells increases with increasing distance from that center. For example, the primary stele is much wider than the segment of meristem from which it arose. Growth is accomplished by cell enlargement and cell divisions.

A T- (or Y-) shaped configuration of cell walls is found at each locus where a longitudinal division followed by additional transverse divisions caused one file of cells to become two. In the central part of the apical mass the tops of the T configurations face the root tip. In the peripheral parts of the root apex similar configurations generally face in the opposite direction. Schüepp divided the apex into *Körper* and *Kappe* on the basis of the orientation of these figures and classified roots according to the location of the boundary between the two.

By microscopic examination of median longitudinal sections most root apices can be divided into *Körper* and *Kappe* regions. In some taxonomic groups the *Körper-Kappe* boundary is distinct and constant in its location, but in others it is indistinct and variable. For example, in taproot apices of young *Fagus sylvatica* seedlings the cortex may be partly *Körper* and partly *Kappe*. In other roots, usually the smaller ones, all the cortex may be within the boundary so that the *Kappe* includes only the epidermis and the rootcap, whereas all the cortex is *Körper*. Individual *Fagus* roots probably also show changes of pattern with time (Clowes 1950). In grasses and a few other angiosperm families (those which have separate rootcap initials) only the cap is *Kappe;* all the rest is *Körper* (Clowes 1961).

The *Körper-Kappe* theory has been too little used and discussed to allow much speculation on its probable future. It may be a good tool if used in suitable combination with others.

Many-Celled Promeristems Versus Central Cells

When longitudinal sections of apices are examined under the microscope the patterns of cells allow deductions to be made about planes of cells division. By virtue of their position in relation to the total pattern, certain groups of cells, or even individual cells, *appear* to be initials. But the cell pattern reveals nothing about the rates of division. Even relatively recently it was assumed, usually without discussion of the point, that all cells of the meristematic region of the tip divide at roughly similar rates (Allen 1947a, b; Clowes 1950; Guttenberg 1947; Bruch 1955).

If, for unknown physiological reasons, cell division were much more rapid in certain regions of the meristem than in others (but

with the plane of division unaffected by rate), the difference would not necessarily be reflected in changes of cell pattern (*p. 19*). In fact, if a central area around the pole of the stele were to become totally inactive, the cell patterns within it would remain unchanged and continue to indicate as initial cells those near the stele pole. Cell patterns are indicators of past events. They do not allow one to distinguish between remote ancestral initials and presently active initials.

Guttenberg (1947, 1955) analyzed the cell pattern in root tips of several species of dicotyledonous plants and concluded that these possessed central initial cells from which all tissues were derived. Combining features of the apical cell and histogen theories, Guttenberg suggested that the central cell was somewhat akin to the single apical cell of pteridophytes, and that the histogen initials were replaced by derivatives of the central cell. The same conclusion was reached with regard to some monocotyledonous roots (Schade and Guttenberg 1951; Guttenberg et al. 1954a, b). A summary of this work and further development of these ideas was presented in a book by Guttenberg (1960).

Guttenberg's (1960) ideas on histogenesis in angiosperm roots beyond the embryo stage can be discussed in terms of the histogen theory. He visualizes two basic types of root apices, *geschlossener* and *öffener*, or closed and open. The closed type has discrete and independent histogens. It is commonly found in the radicles of mature embryos and is retained in the growing roots of many species. In other species the histogens lose their independence during post-embryonic growth and exhibit exchange of cells across previously closed histogenic boundaries. These have open apices. Division of apices into two groups on this basis results in some monocotyledons and some dicotyledons in each group.

Because rootcaps of monocotyledonous species generally arise from separate initials (the calyptrogen), whereas those of dicotyledons do not, Guttenberg distinguishes a total of four root apical types. Apices of two types, closed-monocotyledons and closed-dicotyledons, may be described by the histogen theory. But in the two open types the histogens are not discrete. In these Guttenberg calls the presumed initiating centers *central cells*.

The reputed central cells comprise a very small number of apparent initials occupying an area at the pole of the stele where the single apical cell was formerly thought to be. Divisions of the central cells are not regularly oriented. The cells are totipotent, but according to Guttenberg (1960), not in the sense of pteridophyte apical cells. If destroyed, central cells may be regenerated from the remainder of the meristem. Pteridophyte apical cells are not regenerated. Although central cells themselves divide, the subsequent divisions of their progeny generate most of the cells of the root. The central cell area is not the area of greatest meristematic activity, but Guttenberg believes it to be the generative center. In this view the number of initial cells is very small, sometimes only a single cell.

Clowes (see reviews 1959c, 1961), on the other hand, during the past decade has published a line of evidence supporting the concept of a many-celled *promeristem*. The term "promeristem" refers to

the entire collection of initial cells. Briefly, Clowes believes that the central apical area, including the area of so-called histogen initials or central cells, is the least active meristematically and is quiescent or semidormant. Instead, he believes that the promeristem is made of many cells, not in a compact mass, but located on the periphery of the *quiescent center*. Thus a basic point of difference between the views of Guttenberg and Clowes concerns the number of promeristem cells and their location.

Clowes (1953, 1954) and Kadej (1956) independently performed surgical experiments designed to discriminate between root promeristems having large or small numbers of initials. The technique was the simple one of removing an oblique segment, a vertical sector, or a horizontal wedge-shaped piece from the apex at various distances back from the tip. Roots were allowed to regenerate and grow, if they would, after cutting and were later fixed and sectioned.

The theory was that a few-celled promeristem should regenerate normally or not at all depending upon whether its cells were wounded or not. If the number of initials were large, however, some roots should be found having abnormal sectors regenerated from the damaged part of the promeristem and also normal sectors produced from the undamaged part. Some roots were, in fact, found with partly normal and partly abnormal structure. There were differences in the proportion of normal to abnormal tissues as would be expected if the promeristem consisted of a large number of initials.

Ball (1956), after studying regeneration of split radicles of *Ginkgo* embryos also concluded that the minimal number of initials required for viability is large.

It has, of course, long been known that roots having an undisputed apical cell (as in many ferns) cannot regenerate after decapitation, because all of the promeristem is removed when the apical cell is removed, whereas higher plant roots often do regenerate after the tip of the meristem has been cut away (Prantl 1874). Guttenberg (1960), however, does not accept this kind of evidence as having any bearing upon the number of initials in the normal higher plant root tip. He believes that regeneration after wounding is only an indication of the great powers of restitution inherent in the apical tissue. He does not claim that the root cannot grow without divisions of the central cells, but does say that these few central cells are the normal formative center in unmolested roots.

The results of regeneration studies in angiosperm roots after microsurgery (Prantl 1874; Němec 1905; Clowes 1953, 1954; Kadej 1956; Ball 1956) do not clearly answer the question of active promeristem size in normal roots; but neither do they support the old concepts of apical cells or discrete histogens. They do suggest that the behavior of remaining cells is changed when other cells are cut away or injured (Ball 1956). These experiments also indicate that a large number of cells are potential initials and that the microenvironment may determine whether they actually behave as such (*p. 21*).

The Quiescent Center

When accepted approaches and ideas fail to aid the understanding of a problem, the assumptions upon which those ideas and approaches are based should be reexamined. All the early workers on the apical organization of roots assumed that cell patterns pointed to the actual initials near the pole of the stele. This was based upon a previous assumption that rates of cell division throughout the meristem were roughly the same. If that assumption is false, then cells which appear, because of their position, to be initials may in reality be inactive. The significance of this possibility was realized by Clowes (1954), and that realization led to the development of the concept of the quiescent center within the root apical meristem.

In many grasses the rootcap is separated from the rest of the root by a thick, pectinaceous layer across which there is no interchange of cells. Examination of cell patterns in longitudinal sections of root tips by classical methods suggests that the initials lie between the pole of the stele and the base of the cap. Yet purely mechanical considerations make this difficult to accept. In a review covering his earlier work, Clowes (1959c, p. 511) described the situation in the primary *Zea mays* root as follows:

> At the apex of the stele also, near the root axis, the cell pattern shows that there are again no longitudinal divisions or transverse growth. At this point near the axis, the cortex-epidermis complex consists of a plate of cells, one cell thick, between the pole of the stele and the base of the cap. Hence, if there are no longitudinal divisions and transverse growth in these parts of the stele and cap it is unlikely that there will be any longitudinal divisions or transverse growth in the contiguous cortical cells, because there is no reason for believing that these plates of cells slip over one another. This means that the cells of the cortex-epidermis complex near the axis cannot behave as initials. Nor do they divide transversely since they do not contribute either to the stele or to the cap. They are not meristematic.

Thus on anatomical and mechanical grounds Clowes (1954) advanced the concept of a hemispherical quiescent center in root apices of *Zea*. He suggested that the initials of the meristem are those cells located on the surface of the hemisphere and that the cells within the center itself divide seldomly if at all. With the aid of radioisotopes Clowes (1956a) later demonstrated that a quiescent center could be delineated in which cells have smaller nucleoli, lower ribonucleic acid (RNA) content, and do not synthesize deoxyribonucleic acid (DNA) with incorporation of exogenous phosphate or adenine.

In *Zea* the region thus delineated coincided with that postulated on mechanical grounds. By making autoradiographs of root tips of *Vicia faba* and *Allium ascalonicum* after they had been fed adenine-8-C^{14}, Clowes (1956b) found a well-defined central area of low DNA synthesis (and presumably a low rate of mitosis) in these plants also. The quiescent center concept was further developed by Clowes (1958a, b, 1959a) by radiochemical and other methods and was discussed in detail in a review (Clowes 1959c) and a recent monograph (Clowes 1961).

The elegant autoradiographs of Clowes (1956b, 1959c) show the quiescent center clearly and convincingly. Jensen and Kavaljian

(1958), by careful analysis of frequency of mitosis in different regions of the root tip of *Allium cepa*, were able to confirm the presence of a quiescent center in that species. Additional evidence was provided by Jensen (1957, 1958). Hejnowicz's (1959) analysis of growth and cell division in *Triticum vulgare* root meristems is also compatible with the existence of a quiescent center. Likewise Chouinard (1959b) has reported that root tips of *Pinus banksiana* exhibit a quiescent center.

Clowes (1959c, 1961) believes that the quiescent center will turn out to be of general occurrence except perhaps in very slender roots. Shimabuku (1960) has reported that root apices of *Oryza sativa* show no evidence of a quiescent center. It may be significant that his *Oryza* roots were more slender than the *Zea* roots studies by Clowes.

The general characteristics of the quiescent center (Clowes 1959c, 1961) are of great physiological as well as anatomical interest. The hemisphere or spheroid of meristematically inactive cells, which is the quiescent center, is carried forward passively by the growth of cells below it and around it. All evidence indicates that these cells are inactive only because of their relative position with respect to the active cells and that if the latter are cut away or otherwise nullified the quiescent cells are fully able to become actively meristematic. The size of the quiescent center varies with the width of the tip. Very slender roots may never develop an easily detectable quiescent center, whereas large diameter tips may have a center containing a thousand cells or more.

In the early stages of root development in the embryo, and in primordia of secondary roots, all cells of the apex are meristematic. Radicles of embryos in ripe seeds of *Sinapis* have no quiescent centers, but these appear when the seminal roots are about 5 mm. long (Clowes 1958a). In lateral root primordia of *Pistia* the quiescent center is already well developed while the new root is still pushing through the cortex of the mother root (Clowes 1958a).

The apical area occupied by the quiescent center seldom shows mitotic figures. Buvat and Genevès (1951) noted this in roots of *Allium*, and Buvat and Liard (1953) concluded that the axial apical cells of *Triticum* roots do not divide at all. However, frequency of observation of mitotic figures is not at all the same thing as actual frequency of mitosis because the duration of mitosis (and consequently the chance of observing it) may be variable (Brown 1951), yet it is unlikely that an area which almost never shows mitotic figures is very active meristematically (*p. 46*). More convincing evidence is provided by the inability of quiescent cells to incorporate radioactive adenine or thymidine into DNA, whereas neighboring meristematic cells make liberal use of these compounds in doubling the amount of DNA prior to division (Howard and Pelc 1951: Clowes 1956a).

Exposure of roots to intense beams of X-rays destroys the ability of the meristematic cells to make DNA. Cell divisions are temporarily halted by such treatment. Meanwhile the previously quiescent cells, having been little harmed by the X-rays, begin to synthe-

size DNA, to divide, and eventually to regenerate a new root tip complete with a new quiescent center (Clowes 1959b). The possible reorganization of damaged apices by cells from a reservoir of less susceptible cells makes it invalid to draw inferences concerning the normal behavior of meristems from X-ray induced chimeras. Such results also suggest that cells of the quiescent center are quiescent because of their environment (*p. 21*).

The quiescent center of root meristems has sometimes been likened to the shoot *méristème d'attente* of Buvat (1952, 1953). According to Clowes (1959c, 1961) the root quiescent center ought not to be associated with the *méristème d'attente* because the geometry of root and shoot meristems is quite different, and furthermore the use of adenine-C^{14} reveals a quiescent area in roots but not in shoots (Clowes 1959a). Parranen and Gifford (1958) have shown that cells at the summits of shoot apices do synthesize DNA and are probably meristematic (*p. 19*).

Esau uses the term "quiescent promeristem" in her discussion of shoot apices (Esau 1960, p. 225). This should not be taken to imply that there is a close relation between any inactive center in shoot apices and the quiescent center of roots. In Clowes' usage it is not the promeristem which is quiescent: rather the promeristem is the collection of active initial cells surrounding the meristematically inactive quiescent center. There is, unfortunately, a lack of uniformity and specificity in nomenclature. To Clowes, promeristem means simply "the collection of initial cells" (Clowes 1959c, p. 502). Esau (1960, p. 354), in her glossary, defines promeristem in two ways: (1) "The initiating cells and their most recent derivatives." (2) "The most distal part of the shoot or root." Some of the apparent disagreements in the literature are due to such differences in definitions.

Little is known about the occurrence of quiescent centers in gymnosperm roots. On the basis of the statistical distribution of mitoses Chouinard (1959b) reported a quiescent center in primary roots of *Pinus banksiana*. Quiescent centers have been confirmed in *Libocedrus decurrens* roots with the aid of tritiated thymidine (Wilcox 1962b). Much more information is needed before any generalizations concerning occurrence of quiescent centers in gymnosperm roots are justified.

The concept of a quiescent center surrounded by active promeristem cells, and itself composed of cells which are inactive only because of their environment, is of considerable significance to the study of root growth and development. In my opinion it is also significant to the whole field of developmental morphology. It, in combination with microsurgical and microhistochemical techniques, offers an approach to the general problem of how cell division and growth are favored in one region and simultaneously inhibited in a nearby region (*p. 21*). This problem appears in many aspects during analysis of morphogenesis and histogenesis in shoot apices as well as in roots.

PHYSIOLOGICAL MORPHOLOGY OF SHOOT MERISTEMS AND BUDS

Reactivity of Shoot Meristems

Metabolic Differentiation Within the Meristem

Apical meristems are the loci of a great array of interrelated biochemical reactions. Logically, all metabolites are either synthesized in the cells of the apical meristem or are translocated from the more mature subjacent tissues. Evidence that the degree of dependence upon syntheses in older tissue is less in apices of lower plants than in those of higher plants is discussed below.

Isolated shoot apices of *Equisetum*, *Lycopodium*, and various ferns, even when no foliar primordia are included with the explant, will grow in sterile culture on simple media to produce whole plants. No vitamins, cofactors, or regulators need be added (Wetmore 1954). However, attempts to culture isolated shoot apices (without visible primordia) of higher plants on simple media have repeatedly failed (Ball 1960b). Apices do survive for a time, but fail to grow.

It would be absurd to argue that higher plant apical meristems, the most juvenile, least differentiated, most totipotent tissue of the plant, do not have all the genetic information necessary to synthesize the metabolites and regulatory substances required to maintain meristematic activity. The problem, I believe, is more likely to be one of cell environment being unsuitable for certain essential processes which require such conditions as are normally found in lower regions of the apex (*p. 21*).

Ball (1960b) has suggested that the shoot apices of angiosperms have undergone biochemical differentiation in the direction of loss of synthetic ability, whereas the more primitive shoot apices of lower vascular plants retain complete potentiality for biochemical synthesis. This difference in synthetic ability may be viewed in another way. The inability of higher plant apices to synthesize all essential metabolites from simple precursors may indicate, not lack of genetic information, nor even lack of biochemical mechanisms, but presence of regulating mechanisms or conditions which determine that certain segments of the genetic information and not other segments shall be operative in the apical cells.

Indeed, we must suppose that the genetic information concerning, for example, synthesis of the characteristic pigments and volatile compounds of a plant's flowers, is present in the cells of the vegetative apex. But that information does not become operative until certain conditions have been satisfied. It is logical that those plants having the more highly developed regulatory systems determining the course, activity, and direction of genetically possible processes shall be capable of the greater morphological and physiological specialization in their various organs and tissues. Such specialization may include, for example, the development of elaborate and specific flowering and fruiting structures, but it may also include more control by the maturing plant parts over activity of the apical meristem.

If there were no metabolic differentiation within the various regions of the higher plant meristem, it would be difficult to imagine how any integrating and control systems could operate. Furthermore the isolated apical dome should then have simple nutrient requirements, which is not the case.

Some data concerning metabolic and cytological differentiation within shoot apices of *Lupinus albus*, including up to seven primordia, have been provided by the work of Sunderland et al. (1956, 1957). According to these authors the cells of the embryonic internodes are considerably larger and have a higher absolute protein content than those of the primordia. In the young internodes cell expansion is restricted and division is slow, whereas in the primordia division is more frequent and rapid cell expansion by water uptake is the rule. Respiration per unit of protein content is lower in younger primordia. It increases with increasing development and degree of vacuolization. Growth by water uptake and increasing vacuolization, it should be noted, involves very large increases in vacuolar membrane area and also in volume of vacuolar solution. Both of these factors could influence metabolic reactions.

Sunderland et al. (1956, 1957) have suggested that in *Lupinus albus* metabolites are synthesized in the youngest internodes and are transferred to the primordia where they are incorporated into macromolecular cell constituents. Respiration of internodal tissue declines sharply in the transition from the third to the fourth internode. It is interesting that the primordia in this transition region also undergo a change toward self-sufficiency accompanied by rapid growth and development. The authors cited suggest that a component of the metabolite complex received by the three youngest primordia from their internodes is a differentiation inhibitor.

In further speculations these authors considered the generating system of the stem apex as a central core or corpus of high metabolic activity covered by a mantle or tunica of low metabolic activity. This situation would be similar to, and the precursor of, that supposedly existing between young internodes and primordia, with metabolites transferred from the corpus controlling events in the tunica. In this view, initiation of primordia results from localized concentrations in the tunica of metabolites derived from synthetic reactions in the corpus (*p. 35*). Another kind of interdependence of apical regions is indicated by aseptic culture of isolated apices.

Culture of Isolated Apices

Entire plants of *Lupinus albus* and *Tropaeolum majus* have been grown in sterile culture from explants of shoot apices including the apical dome, the three youngest foliar primordia, and a small amount of subjacent tissue (Ball 1946). The medium must contain essential minerals and sugar, but no added vitamins, hormones, or cofactors are necessary. When the size of the explant is reduced to include only the apical dome, with no visible primordia, a much more complex medium is needed to sustain growth even temporarily and none has yet been developed which promotes normal indeterminate growth (Ball 1960b).

Supplementing a simple nutrient salt and sugar medium with coconut milk delays death of isolated apices for some time but does not promote growth. Additions of auxin, various vitamins, and mixtures of amino acids are largely ineffective. Several nucleic acid derivatives, but not kinetin or adenine, support moderate growth. However, excised apical domes of *Lupinus albus* when planted on nutrient agar medium supplemented with both coconut milk and gibberellic acid have produced as many as nine foliar primordia, and have developed into shoots up to 10 cm. long in a 2-month period. Such development is not normal. It is always followed by cessation of growth. At the same time the apex becomes abnormally large because its component cells become large, highly vacuolate, and divide infrequently. This loss of meristematic characteristics is not prevented or reversed by transferring the cultures to fresh media. Ball (1960b) concluded that such loss of meristematic capacity results from insufficiency or lack of essential substances in both explant and medium.

The work cited above points out several very important questions. What are the substances, essential for growth, which are not synthesized in the apical dome? If apices with only three primordia can be grown to complete plants, why then does not a young shoot cultured from an apical dome become indeterminate in growth after it has developed three primordia? What control mechanisms prevent synthesis of essential substances even though the cells presumably contain the requisite genetic information? Can these controls be overridden? None of these questions can yet be answered.

Morphogenic Regions of the Apex

Starting with the premise that, in spite of manifold differences in detail, all shoot apices show a general homology of organization and morphogenic activity, Wardlaw (1957b) proposed a general system of nomenclature for the various morphogenically distinguishable areas of shoot apices. The scheme, which is partly based upon anatomical interpretations by Schoute (1936), includes five regions (fig. 3):

Distal region.—The distal region comprises the summit of the apical dome. It includes the single apical cells of lower plants and the apical initial cell group (or zone) of higher plants.

Subdistal region.—Inception of growth centers (or loci), the sites of subsequent primordial initiation, occurs in the subdistal region. The growth centers are groups of cells in which concentrations of metabolites conducive to growth have presumably accumulated, but in which no obvious morphological changes can yet be detected.

Organogenic region.—In the organogenic region obvious outgrowth of foliar primordia occurs from the growth centers and internal tissue differentiation becomes detectable or even conspicuous. The boundary between this region and that below is not sharp.

Subapical region.—Characteristically the subapical region exhibits conspicuous primordial enlargement, considerable widening of the axis, continued differentiation of the vascular tissue, and elongation of internodes resulting from cell division and extension in the rib meristem.

Region of maturation.—In the diffuse lower limits of the subapical region meristematic activity declines. There is a gradual transition to the region of maturation. In this region the morphogenic patterns initiated and developed in the upper regions are finally fixed.

The above scheme of nomenclature is very useful in discussing physiological and morphological processes in the shoot apex and will be employed in sections which follow. It must be remembered, however, that the growing apex is a dynamic system. The regions move upward so that individual cells, multiplying as they go, seem to move downward. Actually, of course, the cells also move upward, but with the exception of any apical initials, not as rapidly as the regions. Finally, with their progeny they are overtaken by the advancing front of the region of maturation and become components of less dynamic tissue systems.

The physiology of the superimposed subdistal, organogenic, and subapical regions is of special significance to the problem of growth and dormancy control in woody plants. This is illustrated by a brief consideration of the types of processes which are (and must be) integrated and controlled in order to make bud formation and later outgrowth possible. Growth loci are organized in the subdistal region. In the organogenic region these produce primordia. In most species a first series of primordia must develop into bud scales and a second series into foliar primordia, if bud formation is to occur. This differential development occurs in primordia borne on the periphery of the subapical region. Meanwhile meristematic activity in the axial part of the subapical region is so controlled that there is little internodal elongation.

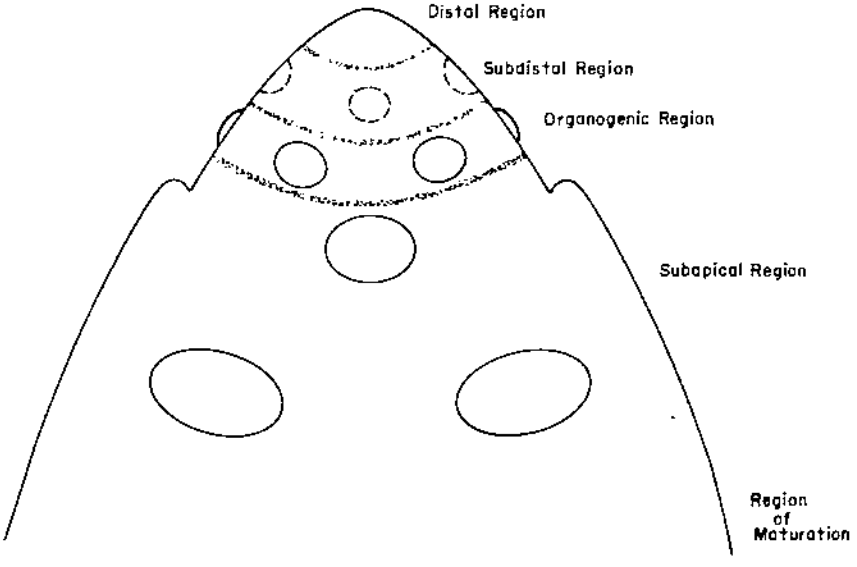


FIGURE 3.—A shoot apex with whorled phyllotaxy, as a system of interrelated regions. Boundaries between regions are diffuse. (After Wardlaw 1957b.)

Generally, inhibition of internodal elongation prevails throughout the winter dormant period, but inhibition of development is not complete. Under mild conditions some development of primordia may continue and additional ones may be initiated in the subdistal region. In spring, after activation of control mechanisms, a rather sudden elongation of foliar primordia and of internodes between them produces the phenomenon commonly called "bud break." Commonly, however, inhibition of elongation of the internodes between bud scales is not released and they remain permanently dormant. Release is both selective and coordinated. Even while the preformed internodes from the subapical region of the winter bud are elongating, scales and foliar primordia of a new bud may be forming above. These phenomena are discussed in more detail, and with literature citations, in subsequent sections (*pp. 35–46, 46–61*).

Special Significance of the Subapical Region

Several important problem areas in the developmental morphology of higher plants are, in essence, only different aspects of the one problem of control of cell division and elongation in the subapical meristem. Some examples are the formation and breaking of buds (*p. 46 ff.*); alterable long shoot versus short shoot growth habit in woody plants (*p. 130 ff.*), and rosette versus cauline habit in herbaceous plants (*pp. 143–144*); habitual auxiliary short shoot development as in *Pinus* (*p. 51*); physiological dwarfing of plants grown from embryos with unsatisfied chilling requirements (*p. 161 ff.*); and lengthy "grass stages" in some *Pinus* seedlings (*p. 132*).

As in the latter example, relative activity of the subapical region may sometimes be related to the degree of juvenility or maturity of the plant. This relationship, however, is a complex one involving other factors and cannot be discussed in detail here. The reader will find comprehensive discussions and references in papers by Robbins (1957), Schaffalitzky de Muckadell (1959), and Brink (1962).

The subapical meristem region is of primary importance in normally caulescent plants because, once restraint upon its activity has been released, it generates most of the cells which make up the mature internodes. True, the ultimate source of cells is the distal region, but these cells are progenitors. Distal cells do not really move downward into other regions. They divide and their progeny, in ever increasing numbers, also divide. Because of cell elongation in the subapical region, movement of cells and regions is upward. In a growing shoot, the greatest increase in absolute cell number occurs as the subapical region passes upward. As it passes through any embryonic, axial segment chosen for examination, short primordial internodes elongate manyfold.

Internodes elongate because their original cells elongate, but also because there is a great increase in cell number. In 1876, J. W. Moll made determinations of cell length and number in internodes of many woody plants. His data, as compiled and republished by Czaja (1929), indicate that short internodes have fewer, but not necessarily shorter, cells than have longer internodes of the same shoot. Sometimes they have only one-tenth as many. Holmsen (1960) found that internodal pith cells of physiologically dwarfed

Prunus persica seedlings are also of the normal length, and that internodal cell number must, therefore, be deficient (*p. 161 ff.*).

After studying stem histogenesis during bolting of rosette plants, Sachs et al. (1959a, b) concluded that the subapical region is almost solely responsible for formation of cells constituting the mature internodes (*pp. 143–144*). Additional data on the source of internode cells are desirable but present information strongly supports the view that the subapical region is of preeminent importance as a cell former.

Origin of Leaves, Cataphylls, and Vascular Tissue

Initiation of Primordia

Shoot apices of higher plants, unlike their root apices, are not normally able to grow for long periods to produce smooth cylindrical axes with no lateral appendages. Furthermore, lateral organs of roots arise in the deep-seated pericycle or endodermis layers at such a distance from the apex that extensive differentiation has already occurred. In contrast, primordia appear on the surface of the shoot apex in the organogenic region where tissue differentiation is not yet obvious. Many questions may be asked concerning these differences, but there are few answers.

Though I invite the reader to entertain other possibilities which may occur to him, it is my opinion that mobilization of metabolites occurs in localized areas in the subdistal region of the apical dome before primordia are detectable. If metabolites and regulators remained uniformly distributed throughout the apical region, why should areas of strong localized growth develop? The inception of growth centers comprising groups of cells which develop into primordia almost certainly results from nonuniform distribution of metabolites and regulators in the apical dome. But how could such nonuniform distribution come about?

In the subdistal region where the presumed growth centers—the precursors of primordia—are organized, there is no microscopically visible pattern. Yet, invisible biochemical patterns may exist. Turing (1952), in his diffusion-reaction theory of morphogenesis, has proposed an explanation of how such patterns may arise. The theory is based upon accepted laws of physical chemistry, which all growing systems obey, but the exposition of it is complex mathematically.

Turing's theory implies that an initially homogeneous system of several reactive and diffusible metabolites and regulators will eventually become unstable, perhaps because of random events. Instability leads to irregular wave patterns which become regularized and may take the form of localized accumulations of metabolites distributed according to a nonrandom pattern. Such pattern formation may occur early in embryonic development and be perpetuated thereafter (*p. 21*).

Wardlaw (1953, 1955a, b) has written nonmathematical commentaries on Turing's theory and has related it to other biophysical and chemical concepts of morphogenesis. Thus far the theory has been of value primarily as an indicator of the direction in which explanations of origins of morphogenetic patterns may be found. Nothing

definite is known about the diffusible metabolites and regulators presumably responsible for initiation of growth centers which become primordia.

Whatever the reason, leaf or cataphyll primordia arise regularly around the circumference of the shoot apex in accordance with a phyllotaxic pattern characteristic of the species (for discussions of phyllotaxis in relation to histogenesis see Dermen 1945; Richards 1956; Cutter 1959). In terms of tunica-corpus terminology, the primordia are apparent outgrowths from the surface of the tunica, although the corpus is commonly also involved (Schmidt 1924; Foster 1936). In terms of cytohistological zonation of the apex, primordia arise from the peripheral tissue zone, but in some gymnosperms only the outermost layers participate (Korody 1937; Cross 1940, 1942). If Wardlaw's (1957b) concept of morphogenic regions is employed, growth centers are organized in the subdistal region and develop to become visible primordia in the organogenic region. Much subsequent enlargement and tissue differentiation occurs in the subapical region.

As the phyllotaxic pattern progresses upward, what determines the site of the next primordium or set of primordia? Aside from the question of why it happens at all, what are the stepwise processes which lead to elevation of a visible primordium in a specific area? Various bodies of thought and speculation regarding these questions have developed, and some have accumulated supporting evidence. Although they cannot be treated in detail, some of these interesting ideas are discussed briefly in the following paragraphs.

Repulsion theory.—Bünning (1952, 1956) has ascribed the patternized distribution of growth centers to the mutual incompatibility of vigorous growth regions of the same type. For example, in a developing growth center, a particular enzyme system may become very active, with resulting deficiency of its substrate in a surrounding field. This deficiency might then prevent inception of additional growth centers nearby. Related ideas were proposed earlier by Priestley and Scott (1933) and others.

Collectively these ideas constitute a repulsion theory of leaf determination, proposing that new primordia arise at the greatest possible distance from the older primordia in the last formed cycle around the apex and also from the summit. Such schemes do not provide for the initiation of a pattern in the embryonic apex, but that deficiency is covered by the diffusion-reaction theory of morphogenesis (Turing 1952) previously mentioned (*p. 35*).

Excessive apical surface growth.—Schüepp (1916) considered the first step in primordium initiation to be fold formation in the outer layers because of greater growth of the apical surface than can be accommodated by interior growth. In this view the outer layers are under compression and are thrown up into folds. This was accepted by Priestley (1928). But if the outer layers actually are compressed, then small cuts made into them should remain closed, not gape open. Gaping of cuts, however, has been observed (Snow and Snow 1947) in apices of several species, suggesting that the layers are under tension, not compression.

Tissue tensions in stem apices were further investigated by Snow and Snow (1951). Though open to the criticism that they may not

reflect conditions in intact apices, microsurgical experiments have weakened the theory that excessive surface growth is a causative agent in primordium initiation.

Theory of prior procambial development.—There is evidence that in some species the procambial strands, precursors of vascular traces, are formed before the leaves which they ultimately serve. Some of this evidence is discussed later (*p. 38 ff.*). If procambial strands are initiated before their primordia, it might be supposed that metabolites transported along the strands would be a factor in initiating growth centers and promoting development of primordia.

It has not been demonstrated that procambial strands are superior to parenchyma as translocation pathways; nevertheless, that possibility prompted Snow and Snow (1947, 1948) to study the leaf-forming influence of procambium by microsurgical experiments. Incisions were made in *Lupinus albus* apices in such a manner that predicted primordial sites were isolated from the procambium below. Yet normal primordia developed in the isolated sites. It was concluded that procambial influences are not important in determining sites of primordia. Similar conclusions with regard to the same species were reached by Ball (1948).

With respect to the behavior of *Sequoia sempervirens*, in which procambial strands are formed before their primordia, and almost always beneath the future primordium sites (Sterling 1945b), Snow and Snow (1948) offered the following explanation: Rudiments of procambial strands arise before the leaves which will be associated with them, but when the primordia do appear they greatly promote differentiation of the strand. Thus traces are strengthened by primordia above them and after such strengthening give off branches into the widest gaps between existing strands. Because primordia also arise in the largest available space it is likely that procambial branches will fall in regular order beneath future sites of primordia. This implies that procambial strands and primordia are initiated independently, but according to similar rules.

An important point made by Clowes (1961) is that the upper part of the apex is very small. The upward path of a procambial branch arising in the wider part below is much the same regardless of which primordium it eventually enters. Clowes also thinks it probable that the uppermost part of the strand is determined only after the site of the primordium has been determined.

First available space theory.—On the basis of ideas first expressed by Hofmeister (1868), Snow and Snow more than 30 years ago advanced the theory that leaves are formed in the first available space on the apical dome (for restatement see Snow and Snow 1947). The first available space theory is often confused with the repulsion theory mentioned above, but it is somewhat more specific. Both theories agree that all primordia in the top cycle influence the *order* in which new primordia arise in depressions and gaps. The first available space theory, however, stresses that the exact *position* above a gap in the preceding cycle where a leaf of the next cycle will be formed depends only upon those leaves which border the gap, not upon others (primordia bordering the gap in which primordium X develops often do not include primordium X – 1). The theory assumes that all superficial tissue of the apex tends to

form leaves. This leaf-forming tendency is inhibited by the distal region and by previously formed primordia. It does not become manifest until a sufficiently large space is available at a sufficient distance from the summit (Snow and Snow 1955). The implication is that the physiological microenvironment determines the position of a primordium, but the significant parameters in that environment are not yet known.

Theory of foliar helices.—Supporters of Plantefol's (1947) theory of foliar helices and of Buvat's (1955) *anneau initial* concept believe that leaves arise along one or several helices, each of which ends in the peripheral *anneau initial* with its own generative center. After a primordium is initiated the center moves onward and upward in its helical path. The nature of the migrating, leaf-forming impulse is vague, and observations that experimental injury to one side of an apex do not change the phyllotactic pattern on the other (R. Snow 1955) are difficult to explain. Critical discussions of these ideas were published by R. Snow (1958) and by Cutter (1959).

If one takes the reasonable position that initiation of primordia occurs whenever space is available on the apical dome and when inhibitions emanating from the summit and older primordia are overcome by distance, then the question of control of primordial initiation becomes one of control of enlargement of the apical surface. Presumably such enlargement would be influenced by gross environmental factors acting upon the whole apex, but more specific mechanisms may also be operative. A possible example of the latter is the previously discussed cambium-like zone (fig. 2, *p. 17*) which has been observed in some shoot apices in mid-plastochron (Popham and Chan 1950; Vaughan 1952). The relative activity of such a zone could easily control elevation of the apical dome and hence generation of additional space for initiation of primordia (*pp. 17–18*).

Obviously many questions about initiation of primordia remain unanswered, but there is enough information to indicate the kinds of problems which confront the researcher who wishes to learn how the all important, but nonetheless micro, events at the apex are controlled.

Procambium

Procambium is that primary meristem or meristematic tissue which differentiates largely or entirely into primary vascular tissue. If all procambial cells differentiate into primary xylem and phloem, meristematic capability is lost and no vascular cambium is formed. Complete differentiation occurs in vascular cryptogams, in a few extreme herbaceous dicotyledons, and generally in monocotyledons.

In most dicotyledons and gymnosperms some meristematic procabium remains after completion of primary growth. This develops into the vascular cambium which produces the secondary plant body. Procambium and cambium may be considered as two developmental stages of the same vascular meristem which first produces primary xylem and phloem, but which may also perpetuate itself to produce secondary xylem and phloem (Esau 1943).

Differentiation of procambium in the shoot apex has a gradual and indistinct beginning. In general, cells in the distal and subdistal regions show little differentiation, but in the organogenic region, in which primordia become visible, cells of the outer layers of the peripheral tissue zone become somewhat larger in size and increasingly vacuolate as the first phase of cortical differentiation. At about the same level rib meristem cells and derivatives which will become pith show similar changes. The inner part of the peripheral tissue zone, however, remains highly cytoplasmic. It constitutes a hollow truncated cone of tissue containing cells which are smaller and more actively meristematic than those in the developing cortex or pith.

This cone, often referred to as a ring because of its appearance in cross sections, has several names. Louis (1935), whose used the term *desmogen* as a synonym for procambium, called it *prodesmogen*, meaning precursor of procambium. Kaplan (1937) called it *Restmeristem*, which should be translated into English, not as "resting meristem," but as "residual meristem." The latter term is used herein in agreement with Esau (1953, 1960), Clowes (1961) and others. Actually, terminology and interpretation of this meristematic zone have long been controversial (see Esau 1943, 1954; Sloover 1958) and points of disagreement must still be expected in the literature.

Procambium is differentiated within the residual meristem. Cells in localized areas divide parallel to the apical axis and gradually form strands of narrow, elongate, procambial cells. In cross section they merely appear smaller than their neighbors. These procambial strands generally arise in association with foliar primordia, but the development of procambium is not totally dependent upon preexisting primordia. For example, systematic removal of leaves from apices does not preclude formation of vascular tissue (Wardlaw 1950), and embryos of *Fagus* have procambial strands in their epicotyls before any leaf primordia are visible (Clowes 1961). This should not be surprising because procambium also develops in roots (Esau 1943) and in stems of leafless vascular plants (Troll 1937–1939).

If the residual meristem is examined in cross section at one of the uppermost nodes, only a few procambial strands will be found. As more and more primordia are initiated above, additional procambial strands may be expected to appear between those formed earlier. This is so because each developing primordium has associated with it one or several strands of procambium. Finally much of the residual meristem has differentiated into procambium, but some may remain to become interfasicular parenchyma.

In higher plants each leaf has vascular tissue connecting it to the vascular system of the main axis. Therefore development of a primordium requires initiation and development of a procambial strand from which a vascular trace arises. Where previously differentiated strands curve outward across the peripheral tissue toward their primordia, new strands diverge in an inward and acropetal direction toward new primordial sites above. There is considerable evidence that procambium normally does develop acropetally (Esau 1942; Gunckel and Wetmore 1946a, b; Sterling 1945b, 1947; see

also Esau 1954) rather than from primordia downward, except possibly in some monocotyledons (Esau 1954). Basipetal differentiation does seem possible, however, in grafts, tissue cultures, and other anomalous systems (Ball 1952; Wardlaw 1952).

In some species new procambial strands begin developing before the primordia they eventually serve are visible, possibly even before their sites have been determined. Such precocious development has been reported in various gymnosperms (Crafts 1943a, b; Gunckel and Wetmore 1946a, b; Sterling 1945b, 1947) and may occur in other groups also (Priestley et al. 1935; Esau 1942). Such behavior increases the difficulty of determining the cause and effect relationships pertaining to initiation of primordia and procambial strands.

If primordia always became visible protuberances before procambium began differentiating toward them it would be logical to suppose that substances emanating from primordia and migrating downward could be responsible for the nonuniform differentiation of residual meristem into procambium. But procambial differentiation often occurs before primordia are visible. Perhaps the growth centers of the subdistal region produce morphogenic substances considerably before any changes are detectable visually. Perhaps, too, initiation of procambium within the apex and initiation of foliar primordia on its surface are both manifestations of nonuniform distribution of metabolites and regulators. Such nonuniform distribution is predicted by Turing's (1952) diffusion-reaction theory of morphogenesis (*p. 35*) and may be a highly significant factor in inception of developmental patterns.

Young (1954) studied the effects of removing single primordia from *Lupinus albus* apices. He concluded that auxin from primordia is operative in maintaining a meristematic state in the residual meristem, but that differentiation of procambium in the latter is induced by some other regulator which he called desmin.

Physiological problems do not end with differentiation of procambium from residual meristem. Why do primary xylem and phloem normally develop from the procambium rather than from some other tissue? Experimental severing of fully differentiated vascular strands can result in restoration of vascular connections across tissues which were never included in any procambium (Jost 1942; Sinnott and Bloch 1944; Jacobs 1952, 1954). Thus cells outside the procambium have the latent ability to become vascular elements, though they do not normally do so.

The first xylem in the procambial traces to leaves often differentiates in the leaf base region. Differentiation then progresses upward into the blade and downward until a connection with older xylem is established (Jacobs and Morrow 1957, Sloover 1958). If we suppose that environmental variables in procambium and adjacent tissue are such that, except for the presence of a specific hormone, all conditions necessary for differentiation of xylem elements are fulfilled, then the pattern of xylem differentiation will be determined by the locus, the amount of production, and the distribution pattern of that hormone. We may further suppose that the procambium and the vascular elements derived from it are a favored pathway of hormone translocation, but that hormone flow may be detoured through adjacent tissue if normal paths are blocked.

Evidence that this thinking may be correct, and that the hormone may be auxin, has been presented by Jacobs (1954, 1956) and by Wetmore and Sorokin (1955). The latter investigators grafted *Syringa vulgaris* shoot apices, with several pairs of primordia, onto callus cultures of the same species. Vascular strands appeared in a pattern suggesting induction of differentiation by a substance diffusing from the scion. Results obtained after addition of auxins to incisions in the callus were similar.

Phloem differentiation, unlike that of xylem, follows rather closely the acropetal course of procambial differentiation (Jacobs and Morrow 1957, 1958; Sloover 1958). Unfortunately almost nothing is known about hormonal or other mechanisms controlling differentiation of procambium cells into phloem elements.

Development of Leaves

Growth of very young foliar primordia is predominantly apical, but that predominance is temporary. In *Drimys winteri* (Gifford 1951) and *Viburnum rufidulum* (Cross 1937a) apical growth continues until primordia are about a millimeter high. Sonntag (1887) gives lengths reached by conifer leaf primordia before apical growth begins to be replaced by intercalary growth as varying from 380μ in *Pinus strobus* to only 200μ in *Taxodium distichum*. In *Clematis ligustifolium* apical growth continues for a relatively longer time, at least until the primordium is 2.5 mm. long (Tepfer 1960). Apical growth generally does not contribute significantly to leaf growth after bud break.

As meristematic activity at the leaf apex declines, cell division and extension in intercalary regions gradually become the major contributors to elongation growth. This normally occurs while leaves are still within the bud. Intercalary growth, which may continue slowly within the dormant bud, also contributes to expansion of the leaf blade. A transient phase of rapid intercalary growth in lamina usually accompanies bud break. Petiolar extension is another aspect of intercalary growth. In some taxonomic groups intercalary growth in leaves may become confined to a basal intercalary meristem which persists long after leaf emergence from the bud. This is especially true of Gramineae and of the genus *Pinus*.

Formation of leaf blades results from marginal meristem activity on the lateral flanks of primordia followed by intercalary growth. Marginal meristems are normally formed while leaves are still almost microscopically small primordia and often before apical growth has ceased (Cross 1937a, 1938; Foster 1936; Gifford 1951). Marginal growth may overlap both apical growth and intercalary growth in time, but it ceases while the leaves are still quite small—only 2 to 2.5 mm. tall in *Cercis siliquastrum* (Slade 1957). Even so, the general pattern of the leaf has by then largely been determined. Subsequent intercalary growth and a final phase of cell expansion and maturation brings it to mature size.

The characteristic shapes of leaves in large part result from nonuniform marginal meristem activity. When the marginal meristem consists of a series of discontinuous segments, compound leaves

develop. This view is probably oversimplified, however. The reader specifically interested in early development of compound leaves is referred to papers by Foster (1935a) and Tepfer (1960). In most conifer leaves marginal meristem activity is of very short duration. Consequently leaves are narrow. They elongate by basally localized intercalary meristems.

The initials of the marginal meristem constitute a band around the edge of the expanding leaf. Derivatives of these initials form a number of tissue layers in the young blade only a short distance within the margin. For the most part these layers remain discrete during further expansion of the blade by intercalary growth because divisions are anticlinal to the leaf surface. Exceptions occur in localized interior areas where divisions in various planes mark initiation of procambial strands which will develop into leaf veins (Smith 1934; Cross 1937a; Foster 1936; Gifford 1951; Pray 1955).

Except for the procambial strands, the internal layers derived from the marginal meristem and extended by intercalary growth differentiate into the spongy parenchyma and palisade layers of the mature leaf. Creation of the air spaces characteristic of these layers results from differential growth duration in the several layers of the blade. Cells in the epidermal layers stop dividing first, but they continue expanding for some time afterward. Meanwhile division ceases first in the spongy mesophyll area and finally in the palisade mesophyll. Continued expansion of the epidermal cells pulls apart the mesophyll cells producing large air spaces in the spongy mesophyll and small ones in the palisade mesophyll (Mounts 1932; Foster 1936; Pray 1955).

Detailed information on vascular development in leaf primordia is available for only a few woody species. In *Cercis siliquastrum* primordia 500μ tall already have procambial midrib strands which are continuous with vascular traces in the internodes below and which may contain developing xylem elements (Slade 1957). Branch veins in this and other species develop within the mesophyll particularly during the period of marginal growth, but also later (Foster 1952; Schneider 1952). The main features of the venation pattern have already been established in *Cercis siliquastrum* leaves when they are only about 3 mm. tall (Slade 1957). Detailed studies on vascular histogenesis in *Liriodendron* leaves have been made by Pray (1955). Earlier literature was reviewed by Foster (1952).

Observations on delineation of procambial strands, and their subsequent differentiation into primary vascular tissue in primordial or embryonic leaves, have not been well correlated with the various phases of bud growth and development in terms of the morphogenic cycle (*p. 46 ff.*). Is there much vascular differentiation in leaves prior to bud break in spring? Does any such activity begin before or after the end of rest (*for definition of "rest" see p. 75*)? Is it possible that the stimulus responsible for cambial activation in spring originates in leaf procambium or in differentiating vascular elements therein? It is because such questions can be asked but not definitively answered that vascular development in young leaves is of interest to physiologists as well as anatomists.

Leaf growth after emergence from the bud need not always be primarily the result of cell enlargement alone. Mounts (1932) reported that cell division in *Catalpa bignonoides* leaves may continue until the blade is as long as 6 cm., but she did not determine the fraction of the total cell number generated by these late divisions. Sunderland (1960) found that most cells present in mature *Helianthus annuus* and *Lupinus albus* leaves are formed after emergence from the bud, and also that cell divisions may continue until leaves are half grown or more. True, there may be more generations while the primordium is still within the bud, but the cell number increases geometrically and the last few generations produced during intercalary growth contribute a large fraction of the total. The situation may be comparable to that in internodes in which most cells are derived from divisions within the subapical meristem (*pp. 34–35*).

The reader should note, however, that Sunderland's conclusions were based upon observations of two herbaceous species which form no buds comparable to the winter buds of woody plants. Emergence of leaves from *Helianthus* and *Lupinus* buds may not be physiologically equivalent to the rapid leaf emergence and growth following bud break of trees in spring. Final judgment of the significance of cell division after leaf emergence should be withheld until more data are available.

In some tree species all the leaves expanded in a growing season are initiated during the preceding season and overwinter in the buds as well-developed embryonic leaves. In numerous other species these "early" leaves are followed by an additional series of "late" leaves. Some of the latter may have been present in the winter buds, but as arrested primordia rather than embryonic leaves. Others are initiated and continue to develop uninterruptedly to maturity during the same growing season.

A very interesting point is that early and late leaves often exhibit easily recognizable differences in a variety of morphological features. Such leaf dimorphism is common in *Populus* (Critchfield 1960). It is often evident in trees which produce lammas shoots (Späth 1912) and in those species having both short shoots, bearing early leaves, and long shoots, bearing late leaves. Examples of the latter are *Ginkgo* (Sprecher 1907; Gunckel and Thimann 1949; *p. 130 ff.*) and *Circidiphyllum* (Titman and Wetmore 1955; *p. 132*).

Critchfield (1960) concluded that in many instances of heterophylly there is a relation between the circumstances of leaf ontogeny and ultimate leaf form. A common feature of seedling leaves, epicormic sprout leaves, and late leaves of heterophyllous shoots is uninterrupted development from early primordial stages to maturity. Such continuous development is much less common in adult than in juvenile woody plants, but it recurs in old individuals in epicormic or adventitious shoots.

Some of the morphological differences between juvenile and adult shoots may be related to differences in continuity of development of primordia. Perhaps, though, environmental differences during specific phases of development are of more basic significance (*pp. 162–163*). Discontinuity of development, however indirect cause

688-803 O—63——4

and effect linkages may be, is almost certainly the consequence of environmental conditions prevailing in the primordium, the bud, and general vicinity of the plant.

In some plants no correlation is evident between leaf shape and external environmental conditions or relative continuity of primordial development. An example is *Hedera helix* (Kranz 1931; Robbins 1960). In this species leaf shape appears to be a function of plant age and the position on the plant of the bud in which the leaf had its origin. Leaf morphogenesis seems to be controlled by persistent internal factors relatively immune to redirection by conditions of the immediate external environment. It is entirely possible, nevertheless, that the pattern prevailing in a plant or shoot was set by environmental influences at some earlier sensitive period in ontogeny (*pp. 162–163*). Though quite persistent, such patterns have been altered experimentally. Robbins (1960) induced adult, arborescent *Hedera* to develop shoots bearing juvenile leaves both by heavy pruning and by treatment with gibberellin.

Many of the questions one might ask about control of leaf morphogenesis (Ashby 1948), or the origin of leaf dimorphism (Critchfield 1960) and other forms of heteroblastic development, encroach upon the problems posed by developmental phase changes (Brink 1962) and juvenile stages in woody plants (Schaffalitzky de Muckadell 1954). These very interesting subjects cannot be discussed in detail in this bulletin.

Development of Cataphylls

The term "cataphyll," literally lower leaf (intended as a translation of the German *Niederblatt*), is commonly used in anatomical literature with reference to bud scales and similar organs. Primordia which develop into scales or cataphylls are initially very similar or identical to those which become foliage leaves, but developmental differences soon become evident (Foster 1931a, b, 1935a, b; Cross 1936, 1937a, b).

Except for more epidermal hairs on cataphylls, it is not possible to distinguish structurally between young cataphylls and foliage leaves of *Viburnum rufidulum* until they are about 500μ tall (Cross 1937a). In *Carya buckleyi* developmental differences become detectable at the 100μ stage. The cataphylls then undergo rapid marginal expansion but foliar primordia first increase in radial thickness (Foster 1935a, b).

In *Morus alba* cataphyll primordia reportedly arise from the tunica only whereas both tunica and corpus contribute to true foliar primordia. In the same species procambium does not appear in cataphylls until they are about 800μ high, whereas it is detectable in foliar primordia much earlier (Cross 1936, 1937b). Early and extensive marginal growth of cataphyll primordia, in *Pseudotsuga* (Sterling 1947), *Pinus lambertiana* (Sacher 1955a, b), and conifers generally, is a distinguishing characteristic because marginal growth of foliage leaf primordia is much less.

In general, cataphyll tissues mature rapidly with less differentiation than foliage leaf tissues. The mesophyll remains poorly developed, often without a distinguishable palisade layer. Vascular development is poor. Stomata are few or absent (Foster 1949). These

are differences of degree and many intermediate stages between scales and leaves are possible. Primordia of many species, in fact, show a gradual transition of development from cataphylls to leaves. Numerous series of transitional forms were illustrated and discussed by Lubbock (1899).

Whether it occurs in spring, summer, or fall, development of a series of scales is normally prerequisite to terminal bud formation. How is the development of primordia controlled? Why do internodes between scales often remain permanently dormant whereas those between leaves usually undergo only temporary dormancy? Why do buds appear in the axils of only some of the scales? These questions are of paramount importance to the problem of growth and dormancy control in woody plants. Unfortunately, present information is too meager to justify any serious attempt to answer them.

Some control over development of primordia has been attained experimentally. As is discussed in detail later, in some species short photoperiods induce primordia at the apex to develop into bud scales rather than additional leaves. Another approach is suggested by Dostál's (1961) report that in *Syringa vulgaris* axillary buds development of primordia which normally become leaves can be altered to yield additional scales by treating the axillant leaf with gibberellic acid.

Dostál believes that bud scales have an influence upon the development of primordia within. For example, removal of the outer scales from *Aesculus hippocastanum* buds just as the development of primordia into leaves is beginning within will cause reversion to scale formation until the normal number is restored (Dostál 1952). In the presence of added gibberellic acid the number of scales which must be present to allow development of primordia into leaves is much greater (Dostál 1961). Thus scales may have a morphogenic influence which can be counteracted by gibberellic acid.

A morphogenic influence of auxin also is indicated by Dostál's (1952) experiments with the auxin antagonist 2,3,5-triiodobenzoic acid. In axillary buds of *Aesculus hippocastanum* auxin promotes development of primordia into scales, whereas 2,3,5-triiodobenzoic acid counteracts the auxin. It promotes development of primordia into leaves even to the extent of inducing elongation and expansion of scales already differentiated.

The work of Dostál (1909, 1926, 1952, 1961), and theoretical considerations (*p. 21*), encourage me to promote the following working hypothesis as a reasonable one: Primordia are not predestined to become leaves or to become scales. They are inherently capable of becoming either. But at an early stage in their development they are very susceptible to morphogenic determination by environmental conditions imposed upon them. These conditions are resultants of internal environmental factors as modified by those external to the plant.

The local environment of the primordium is strongly influenced by neighboring tissues and organs, particularly by the developmental direction which the older primordia have already taken. Because the older primordia collectively often envelop the apex and younger primordia, the development of the former strongly influ-

ences the kinds and amounts of metabolites, regulators, and dissolved gases which the young primordium obtains from or loses to the ambient tissue or gas space. Metabolism and differentiation are not immune to such influences (*p. 148*). The sensitive period during early development, when determination of further differentiation toward either leaves or scales is possible, may be quite short (*p. 163*).

A similar line of thinking reveals the inadequacy of any ideas of predestination in internodes. It is more logical to suppose that internodes between scales are dormant because the primordia they bear, by differentiating into scales, established an environment in those internodes different from that in internodes the primordia of which became foliage leaves.

Vegetative Buds and the Morphogenic Cycle

The Bud Concept

A bud is an unextended, partly developed shoot having at its summit the apical meristem which produced it. The latter is usually covered and protected by primordial leaves and by cataphylls (scales) initiated by the meristem at some earlier time. The subapical region of the meristem includes the internodes between primordial leaves and cataphylls and makes up the mass of the tissue in the central axis of the bud. Internodes in the subapical region are very short.

Bud break and shoot elongation, whenever they occur, are the result of leaf enlargement and subapical meristem activity in the region comprising internodes between leaves. Subapical meristem activity resulting in internodal elongation, however, is not an essential part of bud opening.

Elongating scales may cause buds containing dead shoots to open in a relatively normal manner in spring (Pollock 1950).[5] In such instances bud opening is obviously determined by localized growth of the scales, not by activity within the shoot. In short shoots of *Ginkgo* and *Larix*, for example, little if any internodal elongation accompanies bud opening (*p. 130 ff.*).

Conversely, bud opening may result almost entirely from internodal elongation with the sheath of scales being forcibly ruptured. For example, initial bud opening and shoot elongation in *Pinus* sometimes results from subapical meristem activity in the region of sterile cataphyllary internodes and between points of insertion of short shoots, whereas needle extension by the latter occurs later. Allowing variations of pattern, bud opening results from reactivation of preexisting meristems in the subapical region, in primordia, or in both.

Although bud opening results from renewed meristematic activity, bud formation is not strictly a matter of inhibited internodal elongation or inhibited primordial growth. The first step, bud scale production, involves a specific kind of primordial development. If there were no stimulus for scale formation, inhibited internodal elon-

[5] Pollock, Bruce McFarland. An investigation of the physiology of the rest period in trees, with special reference to *Acer*. 1950. (Doctoral Diss., Univ. Rochester, Rochester, N.Y.)

gation could possibly still cause a rosette to accumulate. But rosette formation is not generally equivalent to bud formation in the sense that the latter term is used with reference to woody plants. Bud formation, therefore, depends upon initiation of primordia by an apical meristem and control of their differentiation (*p. 45*).

In common parlance the bud is spoken of as a dormant structure, and this is correct in a limited way (see *p. 75* for definition of dormant). A bud is dormant in the sense that its potentially active subapical meristem is dormant; hence, there is little elongation between internodes. However, the more distal regions of the meristem are not necessarily dormant and may be initiating new primordia at a rapid rate.

In summer, during and after the main flush of shoot elongation, the dormancy of the new terminal bud applies mainly to its lack of elongation. The apical meristem is quite active. New primordia are being initiated, and in many species lateral buds are initiated in the axils of the primordia within the terminal bud. The dormancy of older lateral (axillary) buds may be more complete and may include inactivity of the apical meristem. Long-term dormancy of lateral buds is supposedly induced and maintained by regulators under the influence of more nearly terminal buds or leaves of the same shoot system.

A knowledge of morphogenesis and developmental anatomy of buds is essential to an analysis of the problem of growth and dormancy control. This is true because the meristems which are controlled are mostly within buds. Vegetative buds are often classified according to their manner of origin as terminal, axillary (lateral), or adventitious. Some of the more significant aspects of the physiological morphology of each type are discussed below. However, relatively few detailed studies have been made and it is likely that exceptions will be found to generalizations based upon present evidence.

A Peculiar Anatomical Feature—The Crown

A peculiar anatomical feature of terminal buds of a variety of species is the crown or collenchyma plate (fig. 4). Schröder (1869), in a paper concerning growth habits of *Acer platanoides*, described a tissue of collenchymatously thickened cells at the base of the young terminal bud. Busse (1893) observed a similar structure in *Abies alba* buds and discussed it in relation to Schröder's report. The structure was rediscovered by Lewis and Dowding (1924), who found it beneath buds of *Picea*, *Pseudotsuga*, *Larix*, and *Abies*, but not in *Pinus*. They described it as a plate of thick-walled cells, giving pectic reactions, dividing the young tissues of the bud from the older parts of the stem.

Lewis and Dowding also noted that a cavity may form beneath the crown, and that this, with remains of the crown, may persist for several years. Korody (1937) described the plate of thick-walled tissue in *Abies concolor* and *Picea excelsa*. She termed it the *Kollenchymaplatte* because the wall thickenings seemed to be of the collenchyma type. Later workers have mostly used the term "crown" after Lewis and Dowding (1924).

FIGURE 4.—Stained, median, longitudinal section of *Abies concolor* bud with distinct crown across the base. Most of the bud scales have been removed. The bud was collected in early spring prior to the period of rapid shoot elongation. (Photograph kindly provided by Dr. Robert V. Parke.)

More recently the existence of the crown has been confirmed in *Abies concolor* (Parke 1959), *Pseudotsuga taxifolia* (Sterling 1946), *Sequoia sempervirens* (Sterling 1945a), *Torreya californica* (Kemp 1943), *Larix decidua* (Frampton 1960), and *Cephalotaxus drupaceae* (Singh 1961). It is relatively certain that a crown is not present in buds of the common pines, for if it were it would be visible in the photomicrographs of Sacher (1954) and Duff and Nolan (1958).

Although Schröder's (1869) original description of the crown was based upon observations of *Acer* buds, occurrence of the crown has not been widely reported in angiosperms. Büsgen and Münch (1931) mentioned a dome of thick-walled cells rich in starch occurring in the basal regions of buds of *Acer* and *Fraxinus*. More anatomical work is needed to establish the relation between structures observed in angiosperms and the crown of gymnosperms. Tolbert (1961) reported a crown to be present in *Hibiscus syriacus*, but it is not as

heavy as that in gymnosperms and disappears during the spring growth flush.

The crown has been described in detail by the authors cited above. In effect it is a plate across the base of the bud consisting of perhaps 5 to 10 rows of somewhat isodiametric cells the walls of which have been thickened with deposits of cellulose, hemicellulose, and some pectinaceous materials. Ligin and lipids are reportedly almost absent. This plate isolates the tissue of the young shoot in the bud from the mature tissue beneath except where it is penetrated by vascular traces.

The nodes bearing bud scales are below the crown and the bud scales are not isolated from the mature cortical tissue. The vascular traces which pass through the crown are much less fully developed above than below and lignification seems to extend only as far as the base of the crown (Lewis and Dowding 1924). The parenchyma below the crown may break down in late summer and autumn to form a cavity which is often partially filled with jellylike material.

The function of the crown is unknown. Lewis and Dowding (1924) did some dye penetration experiments with one-year shoots of conifers cut in January. They concluded that water is prevented from entering the dormant bud and suggested that perhaps the presence of the crown is related to this. In all cases dye penetrated only as far as lignification extended (to the base of the crown) and none ever penetrated into the bud above the crown even though a rudimentary vascular system was present. Such experiments, however, are not conclusive. Buds, being covered with layers of scales, have a very low transpiration rate, and failure of water and dye to enter might be due merely to lack of a water deficit in the bud. Furthermore, xylem vessels still containing living protoplasts would not be expected to be good translocation pathways for dyes although water moves through them freely.

There is no correlation between the presence or absence of a crown and whether or not the winter bud contains a preformed unexpanded shoot. *Sequoia sempervirens* has no preformed shoot, but it has a crown (Sterling 1945a). Buds of *Pinus lambertiana* and *P. ponderosa* have preformed shoots but no crown (Sacher 1954). The relation between crown formation and control of dormancy is completely obscure, but we cannot say that such a relation does not exist.

Terminal Buds

Terminal buds are formed by most, but not all, woody species. Although the new terminal bud may not be noticed by the casual observer until near the end of a growth flush, the first formed elements of the new bud, the scales, are often initiated quite early in spring. In some pines (Sacher 1954) these scales are initiated in the preceding fall, 18 to 20 months before the buds will open. In most temperate zone trees the internodes below scales and transitional forms normally exhibit only traces of subapical meristem activity. These internodes seldom elongate appreciably. Exceptions, of course, are found.

Internodes between bud scales of some *Rhododendron* species do elongate, and in some tropical genera the scale internodes become

much longer than those between true foliar organs (Koriba 1958). In pines also some cataphyllary internodes elongate whereas the internodes of the short shoots bearing the true foliage leaves do not. Such behavior indicates that mechanisms controlling development of primordia and elongation of internodes are not identical.

After a sufficient number of scales has accumulated, the development of additional primordia is modified so that embryonic foliage leaves are produced (*p. 45*). If this change is gradual a series of transitional forms results. The mechanism controlling this is, again, obscure (see Dostál 1961).

The foliage leaf primordia and their unextended internodes constitute the preformed shoot which will be extended during the next growth flush. It is not unusual for a terminal bud in winter to contain primordia of all the leaves which will be expanded the following season, but there are many which do not (Critchfield 1960). The axils of the lower leaf primordia (and sometimes of the bud scales) often already contain partly developed axillary buds or their primordia. Winter terminal buds may thus contain unexpanded shoots complete with axillary buds.

Some woody species form no terminal buds. This is true of many common trees and shrubs having the sympodial growth habit. In these, shoot tips are aborted during late spring or summer and the function of terminal buds is assumed by the uppermost laterals (*pp. 62–65*).

According to Laubenfels (1953), most conifers having scale leaves fail to form true terminal buds. The apparent terminal buds often lack morphologically distinct scales and do not contain preformed shoots to be expanded the following season. In these, winter is merely a period of interrupted growth. This is in agreement with observations on *Sequoia sempervirens* made by Sterling (1945a). Buds of some *S. gigantea* seedlings grown under controlled environments reportedly do contain compacted internodes (Skok 1961).

Terminal buds exhibit variable degrees of anatomical and morphological complexity. Physiological complexity also is indicated by the many different types and loci of meristematic activity, the separate but integrated control of which is the essence of the morphogenic process. In the absence of detailed comparative information from a variety of woody species, the problems and processes involved in the morphogenic cycle of terminal buds can be illustrated by a series of examples.

Abies concolor.—The dormant terminal bud of *A. concolor* contains an unexpanded shoot bearing 50 to 60 needle primordia which are surrounded by 20 to 30 cataphylls (Parke 1959). The bud is separated from the mature tissue below by a crown. At about 4,000 feet altitude in the Sierra near Camino, Calif., subapical meristem activity begins in early April.[6] The new shoot elongates rapidly.

[6] The actual date on which a particular stage of development is reached varies somewhat from year to year. The same comment applies to other approximate date indications in this and subsequent paragraphs.

During the early part of the elongation phase the apical meristem itself remains inactive. After the new shoot has grown a few centimeters the apical meristem begins initiating the primordia which develop into the scales of a new terminal bud.

Elongation of the shoot and development of primordia into scales at the apex continue until mid-June. Thereafter shoot elongation declines. Correlated with declining shoot elongation is increasing size of the apical dome. Primordia continue to be initiated, but they develop into embryonic foliage needles rather than scales. Additional needle primordia are formed until late September when activity ceases and the dormant winter bud is complete (Parke 1959). In *Abies concolor*, then, scale initiation for the next terminal bud is completed while the shoot from the preceding bud is still elongating. The same is true in *Pseudotsuga taxifolia* (Sterling 1946).

Pinus lambertiana and P. ponderosa.—The terminal buds of *P. lambertiana* and *P. ponderosa* contain all the primordia for the following season's growth (Sacher 1954). The axils of many of the primordial cataphylls bear primordial dwarf shoots with small apical meristems. The first sign of spring activity is the beginning of elongation of the main axis. The dwarf shoots also renew their development. The apex of the main shoot itself remains inactive during the first few weeks of shoot elongation. Then, after the new shoot has grown considerably and the needles of the dwarf shoots have burst through their sheaths of scales, the main shoot apical meristem is reactivated.

The first new primordia develop into sterile cataphylls. They are called sterile because they bear no dwarf shoot primordia in their axils. The internodes between these cataphylls will eventually elongate, but not until the next growth flush. Slow production of sterile cataphylls continues throughout the period of rapid shoot elongation.

As shoot elongation slows and ceases the apical meristem becomes more active and new cataphyll primordia are more rapidly initiated. All cataphylls of this second series eventually bear dwarf shoot primordia in their axils. The latter primordia are initiated in the subapical region. In late summer, lateral long shoot bud primordia arise in the axils of a few cataphylls. The apical meristem then enters a period of declining activity during which a new series of sterile cataphylls is produced. These cataphylls are the bud scales of the terminal bud to be formed the next season and expanded the season after that.

Such seemingly precocious terminal bud scale development is not limited to *Pinus*, but occurs in *Carya* (Foster 1931b) and perhaps in other genera also. In addition to the detailed work of Sacher (1954, 1955a, b) several less recent publications contain valuable information on the anatomy and morphology of *Pinus* buds. Especially noteworthy are the monograph by Doak (1935) and the beautifully illustrated, classical papers by Henry (1839, 1847).

Torreya californica.—The winter terminal bud of *T. californica* also contains all the leaf primordia for the following season's growth (Kemp 1943). The undeveloped shoot is enclosed in about eight pairs of cutinized bud scales. The bud is separated from the mature tissue below by a crown. In early spring the bud scales open, the preformed shoot elongates, and the many leaf primordia mature into foliage needles. Although inactive at first, toward the end of this period the apical meristem enlarges and finally, in late April (Santa Cruz Mountains, central California), primordia are initiated which develop into bud scales.

By the end of July all the bud scales have been differentiated, and on external inspection the new bud seems complete and inactive. Within, however, the apical meristem remains active. Primordia which develop into embryonic leaves are initiated in rapid sequence in August. About this time cells of the central core of parenchyma at the base of the bud become thick walled and differentiate into a new crown.

During autumn leaf primordia are initiated at a slower and slower rate until finally, perhaps in October, the apical meristem becomes inactive and the whole bud seems dormant (Kemp 1943). Relative inactivity in the subapical regions has, of course, prevailed since the primordia becoming the first scales were initiated. It is noteworthy that in *Torreya* the apical meristem remains inactive until the elongation of the preformed shoot from the bud is practically complete. Reactivation of the subapical meristem is not immediately followed by activity in the apical region.

Larix decidua.—The dormant terminal buds of *L. decidua* probably contain all the internodes to be extended the following season. Lateral bud primordia, however, are not present. These appear in some axils during the early stages of internodal elongation. *L. decidua* terminal buds are of two anatomically different types, of which one produces long shoots, whereas the other produces short shoots or rosettes with almost no internodal elongation. Long shoot buds have a strongly developed axial part encompassing accumulated foliar primordia and extensible internodes. Short shoot buds lack this (Frampton 1960).

Liriodendron tulipifera.—Unlike the terminal buds of the conifers discussed above, those of *L. tulipifera* do not necessarily contain primordia of all the leaves which will be expanded during the next growing season (Millington and Gunckel 1950). The *Liriodendron* bud usually contains about 8 leaf primordia whereas as many as 14 to 20 leaves are expanded each season.

Dehiscence of the bud scales begins in mid-March (New Brunswick, N.J.). By late March enlargement of the preformed leaf primordia begins and mitotic activity is resumed in the apical meristem. By mid-April young leaves are expanding, the internodes are elongating, and new leaf primordia are being initiated. Internodes between the latter elongate in the same season they are formed. During this period the new shoot usually develops two to five short lateral branches. According to Millington and Gunckel

(1950) these do not arise from axillary primordia present in the bud, but from new bud primordia initiated in spring.

Additional primordia continue to be initiated throughout July and August. Beginning in early July, however, there is a change in the developmental pattern and an inhibition of elongation of internodes between new primordia. A few (usually two) primordia develop into pairs of bud scales. The latter may be interpreted as pairs of stipules belonging to leaves the blade development of which was inhibited. Elongation of internodes below scale pairs is permanently restricted.

After scale formation the developmental pattern reverts to production of primordial foliage leaves and their stipules. By early September the new terminal bud contains eight partly developed leaves. Mitotic activity in the apex becomes very slow and stops in early October (Millington and Gunckel 1950). It should be noted, however, that Moore (1909) reported *Liriodendron* near Wellesley, Mass., to extend only seven to nine internodes per year and therefore concluded that the *Liriodendron bud* contains all the leaves to be expanded in the following season. Local climatic or soil factors may account for this disagreement.

Carya buckleyi var. *arkansana*.—Foster (1932) made a detailed study of the morphogenic cycle of spur shoot terminal buds of *C. buckleyi* var. *arkansana*. Behavior of long shoot terminal buds is similar, but more variable (Foster 1931b). Until mid-March (near Norman, Okla.) the spur shoot terminal bud is apparently dormant. The bud already contains partly developed primordia of two transitional scales of the next terminal bud (Foster 1932).

In late March the transitional scales begin rapid growth and initiation of additional primordia begins. Within the next month seven new scales develop. During the latter part of this period the old terminal bud opens and subsequent extension of the preformed shoot makes the new terminal bud visible. Completion of scale formation for next season's bud generally coincides with cessation of shoot elongation in spring.

Following the seventh and last scale, four additional primordia develop into foliage leaves. This developmental change is remarkably abrupt. By mid-May the next season's terminal bud has been determined. Shortly thereafter two primordia are initiated which become the transitional scales of the terminal bud that will open two seasons later.

By the end of May initiation of primordia at the apex has ceased. Apical and subapical meristem regions of the bud axis both remain inactive until the following spring. Meristematic activity resulting in enlargement and histological specialization of scales and leaves, however, continues (Foster 1932). Initiation of primordia at the apex is thus limited to a fraction of the available growing season. From a long-term viewpoint apical development produces alternate series of scales and leaves. Foster's (1932) discussion of problems posed by development of alternate series of similar primordia into dissimilar organs is still of interest (*see also p. 45*).

Axillary Buds

Axillary buds, the primordia of lateral branches, commonly are initiated while the parent shoots bearing them are still within terminal buds or axillary buds of the preceding generation (Schacht 1853; Hofmeister 1868; Sachs 1875, pp. 131–138). Koch (1893) reported that in some angiosperms lateral buds arise in the axils of the third or fourth youngest primordia.

More recently, initiation of axillary buds has been reported above the second youngest leaf primordia in both angiosperms (Sussex 1955) and gymnosperms (Seeliger 1954). Such early bud initiation is not necessarily the rule, however, as there is considerable variation between species (Garrison 1955; Gifford 1951). Axillary buds of *Pseudotsuga taxifolia* are not initiated until the parent bud is actually elongating in spring (Sterling 1947).

There have been arguments favoring foliar origin of axillary buds (Majumdar and Datta 1946). The prevailing view is that axillary buds arise on the main axis above leaf primordia, in positions determined by leaf positions, but by separate organogenic processes (Garrison 1949a, b, 1955). In some species, butresses of leaf primordia or the embryonic axillary buds themselves grow in such a way that they gain the appearance of foliar origin, though a study of early development reveals these also to be cauline in origin (Kundu and Rao 1955).

Formation of a visible bud primordium results from anticlinal divisions in outer layers of the parent axis coordinated with volume growth in deeper layers. Relative contributions of inner and outer layers are variable and not necessarily the same as for leaf initiation in the same species (Schmidt 1924).

Meristems of bud primordia in the axils of very young leaf primordia can be regarded as having arisen from the organogenic region of the parent shoot meristem itself. They become separate later because of vacuolation and differentiation of surrounding cells. In other instances buds may arise in the axils of older leaf primordia in the subapical region where some differentiation in the cortical area of the internodes has already occurred. How are meristems established in these bud primordia? They are established by a process of dedifferentiation.

By unknown means maturation and differentiation in certain cells is reversed, renewed cell division is evoked, and a meristem is organized. In a sense, there is no sharp demarcation between late initiation of normal axillary buds and initiation of adventitious buds on shoots although the extent of dedifferentiation preceding the latter is usually greater.

If bud primordia appear in the axils of very young leaf primordia, initiation of the bud and its procambial connections with the main axis may appear simultaneous, as in *Syringa* (Garrison 1949a). Buds initiated above older leaf primordia may at first have no procambial connection with the main axis, but as the first foliar appendages of the new branch are initiated procambial strands develop, as in *Drimys winteri* (Gifford 1951).

The first foliar organs of gymnosperm and dicotyledonous angiosperm axillary buds are usually a pair of opposite prophylls (Troll

1937–1939, pp. 333 and 447). Procambial strands differentiate in and below these and across intervening tissue to merge with the developing vascular system of the parent shoot. These procambial strands become branch traces. As additional leaves are initiated the traces are strengthened (Garrison 1949a, b) and eventually a complete vascular cylinder is formed.

More than one bud may be initiated in a single axil. In many species axillary buds obvious to the eye are subtended by a series of progressively less developed and less conspicuous supplemental buds. Occurrence and behavior of these buds was discussed in detail by Sandt (1925). Interesting problems of inhibition and control of development are involved.

Why do bud primordia develop when and where they do? Again, as in the case of leaves, we must suppose that strong localized growth activity is the result of nonuniform distribution of metabolites and regulators (*p. 35 ff.*). There is some evidence of localized areas of enhanced peroxidase activity in axils of leaf primordia prior to bud initiation (Van Fleet 1959). Additional work in this area is much needed.

Axillary buds, unlike leaves, do not arise in the largest available space between other primordia and the apex (*p. 37*), but above the center of a leaf. If leaves are displaced from their normal phyllotaxic positions by surgical operations, buds still arise in their axils. Removal of leaf primordia when very young will sometimes prevent initiation of axillary buds, and if incisions are made between leaf primordia and the summit, buds always appear on the leaf side, never on the apical side. Such observations strongly suggest that in many species local metabolic-environmental conditions (*p. 21*) determined by the leaf are a major factor in determining bud position. A confusing note is provided by some, probably exceptional, species in which bud primordia appear (mostly in inflorescences) before the leaves subtending them (Snow and Snow 1942).

Development of bud primordia differs from that of leaf primordia in several basic respects, though both are lateral outgrowths of the same parent structure. The leaf develops with dorsiventral symmetry, the bud with radial symmetry. The growth of the leaf is determinate, but that of the bud is potentially indeterminate. What are the causal factors underlying these differences? What is the origin of these factors? When does their influence upon a young primordium become irreversible? These questions can be approached experimentally.

The reader specifically interested in these problems may wish to consult papers discussing experimental work with pteridophytes (Wardlaw 1955c; Cutter 1956; Steeves 1961). In *Osmunda cinnamomea*, at least, young primordia can be excised and cultured aseptically while they are still capable of becoming either leaves *or* shoots (Steeves 1961).

Literature on the origin and development of lateral buds in angiosperms has been reviewed by Sifton (1944), Philipson (1949), and Garrison (1955). Koch (1891), Doak (1935), and Korody (1937) made contributions to and reviewed the work on axillary buds of gymnosperms, but research in the latter field was never very active. Henry in 1839 accurately illustrated and described needle bundles

of *Pinus* as dwarf axillary shoots, but it was 1955 before a detailed ontogenetic study of these dwarf axillary shoots was published (Sacher 1955b).

In the following paragraphs brief accounts are given of the origin and development of axillary buds in a few species. These are merely examples. The number of species studied is still too small to warrant saying that these are representative, but the available information is of value in locating the major physiological problems.

Betula papyrifera.—Axillary buds of *B. papyrifera* are initiated during spring and summer just after leaf primordia are initiated in the parent axillary buds (the species lacks terminal buds). Axillary bud meristems and their procambial traces are organized from detached groups of meristematic cells left behind by nonuniform vacuolation and differentiation in the second to fourth node region. After this early initiation the primordium may develop into a small mound of tissue, but there is no further activity until the following spring. By late April (Jamaica Plain, Mass.) two primordial scales or prophylls appear. These may be interpreted as the stipules of an abortive leaf.

Beginning in mid-May a series of about seven foliage leaf primordia are initiated. As each leaf primordium enlarges, a bud primordium of the next generation appears in its axil (Garrison 1949b). Thus an axillary bud primordium is laid down in one season, and its scales and leaves are initiated the next season. The bud may open the third season (perhaps 22 months after initiation) or it may remain dormant for many years. Morphogenic cycles of axillary buds in *Syringa vulgaris* (Garrison 1949a) and *Euptelea polyandra* (Garrison 1949b) are similar. Axillary buds in *Magnolia stellata and Liriodendron tulipifera* may undergo slightly more development during the first season but otherwise their behavior is also similar.

Pinus lambertiana.—In the genus *Pinus* the main stem axis bears cataphylls or scale leaves. In young trees the cataphylls may elongate and serve as primary or juvenile foliage leaves. The foliage leaves in older individuals, however, are borne on dwarf lateral shoots which arise in the axils of cataphylls. In *Pinus*, then, foliage leaf primordia are not initiated by the apical meristem of the main axis, but by meristems of axillary buds which develop into dwarf (or short) shoots.

According to Sacher (1955b) initiation of dwarf shoot primordia in *Pinus lambertiana* occurs in the axils of cataphylls a few internodes below the apical meristem within terminal buds or lateral long shoot buds. Initiation begins in mid-May (Berkeley, Calif.) and continues through August. Each primordium is at first a small mound of uniformly meristematic tissue, but soon develops well defined cytohistological zonation at its apex. All appendages and vascular tissue arise from the peripheral zone.

The first primordia develop into 2 opposite prophylls, followed by 11 cataphylls (see also Sacher 1955a). After the last cataphyll, five additional primordia develop into embryonic foliage leaves. Some unknown mechanism then brings a halt to further activity of the apical meristem for the season. The size of the apical dome is reduced and the cells become vacuolate. Dwarf shoot buds thus spend the winter within the parent long shoot buds.

In spring, as the parent bud elongates, growth of dwarf shoot buds is resumed in the form of needle elongation. By the time needles have become a millimeter long the apex of the dwarf shoot has temporarily resumed meristematic activity. This activity results in some increase in the size and number of cells in the apex, but usually no more primordia are initiated and the apex again becomes quiescent. During the following year its outer layers often become desiccated.

In a few instances the apices of dwarf shoots produce cataphyll primordia after the needles have matured. This has been interpreted as the beginning of interfoliar bud formation (Borthwick 1899: Doak 1935). Whereas the dwarf shoot is normally a determinate branch system, it has a latent ability to proliferate into a long shoot. Such proliferation may often be induced by removal of the apex of the long shoot on which the dwarf shoot is borne.

Pinus resinosa.—Sacher's (1955a, b) work with *P. lambertiana* is not applicable to the entire genus. Duff and Nolan (1958) found important differences in the pattern of bud morphogenesis in *P. resinosa*. In the latter species the new terminal bud begins to form during July (Chalk River, Ontario), after the period of maximal shoot elongation has passed. As cataphylls are initiated a small mass of meristematic tissue persists above each. These areas remain meristematic after isolation by younger primordia and by surrounding vacuolation. They develop into mounds of tissue which are the axillary dwarf shoot primordia. Development after initiation is slow. Usually only a few scale primordia are produced during the late summer and autumn.

No leaf primordia are initiated until spring. Their initiation can be induced by application of growth substances, but when this is done the dwarf shoot axis also elongates and projects through the bud scales in fall. This is followed by distorted development in spring. The lack of late summer and fall development of dwarf shoot bud primordia cannot be blamed entirely upon the cool climate (Chalk River, Ontario) because the megasporangiate cone primordia, which are formed later in summer than dwarf shoot primordia, do develop throughout the fall and winter (Duff and Nolan 1958).

Sequoia sempervirens.—In most woody species axillary buds are initiated within older buds. *S. sempervirens*, in its natural habitat, does not form winter buds containing preformed, unexpanded shoots (Sterling 1945a). Irregularly throughout the growing season lateral bud primordia appear between the uppermost leaf primordia and the apex. The bud primordia are initially almost indistinguishable from leaf primordia but their development soon diverges. Bud primordia become spindle shaped prior to initiation of two opposite prophylls. The leaf primordia acquire a pointed apex when only five or six cells high (Sterling 1945a). Are the buds truly axillary in the sense that their position is determined by leaf position? This has not been studied in detail. Likewise no detailed information is yet available concerning further development of the lateral shoot buds.

The above examples and others in the literature illustrate the great variation in first-season development of axillary buds. At the approach of winter new axillary buds of *Torreya* (Kemp 1943), *Syringa*, *Betula* (Garrison 1949a, b), *Liriodendron*, and *Juglans*

(Garrison 1955) are only small mounds of tissue, possibly with primordial prophylls; those of *Alnus*, *Magnolia*, and *Pterocarya* may be only primordia or may have several leaves; and those of *Akebia* and *Schisandra* may range from primordia to buds with many leaves.

Axillary short shoots of *Pinus lambertiana* produce all their foliar organs during the first season (Sacher 1955b), although this is not necessarily true of other pines (Duff and Nolan 1958). Axillary short shoot buds of *Pinus*, however, are not directly comparable to axillary buds of other genera mentioned above. The *Pinus* short shoot is a determinate branch system lacking subapical meristem activity. It normally extends leaves the season after its own initiation. Axillary buds of many other genera are initiated one season, mature in a second, and expand in a third, or later, season. A good general discussion of the origin and development of axillary buds is that of Holthusen (1940).

Consideration of morphogenic cycles of terminal and axillary buds reveals the inadequacy of a whole plant, whole shoot, or whole bud concept of dormancy. The summer bud with a dormant subapical meristem is apt to have an active apical meristem and developing leaf primordia, in the axils of which lateral buds are being initiated by small meristems there. The problems posed by a study of axillary bud formation are, as in the instance of terminal buds, problems of control—control of rate and orientation of cell division, control of cell enlargement, and control of differentiation.

Adventitious Buds

Unlike terminal or lateral buds, adventitious buds arise without benefit of a connection with the apical meristem or tissue recently derived from it. Adventitious buds often appear near wounds or in callus tissue but are not limited to such loci. They may form on stems, hypocotyls, leaves, or roots. Long dormant axillary buds are often mistaken for adventitious buds when they finally become active. This problem has been discussed by Priestley and Swingle (1929), Stone and Stone (1943), and Stone (1953).

Many of the new branches which appear after pruning probably originate from dormant buds already present at the time of pruning rather than from adventitious buds. This is probably also true of root collar sprouts of *Pinus* (Stone and Stone 1954). True adventitious buds, however, do occur and are particularly common on roots of *Robinia pseudoacacia*, *Ailanthus altissima*, and some species of *Rhus* and *Populus*.

Seeliger (1959a, b) studied the developmental anatomy and morphology of adventitious bud formation on cultured roots of *Robinia pseudoacacia*. The buds arise within the pericycle and there is no direct connection between root growth and adventitious bud formation from the roots. The physiology of adventitious bud formation on roots of *Populus tremula* with respect to growth regulating chemicals has been studied by Eliasson (1961). Earlier literature on shoot bud formation in roots was discussed by Beijerinck (1887) and by Priestley and Swingle (1929).

Appearance of presumably adventitious buds on shoots anywhere but in leaf axils or in masses of callus tissue is rare. When they

appear in axils it is difficult to be sure that they are in fact adventitious and not identical with the poorly developed supplemental axillary buds discussed by Sandt (1925). Anatomical aspects of adventitious bud initiation in wound callus and on leaves were discussed by Priestley and Swingle (1929). In callus tissue, buds may arise from superficial cells or from the cork cambium, if present (Simon 1908). Initiation of adventitious buds on shoots of trees beyond the seedling stage may be uncommon. MacDaniels (1953) was unable to force adventitious bud formation in *Malus* scions. All shoots which appeared were from dormant buds.

Once initiated, adventitious buds usually develop into shoots without a dormant period. As the first leaves are formed vascular connections are established between the bud and the vascular system of the parent structure. It appears that substances produced in the bud cause dedifferentiation and renewed cell division in the tissues in the path of the vascular connection to be established.

Physiological Processes in Buds

Buds appear superficially dormant during most of their existence and once dormancy is broken they usually lose their identity as buds and become shoots. But dormancy is, however, often confined to the subapical meristem and does not imply complete inactivity even there. Considerable physiological activity occurs within dormant buds. Geleznoff's (1851) observations showed that growth and development is possible within buds of *Ulmus* and *Larix* even in midwinter, perhaps occurring when buds are warmed sufficiently by the sun. Supporting observations were made by Askenasy (1877) and Küster (1898).

Continued development of reproductive buds in winter has also been reported more recently in both deciduous (Victorov 1943; Tyrina 1958) and evergreen (Duff and Nolan 1958; Gifford and Mirov 1960) tree species and may be common. Cell division and differentiation occurs, of course, in so-called dormant vegetative buds during summer and early fall. It is much less certain that it continues during the winter. In the interpretation of observations it is important to distinguish between rest, quiescence, and correlated inhibition.[7]

It would be very interesting to know in detail how major physiological processes within bud tissues wax and wane or take on completely different aspects as the bud goes through its morphogenic cycle. Such detailed information is not yet available, but Gaümann (1935) has provided an outline of at least some of the gross metabolic changes occurring in *Fagus sylvatica* buds during the year (*see also pp. 78–80*).

Fagus sylvatica buds decline in volume and dry weight during autumn and early winter. This happens because respiratory oxida-

[7] Rest is a type of dormancy maintained by conditions within the affected tissue itself. Rest in buds usually implies a chilling requirement, the fulfillment of which breaks rest. Other types of dormancy are *quiescence*, imposed by unfavorable environmental conditions, and *correlated inhibition*. An example of the latter is inhibition of bud growth by leaves or other buds on the same shoot (*see also pp. 73–76*).

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tion of reserve carbohydrate, mostly in the form of cell wall hemicellulose, is not compensated for by translocation from the twigs. Content of lipid and nitrogenous materials changes little during the same period. By mid-December (in Switzerland) translocation of reserve metabolites from the twigs becomes substantial, and by late January it is sufficient to halt further dry weight loss in the buds. Thereafter buds show a dry weight increase although lipid content continues to decline.

The total dry weight of a twig-bud system decreases, of course, because of respiratory carbon loss even when the buds themselves are gaining. During late winter and spring bud volume increases faster than dry weight. This results partly from an increase in tissue volume because of water uptake and partly from loss of bud compactness. Protein content varies little from January to April, suggesting that cell division is minimal. Bud break and young shoot elongation are accompanied by greatly increased respiration and temporary loss of dry weight, but a gain in fresh weight. The new shoots soon become self-sufficient, and new buds develop upon them.

New buds of *Fagus sylvatica* grow slowly at first, reaching a dry weight of about a milligram by early June. Between mid-June and mid-October dry weight increases about fiftyfold. Maximum growth rate is reached in late September, but growth stops while the leaves are still photosynthetically active. During the period of rapid growth, protein content increases to about 9 percent and lipid to about 2 percent of total dry weight. Reserve carbohydrate content, mostly hemicellulose, sucrose, and glucose, increases considerably during the period of leaf senescence and fall.

Gäumann's (1935) data indicate that winter buds of *Fagus* derive much of their metabolic energy from hemicellulose. Confirmation of hemicllulose oxidation in buds of other species, and determination of the fate of pentoses and other constituents involved, would be of interest.

Buds of many woody species have a well-defined winter rest period during which respiration is considerably slower than during the preceding period of correlated inhibition or during the quiescence which often follows winter rest. In the past some authors have failed to distinguish between the different types of dormancy, but there is general agreement that dormant buds respire slowly, and that a pronounced increase in respiration accompanies bud break (Pollock 1953; Kozlowski and Gentile 1958; Neuwirth 1959).

The mechanisms controlling respiration at a low level during rest remain unknown. Thom (1951)[8] found no evidence that respiration of resting pear buds is controlled by inadequate oxygen permeation through the scales. She reported the RQ[9] of resting buds to be consistently less than unity. Such results are consistent with oxidation of some fat or protein in addition to carbohydrate.

Results of oxygen partial pressure experiments by Pollock (1953) indicate that respiration of *Acer platanoides* buds is severely limited

[8] Thom, Lucy Chan. A study of the respiration of hardy pear buds in relation to the rest period. 1951. (Doctoral Diss. Univ. California, Berkeley.)

[9] RQ refers to respiratory quotient—the ratio of volumes of carbon dioxide produced to oxygen consumed per time unit.

during summer by slow oxygen diffusion through the scales. Scale removal results in a much higher rate. This apparent oxygen deficiency within the buds is most severe during August and September. Carbon dioxide production is high at the same time. An RQ value greater than unity results, suggesting partially anaerobic or fermentative respiration. A possible role in the induction of rest was ascribed by Pollock (1953) to products of such anaerobic respiration. Once rest has been induced, respiration is repressed by some factor other than oxygen deficiency (Thom 1951;[8] Pollock 1953).

Under natural conditions repression in *Acer* is relieved by fulfillment of the chilling requirement. By direct measurement of respiration of primordia from chilled and unchilled *Acer saccharinum* buds, Pollock (1960) found that oxygen uptake rises as a result of chilling whereas it declines with time in primordia from unchilled buds. Chilled primordia are also less responsive than unchilled to 2,4-dinitrophenol, which uncouples respiration from oxidative phosphorylation. According to Pollock, this means that chilled primordia utilize a greater proportion of the total respiratory enzyme capacity than do unchilled primordia (*p. 161 ff.*).

In my opinion evocation of higher respiratory rates by treatment with 2,4-dinitrophenol is evidence against repression of respiration in unchilled primordia by simple inhibition of enzymes at the substrate level or in such systems as the tricarboxylic acid cycle. Metabolic control by mechanisms modulating synthesis and utilization of compounds containing energy-rich phosphate bonds seems more likely. When compounds containing such bonds are utilized in work processes, phosphate acceptors are regenerated. These can again participate in oxidative phosphorylation.

Laties (1957) proposed that the supply of phosphate acceptors can regulate the respiratory rate in the normally coupled system. If this is so, then lack of demand for energy in synthetic processes can result in respiratory inhibition. Thus the question of respiratory inhibition during dormancy may really be one of relative inactivity of energy-requiring processes such as biochemical syntheses and ion accumulation.

Though the work discussed above associates high RQ values with oxygen deficiency, it is possible that active plant meristems normally have RQ values greater than unity because of what Ruhland and Ramshorn (1938) called aerobic fermentation. They postulated that oxygen consumption of dividing cells is always less than that of elongating, differentiating, or mature cells, and that low oxygen consumption is not necessarily a result of oxygen deficiency. The effect which this might have upon buds at various seasons has not been studied.

The possible roles of inhibitors and other regulators of growth and metabolism in control of metabolic processes in buds are discussed later in several separate sections. A discussion of chilling requirements is included with that of nonperiodic temperature effects (*p. 157 ff.*).

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[8] Thom, Lucy Chan. A study of the respiration of hardy pear buds in relation to the rest period. 1951. (Doctoral Diss. Univ. California, Berkeley.)

Shoot Tip Abortion

Inability to Form Terminal Buds

Many common angiospermous trees and shrubs never form persistent terminal buds. Their shoot tips die and are abscised each season. The uppermost surviving axillary buds then become pseudoterminal buds, and growth proceeds from them the following season. Mohl (1844, 1848, 1860), and others before him (see Lubbock 1899), already knew that shoot tip abortion cannot be ascribed to late spring or early autumn frosts and that it is a natural, nonpathological phenomenon. Lubbock (1899, pp. 9–10) had this to say:

> There is a remarkable point about the Lime and some of our other forest trees and shrubs, which Vaucher [Soc. Phys. et Hist. Nat. Genève 1: 296, 1822] seems to have been the first to notice, namely, that the terminal buds die, and that very early. . . . If a branch be examined a little later, it will be found to be terminated by a scar, left by the true terminal bud, which has dropped away, so that the one which is apparently terminal is really axillary.
>
> The same thing occurs in the Elm, Birch, Hazel-Nut, Lilac, Willow, &c. In these and many other species the bud situated apparently at the end of the branchlets is in reality axillary, as is shown by the presence of a terminal scar, due to the fall of the true terminal bud. I have found that even at the end of May the terminal buds of the Lime have almost all died and fallen away.
>
> But why do the terminal buds wither away? In some cases the bud contains a definite number of leaves, but in the genera above mentioned the number is indefinite—more than can come to maturity; and yet the rudiments, which are constructed to produce true leaves, cannot modify themselves into bud-scales. Thus, in the Ash, Maple, Horse Chestnut, and Oak, which have true terminal buds, there are comparatively few leaves; while in the Elm there are about seven, Hornbeam eight, Lime eight, Willow fifteen, and Lilac fifteen.
>
> In the above species it is generally the uppermost lateral bud or buds which develop, but in some cases, as in *Viburnum Opulus* (the Guelder Rose), *Gymnocladus*, &c., these also perish, and as a rule only the lower ones grow, and the upper part of the stem dies back.

Since Lubbock wrote the above, a little progress has been made in understanding shoot tip abortion, but the question of why it happens cannot yet be answered. The unadorned statement, "terminal buds lacking," which occurs in botanical descriptions of many genera [10] of trees and shrubs, glosses over a great deal of interesting physiology. It implies lack of control mechanisms able to direct development into scales of primordia initiated by the apical meristem.

Yet the first series of primordia initiated by axillary meristems on the same shoot do develop into bud scales. A second series of primordia initiated by each axillary bud meristem develops into leaves. What is lacking is the ability to revert the developmental pattern back to scale formation after a series of leaves has been produced (*p. 45*). Finally formation of additional leaves is halted by loss of the entire apex with some of the younger leaves and internodes. Thus apical growth of each shoot is determinate, but growth of the whole shoot system is indeterminate because axillary meristems can produce buds.

[10] A partial list of temperate zone genera follows: *Salix, Betula, Carpinus, Corylus, Castanea, Ulmus, Celtis, Platanus, Gleditsia, Gymnocladus, Robinia, Ailanthus, Rhamnus, Tilia, Diospyros, Syringa,* and *Catalpa*. Abscission of shoot apices occurs in some tropical genera also (Koriba 1958).

Physiology of Apical Abortion

Is it possible that under suitably controlled conditions apical abortion can be prevented in those species which normally undergo it? Wiesner (1889) did some experimental work on the problem, using *Rhamnus cathartica*, and found that abortion of the apex could be prevented by timely removal of lateral buds. Apical growth then continued if plenty of water was supplied.

Later Mogk (1914) studied apical behavior of *Tilia ulmifolia*, in which the apex and several of the youngest internodes are abscised in May (Central Europe). Mogk found no evidence to support the then current ideas that apical abscission was due to severe competition for water and nutrients between the apex and expanding leaves and internodes below. His results led him to suggest that apical regions cease growth and abort because a constitutional change has been induced in them which prevents utilization of available nutrients and water.

Klebs (1917) attempted unsuccessfully to discover the basis of the constitutional changes suggested by Mogk. He was, however, able to maintain growth and prevent abscission of the apices of well-fertilized and watered *Robinia pseudoacacia* seedlings for as long as 10 months by bringing them indoors under continuous artificial light during winter. Klebs concluded that removal of leaves and lateral buds is not necessary to prevent apical abortion when the seedlings are exposed to summer daylight or to continuous artificial light and when water and nutrient supply is optimal.

After development of the photoperiodism concept (*p. 84 ff.*), later workers demonstrated that apical abortion in *Robinia* (Wareing 1954; Wareing and Roberts 1956) and *Catalpa* (Downs and Borthwick 1956a; Downs 1958) can be markedly hastened by short photoperiods and delayed by long photoperiods. Photoperiodism is certainly a valuable experimental tool, but the degree to which it controls apical abortion under natural conditions remains to be determined.

Excision of young lateral buds from shoots may delay apical abortion (Wiesner 1889). Removal of very young leaves may also result in additional leaf development at the apex and delayed abortion, but only if a vigorous shoot is chosen for the experiment (Berthold 1904). Axillary bud removal from developing long shoots of *Cercidiphyllum japonicum* promotes formation of leaves beyond the normal number, but internodes between them gradually become shorter (Titman and Wetmore 1955).

In vigorous shoots of *Syringa vulgaris* destruction of the uppermost axillary buds promotes renewed growth and delays abortion of the apex. Weak shoots give no such response (Garrison and Wetmore 1961). Obviously young leaves and axillary buds do have an influence upon growth at the apex, but this is probably more subtle than mere competition for water and nutrients.

Syringa vulgaris shoot tips put into nutrient culture medium grow for a time and expand a few leaves, but their apices ultimately abort just as those on intact plants. Abortion occurs even though water stress is not a factor and competition for nutrients can hardly be severe. The first step in abortion is not tissue necrosis, but cessation of growth. In the final stages cellular disinte-

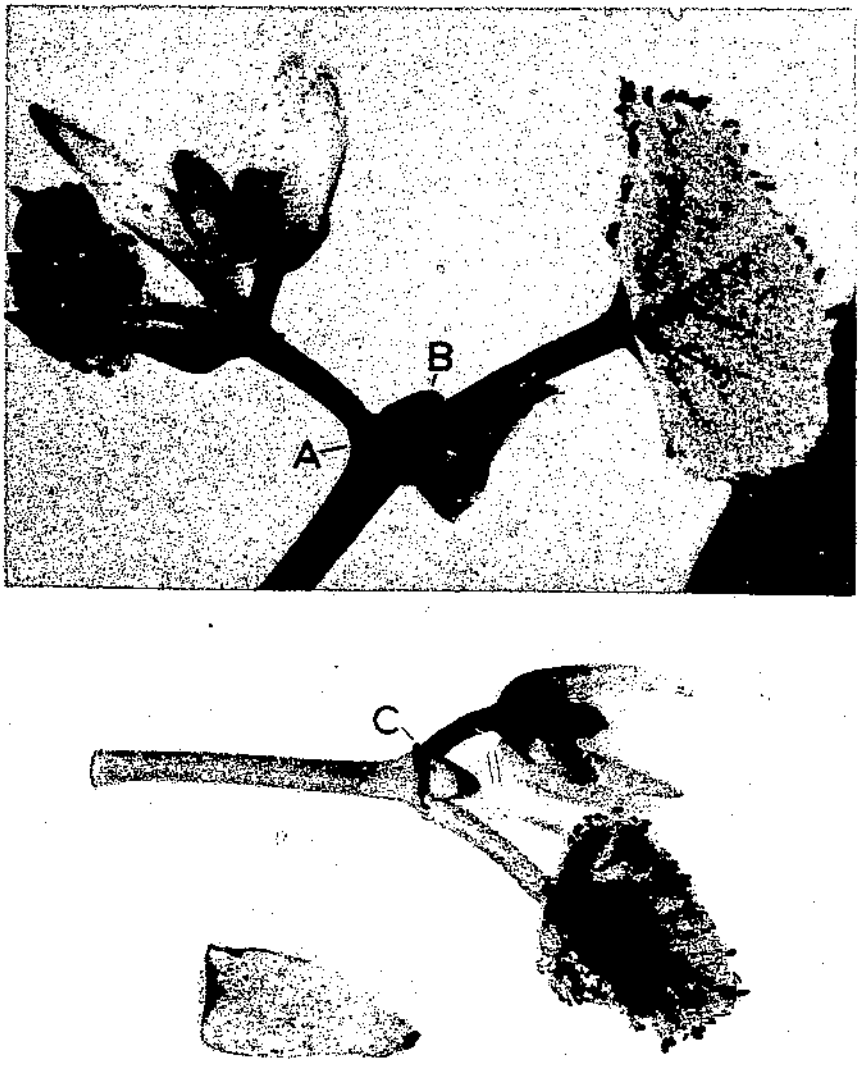

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FIGURE 5.—*Top*, Shoot tip of *Tilia americana* just prior to abortion of the part to the left of *A*. Abscission will occur at *A*, already marked by an abrupt transition from pale yellow above to green below. After abortion the uppermost surviving axillary bud *B* will become the pseudoterminal bud. (Enlarged about 2 X.) *Bottom*, Aborted parts of a *T. americana* shoot. The stipule below was cut away at *C* to improve visibility. The part to the right of *C* includes several small leaves with their stipules and well-developed axillary buds. Total fresh weight of the aborted parts was about 90 mg.; dry weight was about 20 mg. (Enlarged about 2.3 X.)

gration occurs and a cork cambium forms across the axis just above the uppermost pair of lateral buds. The tissue above drys up and eventually falls away (Garrison and Wetmore 1961).

I was able to watch abortion of *Tilia americana* shoot tips near Beltsville, Md., May 22 to 25, 1962. A considerable amount of young shoot tissue is normally aborted by *Tilia*, and this is not the result of water stress (see Mogk 1914). Prior to abortion tips turn yellow but do not wilt perceptibly. Tips collected just after abortion may still have a water content to 75 to 80 percent. The aborted part includes several partially elongated internodes and partially expanded leaves with stipules and plump, well-developed axillary buds (fig. 5).

An abscission layer is formed just above the uppermost surviving axillary bud and the shoot tip drops away while still alive and well hydrated. Seedlings of *Tilia americana* occasionally retain their apices and form persistent terminal buds (Ashby 1962). *Tilia* should provide ideal material for physiological and biochemical study of apical abortion.

Shoot tip abortion is a phenomenon of little practical significance but one of theoretical interest. How did this peculiar method of closing off a season's growth evolve? Or is it perhaps no more peculiar than formation of a terminal bud? What determines the location of the abscission layer or lower limit of abortion? In the terms of Mogk (1914), what constitutional changes prevent utilization of available water and nutrients? These fascinating questions deserve much more attention than they have received so far.

PHYSIOLOGICAL ANATOMY AND DEVELOPMENT IN THE CAMBIUM

Developmental Anatomy

The vascular cambium is derived from procambial cells (*p. 38 ff.*) which did not differentiate into primary xylem or phloem during development of the primary plant body. In gymnosperms and in most woody angiosperms the cambium constitutes a meristematic sheath around stems, roots, and their branches. With few exceptions, the major part of the bulk of a mature woody plant is a product of the cambium.

The anatomy of cambium is quite different from that of apical meristems. Cells in mitotically active regions of apical meristems are relatively small, densely cytoplasmic, and often nearly isodiametric. Cambial cells are larger and are highly vacuolate when active. Two different forms of initial cells, fusiform initials and ray initials, exist in the cambium. Fusiform initials are long and slender, whereas ray initials are nearly isodiametric. Both kinds of initials are usually present at all times, but not in equal numbers.

Microscopic examination of the tangential surface of a sample of cambium will probably reveal one of two basic patterns of cell arrangement. In storied or stratified cambium the fusiform initials occur in horizontal tiers; i.e. groups of cells are arranged side by side with their ends at about the same level. This pattern is characteristic of plants with short fusiform initials. In the second type,

nonstoried or nonstratified cambium, the fusiform initials show a more random arrangement and their ends overlap. This type is more common in plants with long fusiform initials. Intermediate types are also found.

Nonstratified cambium is found in all gymnosperms and in most woody angiosperms. The stratified type is less common and is characteristic only of those dicotyledonous genera usually considered to be most advanced. Examples are *Grewia*, *Kleinhovia*, *Robinia*, *Diospyros*, and *Wisteria* (Bailey 1923). Whatever the arrangement of the vertically elongate fusiform initials, scattered between them are small groups of more nearly isodiametric ray initials. Number, shape, size, and arrangement of these show great variation in different plant groups.

When the cambial meristem is very active, new cells are produced so rapidly that differentiation does not keep pace and several layers of meristematic cells may be present. According to the usage of Bailey (1943), only the initial cells themselves constitute the cambium. This was partly based upon the classical work of Sanio (1873) and Mischke (1890) which supported the idea of a single layer of cambial cells in conifers. In practice, however, it is difficult to distinguish derivatives from initials.

Derivatives often divide periclinally once or several times before they become nonmeristematic and differentiate into xylem or phloem cells (Raatz 1892; Bannan 1951). Because of this, the term "cambium" has also come to mean the zone of meristematic activity including the initial cells and all of the undifferentiated derivative cells (Bannan 1955, 1957a). In this sense it is correct to speak of the undifferentiated derivatives of the true cambial initials as xylem or phloem initials, depending upon their position.

The cambial zone is thickest during the period of most rapid growth. During the autumn and winter months cell division becomes very slow or stops. The xylem and phloem initials, however, continue to differentiate until sometimes only a single layer of undifferentiated cells (the cambial initials) remain between mature xylem and phloem (Esau 1948). In *Larix decidua* the dormant winter cambium is about six rows of cells thick (Knudson 1913), in *Thuja occidentalis* two or three rows (Bannan 1955), and in *Robinia pseudoacacia* three or four rows (Wareing and Roberts 1956).

Divisions do occur among cambial derivatives. It is even possible that sometimes mitotic frequency of the initials may be less than that of derivatives. Yet, derivatives do not usurp the functions of initials. The initial function is retained by only one daughter of an initial cell division, but the polarity of this apparent inheritance is not fixed. Sometimes the phloem-facing and sometimes the xylem-facing daughter retains the initial function. This raises important questions concerning the concept of initials function and its inheritance or control by microenvironment (*p. 21*). An interesting discussion of these problems in cambial and apical meristems was published by Newman (1956).

During the period in which fusiform initials are producing new cells, which differentiate into elements of the vertically oriented vascular tissue, the ray initials also produce new ray cells. These elongate somewhat in the radial direction. The origin and develop-

ment of rays has been treated extensively by Barghoorn (1940a, b, 1941a, b). Bannan (1953) has made more recent contributions.

Most of the divisions of the cambial initials are periclinal (tangential). It is obvious, however, that exclusively periclinal divisions would provide no means for increasing the number of files of initials as the girth of the stem increases. This is accomplished by an interesting mechanism. It involves a small but variable number of anticlinal (actually pseudotransverse or oblique) divisions per file of fusiform initials, usually during the last part of the growing season.[11] The pseudotransverse divisions are almost entirely limited to a single layer of initials (Bannan (1957b). This fact can be used in support of the concept of a single initial layer (Sanio 1873) even though the layer is not always obvious.

Characteristically the pseudotransverse divisions are so acutely oblique that each daughter cell has a long, sharp point. The cells grow in length during autumn (and perhaps early winter) and thrust their points between other cells. According to Bannan and Bayly (1956) there is considerable competition and accompanying mortality during this intrusive growth stage. Apparently the largest cells usually survive, but more basic is the fact that those which have the largest ray contact (often the largest cells) persist and those with little or no contact are crushed or may undergo further divisions and initiate a new ray.

Competition between cells after pseudotransverse divisions may explain why the fusiform cambial initials and their derivatives become longer instead of shorter as trees become older (Sanio 1872; Bailey and Shepard 1915; Bailey 1920). Results obtained by Neeff (1920) with *Tilia tomentosa* indicate that cambial girth growth in roots proceeds via a mechanism similar to that in stems.

The rate of pseudotransverse division of fusiform initials is itself related to the rate of stem growth. In the early years of rapid perimeter growth there are many pseudotransverse divisions, and many of the progeny survive to initiate new files. With increasing age of trees there are fewer such divisions and perhaps also lower survival rates of the daughter cells. These changes are accompanied by a rapid increase in cell length during the early years and a slower rate of increase later (Bannan 1960a, b).

Discussion of the very interesting physiology associated with development of reaction wood from cambial derivatives cannot be undertaken here. This subject has been admirably covered by Gessner (1961). However, the fact that reaction wood forms on the lower sides of branches on leaning stems of gymnosperms, but on the upper sides of similar branches and stems in angiospermous trees, is worthy of special mention. Does this indicate a basic difference in growth control mechanisms between angiosperms and gymnosperms? Further research on the physiology of reaction wood formation in the two groups may be very rewarding.

Aside from any role they may have in the development of reaction wood, mechanical pressure and spatial relationships must be included among the factors controlling normal differentiation of cambial

[11] See Klinken 1914; Bailey 1923; Bannan 1950; Whalley 1950; Bannan and Bayly 1956; Bannan 1960a, b.

derivatives into xylem and phloem. Longitudinal bark tongues of *Populus trichocarpa* and *Pinus strobus* have been separated from the wood in spring and maintained in a humid atmosphere while still attached to intact bark at their acropetal ends. Under such conditions the cambial zone along the inner surface produces parenchymatous callus. But if similar bark tongues are isolated from the wood by a plastic film, while held tightly against it by external mechanical pressure, the cambium produces normal elongate xylem and phloem elements (Brown and Sax 1962).

Morphogenic Cycles in the Vascular Cambium

Cambial activity may sometimes be continuous, though not necessarily uniform in rate, in trees growing where winters are mild (Oppenheimer 1945). But even in the tropics it is more likely to be seasonal or episodic (Koriba 1958). Cambial growth in temperate zone trees is definitely seasonal, and the term "dormancy" may be applied to the state of inactivity usually coincident with the low temperatures and short days of the winter months.

Inception of cambial dormancy is gradual and poorly defined. Its relation to the dormancy status of buds in late summer and autumn is uncertain. Breaking of cambial dormancy, however, is closely related to renewed growth of buds in spring (Ladefoged 1952), and may normally be contingent upon prior breaking of dormancy, at least to the extent of renewed provascular development, in the buds. The physiological aspects of cambial reactivation in spring are discussed in more detail in a later section (*p. 133 ff.*).

In the dormant cambium all cells are narrow in the radial dimension. Radial walls are thick and the protoplasm is dense. In spring, increasing vacuolation, thinning of the radial walls, and an increase in radial diameter results in obvious cell swelling.[12] With these changes the bark becomes peelable. The buds may also swell, but bud break and renewed cell division in the cambium do not necessarily follow immediately. Bark peelability may precede actual meristematic activity by as much as a month (Huber 1948; Wilcox et al. 1956).

The disagreement in the literature as to when cambial activity begins in relation to bud break is undoubtedly partly a consequence of frequent use of bark peelability as a criterion of meristematic activity and failure to recognize the error thereby introduced. The time relations between bud break and inception of cambial cell division were discussed in detail by Ladefoged (1952). Though more work is needed, it seems likely that cambial activity is initiated in the base of the bud and is influenced by conditions within procambial and primary vascular tissue there. In many species there is appreciable primary growth in the embryonic shoot tissues before bud break. These growing tissues may supply the regulators which induce cell divisions in the cambium below.

There can be little doubt that renewed meristematic activity in the cambium is propagated downward along twigs and stems after it is initiated in, or just below, the buds. But how does reactivation

[12] See Bailey 1930; Cockerham 1930; Priestley 1930; Wight 1933; Fraser 1952; Ladefoged 1952.

proceed at any specific level selected for observation? Somewhat surprisingly, the first cambial divisions do not usually occur in the actual cambial initial layer (Bannan 1955, 1962; Grillos and Smith 1959). Partly differentiated cells adjoining the latewood of the preceding season are more likely to divide first. These are called xylem mother cells.

In *Thuja occidentalis* (Toronto, Canada) divisions in the initiating layers do not become widespread until the xylem mother cells have undergone approximately two mitotic cycles (Bannan 1962). Spring initiation of cell division in layers nearest to the mature xylem, if a general phenomenon, is of interest because of its possible relation to supply of growth regulators, water, and nutrients.

Earlywood formation in some species may largely result from repeated divisions of xylem mother cells which were already present in the dormant cambium. In others the xylem mother cells themselves may first be derived from the cambial initial layer. There is probably also considerable variation within species. Intervals between cell divisions are long early in the season. They become shorter during the main vernal surge of xylem formation, but divisions in cambial meristems are generally less frequent than in apical meristems. This may be related to the great length of the fusiform initials. Phragmoplasts of these cells must sometimes migrate several millimeters before cell division is complete. During active growth successive divisions probably occur at 4 to 7 day intervals (Raatz 1892; Bannan 1962).

In spring, frequency of cell division is highest in the xylem mother cell zone and considerably lower in the cambial initial layer and the phloem mother cell zone. By midsummer, however, there has often been a drastic reduction in frequency of division in the xylem mother cells. The zone of these cells is reduced in thickness and new xylem-facing derivatives of the cambial initials divide only once or twice before maturing into tracheids.

The vernal surge and the mid- to late summer lag in xylem production has been well documented.[13] It is probably general. These changes, and accompanying differences in cell wall development have given rise to the popular terms "earlywood" and "latewood." Wood produced during the vernal surge, the earlywood, may account for much of a season's diameter growth. According to Gäumann (1928), *Picea* and *Abies* have produced 95 percent and 86 percent, respectively, of their growth rings by mid-July. Bannan (1955) reported that *Thuja occidentalis* produces 70 to 80 percent of its growth ring by the beginning of July.

The time of transition to latewood production is highly variable within species (Bailey and Faull 1934) and between species (Eggler 1955; Gäumann 1928; Ladefoged 1952). Possible causes of the transition are discussed in a later section (*p. 137 ff.*). Extent of latewood production also is variable (Lagefoged 1952; Studhalter 1955; Bannan 1962) and shows influences of local climatic conditions. Unusual environmental conditions can cause reversion to earlywood formation and production of so-called false rings (Glock

[13] See Buckhout 1907; Korstian 1921; Gäumann 1928; Fowells 1941; Daubenmire 1945; Fraser 1952; Ladefoged 1952; Eggler 1955; Fritts 1958; Grillos and Smith 1959; Bannan 1962.

1955). Such reversion may also accompany development of lammas shoots (Späth 1912).

Phloem cells are derived from cambial initials following divisions in which the initial function is retained by the xylem-facing daughter cell. There is no evidence of a vernal surge of phloem production. Variability is great, but it is likely that phloem production begins later than that of xylem and continues at a rather steady rate for the remainder of the season (Rees 1929; Esau 1948; Fraser 1952; Bannan 1955; Grillos and Smith 1959). Phloem elements formed late in fall may not mature until the following spring (Strasburger 1891; Elliott 1935; Ladefoged 1952; Grillos and Smith 1959). It is possible that some of the older phloem is also functional in spring (Raatz 1892; Huber 1939; Esau 1948).

Cambial meristems, because of their less complex anatomy and physiology, may offer readily available experimental material for the study of some growth control processes in woody plants. However, methods must be devised to allow circumvention of traumatic effects induced by sample taking. A further difficulty is that cultures of cambial cells tend to produce only masses of callus. Present techniques do not allow one to expect normal differentiation into xylem and phloem (*pp. 40–41, 68*).

PART II. EPISODIC GROWTH AND DORMANCY OF SHOOTS

CONCEPTS, NOMENCLATURE, AND DEFINITIONS

The Dormancy Concept and Its Development

A completely accurate definition and delineation of dormancy is difficult to achieve. In common usage of the term "dormancy," without reference to causal factors, means a temporary suspension of visible growth and development. Thus the annual rhythm of higher plant development, resulting in spring and autumn changes in aspect of the landscape, is thought of as an alternation of a period of growth and development with a period of inactivity or dormancy. The actual situation is, however, much more complex.

Not all parts of the plant are dormant at the same time. Correlation between root and shoot activity is often indistinct, and several levels or types of dormancy or growth may prevail within the organs of a single twig or bud at the same time (*pp. 49–58*). The apical meristem may be inactive during the period of most rapid shoot elongation in spring (Kemp 1943; Sacher 1954). Cambial growth may continue into the autumn, after the shoot seems to be dormant in other respects (Priestley 1930). In late summer when the new buds appear dormant their subapical meristems are, indeed, inactive in the sense that internodes are not elongating, but intiation and development of primordia may continue (Kemp 1943; Millington and Gunckel 1950). Growth and development of embryonic axillary buds within the seemingly dormant older buds may continue during most of the winter while outward appearances of general dormancy are maintained (Küster 1898; Chandler and Tufts 1934; Bell 1940).

The term "dormancy" is useful in general discussions concerning annual rhythms of activity, but it does not adequately define specific physiological states or conditions as they exist in the several potentially meristematic areas during various seasons. A more specific terminology is needed. For example, if twigs of *Tilia* are brought into a warm greenhouse immediately after leaf fall, the buds will not open for many months in spite of favorable conditions, but if twigs from the same plant are taken indoors in mid- or late winter they will sprout almost at once (Molisch 1922).

Another example is provided by the old German tradition of taking cherry twigs indoors on St. Barbara's Day (December 4). If kept in a warm room the cuttings will flower by Christmas Day. However, twigs brought indoors in November rather than December frequently will not open their flower buds at all (Molisch 1922). Evidently the kind of dormancy prevailing in flower buds early in winter is different from that prevailing later. Leaf buds of many woody species behave similarly (Howard 1910). This behavior is

explicable in terms of a chilling requirement which must be satisfied before bud break can be induced by mere exposure to warmth and light (*p. 157 ff.*).

Why do axillary buds and young terminal buds usually grow out after branch defoliation in early summer, but not after natural or experimental defoliation in late summer or fall? Why does a large fraction of the axillary buds remain dormant even under the most favorable conditions for growth (*pp. 81–89*)? Such behavior also is understandable only if several physiological types of dormancy exist and if control mechanisms involve reactions more complex than mere reception of and response to stimuli provided by the immediate external environment.

Shoots may sometimes become dormant, in the sense that they cease elongating and form terminal buds, and then break dormancy again, even though the environment is continuously suitable for growth. This phenomenon, along with observations such as those mentioned above, led to controversy as to whether dormancy was primarily autonomic (also autogenic) or aitonomic (also aitogenic). Autonomic dormancy was presumably controlled by internal factors whereas aitonomic dormancy was induced and controlled by environmental factors.

This controversy was very active during the last decades of the 19th century and the opening decades of the 20th. Grisebach (1872) took the extreme position that the yearly cycle of growth and development in plants is controlled by its heredity and that environmental stimuli are suppressed whenever their indications do not serve the plants well being. Askenasy (1877) took the opposite position that growth and dormancy are controlled by mechanisms responding to external conditions. By the turn of the century there was considerable doubt that autogenic dormancy was a fixed hereditary property because of increasing evidence that relatively constant external conditions could interfere with the normal cycle of growth and dormancy.

It became of interest to observe behavior of temperate zone trees in the relatively uniform climate of tropical highlands. For example, the plant geographer, Schimper (1903) pointed out that *Quercus pedunculata* and *Liriodendron tulipifera* transplanted to the botanic garden at Tjibodas at about 5,000 feet elevation in western Java appeared to be growing as evergreens. Actually each twig continued to show alternate growth and dormancy, but not in synchrony with other twigs. The periodicity or autogenic dormancy of the plant as a whole was lost. Because of accumulating evidence against strictly hereditary control over dormancy, Pfeffer (1903) took the intermediate position that buds appear to have an inherent rhythm which can, however, be modified by environmental conditions.

Much of the literature arising from the controversy mentioned above had little lasting value. An exception is the work of Klebs (1911 to 1917). His extensive work on the role of environmental factors in growth control led him to postulate that dormancy was, indeed controlled by environment, but only indirectly as a result of interactions of genetically determined processes within and the environment without. Consequently, he believed that dormancy could be prevented if one had complete control of the environment.

In some of his work, discussed later (*p. 90*), Klebs came very close to discovering the great importance of the photoperiod in dormancy control. He also suspected the importance of the spectral quality of light. Klebs had an outlook which today would still be almost modern.

The discovery of photoperiodism, a long unappreciated environmental variable, as a potent factor in control of growth and development; the detection of naturally occurring biochemical growth regulators; the development of the concept of endogenous rhythms; these were breakthroughs which overshadowed the old controversy concerning the relative importance of genetic versus environmental factors in regulating episodic growth and dormancy. It became obvious that the environment is very complex and that changes in many of its component factors are detectable by genetically determined biochemical mechanisms within the plant. The many implications of these advances must be discussed separately, but it can be said here that the concept of shoot dormancy has become only a little less vague and unsatisfying.

Kinds of Dormancy—Definitions

In spite of some progress, confusion and vagueness about the nature and meaning of dormancy is still present. This is in part due to nonstandardization of nomenclature. Some authors have not distinguished between types of dormancy. Others have introduced new and specific terms. Some have assigned new and limited meanings to old terms. Here the nomenclatural situation is examined (table 1) and those terms adopted which show signs of gaining wider acceptance and which appear least likely to cause confusion and inconvenience.

Doorenbos (1953) used the term "dormancy" in its widest sense to apply to "any case in which a tissue predisposed to elongate does not do so." This usage was followed by Wareing (1956), Richardson (1958a), and others. This is equivalent to the general use of *Ruhe* by Molisch (1922) and other German writers. The usage of Doorenbos (1953) is adopted here.

The simplest type of dormancy, or failure of predisposed tissue to grow, is that of inactivity imposed directly by cold, drought, etc. Growth is resumed as soon as environmental conditions are again favorable, there being no internal mechanisms to prevent it. Dormancy of this type was called *erzwungene Untatigkeit* by Johannsen (1913), *unfreiwillige Ruhe* by Molisch (1922), quiescence by Samish (1954), and imposed dormancy by Doorenbos (1953). The term "quiescence" is used here. It is considered to be entirely synonomous with "imposed dormancy."

Dormancy which is not the result of the immediate external environment has been called *freiwilliger Ruhe* (Molisch 1922), physiological dormancy (Richardson 1958a), and rest (Chandler 1942; Samish 1954). Tissues in which such dormancy prevails may be predisposed to grow, and the external environment may be propitious, but growth cannot proceed because of unfavorable internal physiological conditions. Such physiological dormancy is of two types depending upon whether the unfavorable conditions have their origin in the dormant organ itself or are imposed by influences or

TABLE 1.—*Nomenclature of dormancy*

Reference	Approximate definitions and equivalence of terms		
	Dormancy imposed by the environment—no internal control	Physiological dormancy	
		Dormancy imposed by agents or conditions within the plant, but outside the dormant organ	Dormancy maintained by agents or conditions within the organ itself
Johannsen (1913)	*erzwungene Untatigkeit*	*Ruhe* (divided into 3 time phases: *Vorruhe, Mittelruhe, Nachruhe*)	
Molisch (1922)	*unfreiwillige Ruhe*	*freiwilliger Ruhe*	
Stiles (1950)	imposed rest	spontaneous rest	
Curtis & Clark (1950)	(no recognition of different types of dormancy, rest and dormancy considered synonomous)		
Doorenbos (1953)	imposed dormancy	summer-dormancy	winter-dormancy
Samish (1954)	quiescence	correlated inhibition	rest
Wareing (1956)		summer-dormancy	winter-dormancy
Richardson (1958a)	imposed dormancy	physiological dormancy	
		summer-dormancy	winter-dormancy
Kramer & Kozlowski (1960)		temporary dormancy	permanent dormancy
This review	quiescence	correlated inhibition	rest

agents emanating from some other organ on the same plant. For example, lateral buds are presumably held dormant by substances produced in terminal buds or in leaves.

The mechanism of such control may be very indirect, but the ultimate control does not lie within the lateral buds tnemselves and dormancy cannot usually be broken by treatments limited to the dormant buds. More systemic treatments are required. Conversely, dormancy may be maintained by conditions within the dormant organ, as is commonly the case with winter buds having unsatisfied chilling requirements. This dormancy cannot be broken by systemic treatments from which the dormant organ is shielded. It must itself be treated.

Doorenbos (1953) used the terms "summer-dormancy" and "winter-dormancy" to differentiate between the two types of physiological dormancy. Wareing (1956) also recognized the need for such a distinction. "Summer-dormancy" is in large part synonomous with the term "correlated inhibition" (Samish 1954). The term "correlated inhibition" is used here. That type of dormancy which is maintained by conditions within the dormant organ itself and which can usually be overcome by adequate cold treatment is the "winter-dormancy" of Doorenbos (1953) and is included in the concept of "rest" by Chandler (1942) and Samish (1954). The term "rest" is used herein in the narrow sense, indicating a type of physiological dormancy maintained by factors or conditions within the dormant organ itself.

The scheme of nomenclature employed here is summarized below:

Dormancy.—A general term for all instances in which a tissue predisposed to elongate (or grow in some other manner) does not do so. (After Doorenbos 1953.)

Quiescence.—Dormancy imposed by the external environment. Synonomous with the term "imposed dormancy" as used by Doorenbos (1953). (After Samish 1954.)

Correlated inhibition.—A type of physiological dormancy maintained by agents or conditions originating within the plant, but not within the dormant organ itself; includes "summer dormancy" of Doorenbos (1953). (After Samish 1954.)

Rest.—A type of physiological dormancy maintained by agents or conditions within the organ itself. Synonomous with "winter dormancy" (Doorenbos 1953) and "rest" in its narrow sense as used by Samish (1954).

It is important to recognize that the three kinds of dormancy differentiated above may overlap in time and that all may exist in turn within the same organ. In summer a bud may be held dormant by influences of leaves or more apically situated buds. It is then in a state of correlated inhibition. Removal of leaves in summer may allow the buds to grow out. Approach of autumn is accompanied by a gradual transition of buds of many species into rest which is not usually broken by mere removal of leaves or superior buds. Duration of rest is extremely variable. In some species it may not exist at all. In many species rest is broken by the cold of early winter and the buds then are merely quiescent until the external environment becomes permissive of growth in spring.

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The nomenclature discussed above is the result of realization that different kinds or levels of dormancy do exist. It is useful in a general analysis of physiological problems related to episodic growth and its control. But this nomenclature should be regarded only as a temporary expedient because its inadequacies are already evident. It assumes dormancy to be a property or condition of the whole bud without recognizing that several types of meristems exist within it, and that the same kind of dormancy does not necessarily prevail in all at the same time. More detailed physiological studies are prerequisite to the development of a more satisfactory system of nomenclature.

ALTERNATE GROWTH AND DORMANCY

Implications of Episodic Growth

Episodic rather than continuous growth is almost universal among woody plants. Periods of rapid shoot elongation alternate with periods of little or no elongation. Whereas one might expect growth to be continuous under favorable conditions, especially in young plants. this is true of only a minority of species even in the tropics (Klebs 1911, 1912; Koriba 1958). Most tropical trees grow in flushes, often more than one per year (Klebs 1915; Quetel 1939). *Camellia sinensis*. the tea plant, may exhibit as many as five flushes per year in northeastern India (Wight and Barua 1955). Woody plants of the temperate zones also seldom grow continuously throughout the warm months. Many mature trees show shoot elongation during only a few weeks in spring and early summer. Young individuals may grow continuously for longer periods, but a common response to highly favorable conditions is production of a second, and even a third, growth flush by precocious shoot expansion from recently formed buds (Späth 1912).

In the organogenic region of the shoot apex, initiation of primordia proceeds at a much less erratic rate than elongation of internodes between primordia. Following initiation, development of primordia usually occurs in such a way that a series of scales follows a series of leaves and vice versa. If growth is to be continuous, development of primordia must be controlled so that scales are not formed or do not accumulate. In addition meristematic activity must persist in the subapical region. Continuous growth requires a delicate balance between initiation and development of primordia and elongation of internodes.

Is it possible that substances produced in maturing leaves. particularly when those leaves are close to the apex, are operative in promoting scale differentiation and inhibiting internodal elongation? Leaves certainly can prevent development of their axillary buds (*pp. 82-83*). It is not unreasonable to look at maturing leaves on growing shoots as sources of regulators which may influence development at the apex. When conditions favor continued growth, maturing leaves are some distance below the apex and the latter may be outside their sphere of regulatory influence. However, when

stress conditions prevail,[14] or when temperature or photoperiodic regimens are unfavorable, retarded elongation growth results in leaf maturation closer to the apex.

Unfavorable growth conditions could thus favor increased foliar control over apical development. In my opinion, this thinking offers a way of interrelating several sets of otherwise seemingly unrelated dormancy-inducing conditions. This line of thinking may be helpful only with reference to those species in which growth is theoretically indeterminate, i.e., not limited to the number of leaves and internodes preeixsting in the bud (*apply, for example, to behavior of Weigela florida, p. 94, and Cornus florida, p. 95*).

Some woody species can be forced to grow continuously for many months by exposing them to artificially extended photoperiods or to continuous light and suitable temperature conditions (Klebs 1914, 1917; Dostál 1927; Bałut 1956; Downs and Borthwick 1956a). But it is not certain that dormancy could be postponed indefinitely by such treatment. Bałut (1956) found that continuous uniform conditions of light and temperature result in eventual death of young *Fagus sylvatica* and *Abies alba* trees. He regards a dormant period as essential to normal ontogenesis.

Bałut's idea, I believe, merits consideration. It is not at all uncommon for primordia developing continuously to produce leaves which are morphologically different from those developed discontinuously (*pp. 43–44*). Is there any reason why continuous development could not induce biochemical changes as well as morphological ones? If such changes are possible, on what basis can we deny that some of them could be potentially lethal?

The dormancy prevailing between successive flushes in the same season and immediately after the last flush may be correlated inhibition of buds by leaves. How this inhibition is overcome in instances of natural production of additional growth flushes beyond the first is not yet known, but it can usually be broken artificially by defoliation. In late summer and autumn, however, defoliation is no longer effective because the buds have entered rest and remain dormant after correlated inhibition is lifted (see Molisch 1908–1909).

Shoots of woody plants of the temperate zones generally exhibit a definitely periodic growth cycle, including physiological dormancy different from correlated inhibition of buds by leaves. This is the rest period, the induction of which may be influenced by leaves, but which, in many species, is broken by exposure of the buds to low temperatures. Exceptions, of course, exist. *Sequoia sempervirens* does not form a typical dormant bud structure and is reportedly only quiescent during winter (Sterling 1945a). Other species which do form dormant winter buds may, nonetheless, lack rest periods. Dormancy in these is only quiescence imposed by an unfavorable environment. Examples are *Spiraea sorbifolia* (Howard 1910) and *Weigela florida* (Downs and Borthwick 1956b).

Until about 1925 lack of general recognition of the photoperiod as a significant factor in the natural environment resulted in much confusion in the literature concerned with the relation of low tem-

[14] Readers specifically interested in the role of water stress in growth inhibition and dormancy induction are referred to Zahner (1962).

perature and other factors to episodic growth. Variations in length of the photoperiod follow an annual cycle which is regular and predictable. This is a source of strong periodic stimuli outside of the tropics. In the tropics seasonal fluctuations in the photoperiod are small to nil and are probably not an important regulator of episodic growth.

Temperate zone trees grown in the tropics at altitudes where moderate temperatures prevail throughout the year may lose their overall periodicity but maintain a nonsynchronized episodic growth in the various branches (Schimper 1903; Dingler 1911; Coster 1926; *p. 72*). In such cases neither low temperatures nor unfavorable photoperiods can be responsible for dormancy between growth flushes. It is more likely that relations between leaves and apices on the same shoot are of significance (*p. 76*). Any general conclusions about the nature of episodic growth and dormancy must be consistent with the behavior of native and temperate zone plants growing in the tropics also.

Episodic growth implies existence of a state different from the growing state. In the context of the present discussion that state is the dormant state. Buds, containing the dormant shoots, are characteristic features of the dormant state. Subsequent sections largely concern the manner in which a growth episode is begun by renewed growth in a dormant bud and the way in which it is ended by formation of a new terminal bud or by apical abortion (*p. 62 ff.*).

Associated Anatomical and Cytological Changes

Without reference to the specific kind of dormancy prevailing or to the manner in which it is controlled, it may be said that dormant meristematic tissues frequently are anatomically and cytologically different from similar tissues in the active state (*see also pp. 59–61*). According to Swarbrick (1927) and Priestley (1930) protoplasts of cells within meristematic tissues contract during dormancy and assume an opaque appearance and gel-like properties distinctly different from the translucent sol state characteristic of active tissue.

Genkel' and Oknina (1948) reported that protoplasts of dormant cambial cells of *Betula*, *Pinus*, and *Taxus* are contracted into rounded globules each covered by a visible lipoidal layer. Plasmodesmata are ruptured when this occurs. In spring the protoplasts again swell and the plasmodesmata are reestablished (Oknina 1948). In the opinion of Meeuse (1957) these observations are plausible. The plasmodesmaducts in the cell walls probably remain intact during dormancy and again become filled with protoplasmic strands before growth is resumed.

The lipoidal layer mentioned by Genkel' and Oknina (1948) may under some conditions be an essential part of the dormant protoplast. Kydrev (1959) stressed the importance of fats in the ability of wheat embryos to return to dormancy after germination has begun. Reportedly, cells not containing significant amounts of

lipids are not able to reenter dormancy under unfavorable growing conditions. Seedling roots lose their fats, and their ability to return to dormancy, very quickly; hence they are quite intolerant of drought etc. Actual shrinking of protoplasts away from cell walls may not be associated with shoot dormancy in all species. Genkel' and Oknina (1948) were unable to confirm it in *Juglans regia*, and numerous authors who have examined dormant cambium do not mention it. A complicating factor is the possibility that some of the observed changes are more closely related to frost hardiness than to dormancy per se.

Mention is frequently made in the literature of a relation between bound water and dormancy. Bound water changes are detectable during induction and breaking of rest in some buds, but this has contributed little to our understanding of dormancy control. In the view of Bünning (1953, pp. 44–45) hydration of the protoplasm by increasing amounts of bound water during the late summer is a factor in inducing rest. This point was also discussed by Samish (1954).

Some of the cytological changes physically associated with winter shoot dormancy are not limited to the potentially meristematic tissues. The chloroplasts of many conifers undergo aggregation during winter and reappear as individual bodies again in spring. This subject had already been discussd by several authors before the turn of the century (see Pfeffer 1900, p. 335).

Lewis and Tuttle (1920, 1923) examined the leaves of *Picea canadensis* during the rigorous winter at Edmonton, Alberta, and found a distinct localization of mesophyll cell contents around the nucleus. The identity of individual chloroplasts was completely lost and the major part of the cell was occupied by a fat-filled vacuole. All starch had disappeared in early autumn. In early April distinct chloroplasts quickly reformed and cells soon displayed their normal summer appearance. Enclosing the twigs in lightproof bags in spring did not prevent or delay conversion of fat to starch or reappearance of individual chloroplasts. This suggests temperature as the controlling factor.

The work of Ryanstev in 1930 (cited by Vasil'yev 1961, p. 169) with *Pinus*, *Cedrus*, *Juniperus*, and other genera near Molotov, U.S.S.R., also suggested that changes in conifer chloroplast condition are temperature controlled. The shift of chloroplasts from the aggregated condition in the nuclear region to the normal summer distribution occurred each time winter twigs were brought indoors for 15 to 48 hours. Subsequent exposure to temperatures between +1° and –4° C. for 20 to 48 hours induced a return to the winter condition.

The importance of temperature is likewise indicated by Parker's (1957) report that *Pinus monticola* leaves obtained from a heated greenhouse in midwinter contained normal summer type chloroplasts whereas they were absent in leaves of the same species outdoors at the same time. According to more recent work by Genkel' and Barskaya (1960), however, low temperature alone is ineffective in inducing the change from summer to winter chloroplast condition

in *Picea excelsa* leaves. Photoperiod and light intensity may also be involved.

A detailed study of seasonal changes in chloroplast condition and arrangement in *Pinus cembra* and *Picea excelsa* near the Alpine timberline was published by Holzer (1958). Chloroplasts of these species are oriented along the cell walls in summer. After several frosts in autumn the plastids collect in groups, around the nucleus in *Picea*, but in folds or bays of the cell membrane in *Pinus*. Holzer believes that chloroplasts retain their identity throughout the winter, though they are aggregated. Bringing plants into a heated room results in a return to summer conditions and active photosynthesis in about 8 days. Holzer (1958) also discussed low temperature induced changes in cell protoplasm.

Pinus strobus chloroplasts apparently behave similarly to those of *P. cembra*. They collect in folds of the cell membrane in winter, but by means of electron microscopy can be shown to retain their identity. In winter, too, the protoplasmic reticulum becomes more extensive and appears to enmesh mitochondria as well as chloroplasts (Parker and Philpott 1961).

The relation, if any, between the state of dormancy in meristematic tissues and cytological changes in nonmeristematic cells is unclear. The whole subject of seasonal anatomical and cytological changes needs additional study with recognition that the concept of whole plant or whole shoot dormancy is inadequate. If cytological changes are related to dormancy at all they are probably related to a specific kind of dormancy in a specific type of meristem, otherwise they may be independent responses to environmental stimuli.

ANALYSIS OF THE CONTROL PROBLEM

Internal Physiological Factors

Why Summer Growth Inhibition?

Many temperate zone tree species undergo only one flush of growth per season, though under unusual conditions the newly formed buds may open and produce a second flush. Other species under favorable conditions frequently exhibit two or more flushes per season (Späth 1912; Klebs 1914; Wareing 1949; Kraevoi and Eskin 1957). Subapical meristems of both groups become dormant temporarily, as between flushes, or more permanently, as at the end of a single flush; and this entry into dormancy often occurs in early summer while environmental conditions are seemingly still highly favorable for growth.

During and after shoot elongation the apical meristems of such plants continue to initiate primordia, but primordial development is modified and internodal growth arrested so that a bud is formed. What prevents further elongation growth when conditions seem to be favorable? This is the basic problem in the physiology of episodic growth. It raises further questions which physiologists have attempted to answer in various ways (*p. 76*).

Possible Root Influences

Does rapid stem elongation after bud break use up the supply of a root-produced stem-growth hormone such as the caulocaline postulated by Went (1938)? Went explained the observed effects of optimal nutrition in prolonging stem growth (Klebs 1911) as being the indirect result of greater production of caulocaline in the roots and transport to the stem. By the same argument the observed increased and prolonged growth of remaining branches after heavy pruning would be expected because of less dilution of available caulocaline. There exists, however, evidence that some stems can grow without attached roots (Skoog 1944; Loo 1945), though such growth is much less than normal. Went (1951) explained these as exceptions in which some caulocaline is synthesized in the stem itself. Howell and Skoog (1955) found that growth stimulation of pea epicotyls in vitro by adenine and coconut milk required the presence of roots. This supports the hypothesis that a stem growth factor is produced in the roots. Caulocaline has not been isolated, and its existence as a specific hormone is still speculative.

Kraevoi and Eskin (1957), after studying the multiple growth flushes of *Quercus rubra*, suggested another way in which roots might induce temporary dormancy in shoots. They found that episodic shoot growth was accompanied by episodic root growth with shoot flushes lagging slightly behind root flushes. Nucleic acid content was high just before bud break and low when growth ceased. This led them to postulate control of nucleic acid synthesis in shoots by root-produced hormones. The latter were not identified. This does not aid in explaining episodic growth. It merely transfers the problem to the roots. Like Went's caulocaline hypothesis it must be considered speculative until more evidence is available.

Correlated Inhibition and Apical Dominance

Do leaves produce substances which inhibit shoot elongation? The idea that they do gains support from the long known and often demonstrated fact that terminal and axillary buds can usually be made to open precociously by defoliating the branch in spring or early summer. This happens naturally when insects or hailstones defoliate trees. It may be argued that lateral buds are inhibited by terminal buds rather than by leaves, but this does not change the problem. Terminal buds also seem to be inhibited by leaves.

Goebel (1880) was able to cause axillary buds to grow out by removing leaves but allowing the terminal bud to remain. Nonetheless, he found that the terminal buds still had some inhibitory effect. Such effects have been confirmed by Sandt (1925) and Dostál (1909, 1926, 1927). Dostál noted that after removal of the shoot apex, the leaves still prevented axillary buds from growing as rapidly as those of defoliated controls.

Dostál (1927) also grew seedlings of *Fagus sylvatica* and *Quercus pedunculata* under continuous light and constant temperature and studied the effects of various additional treatments upon length of alternate periods of growth and dormancy. He concluded that episodic growth in a constant environment is not under control of

the roots but is greatly affected by position, size, and number of leaves on the shoot. Dostál interpreted dormancy between growth flushes as being a result of foliar inhibition of growth of primordial leaves and of the internodes between them. These ideas are still plausible.

The physiology of correlated inhibition of buds by leaves is inextricably entwined with that of apical dominance. Divergence of views has been prominent among those seeking causal explanations of these phenomena. One view is that inhibition is caused by deficiency of nutrients for which the meristems compete, with the possibility that the most active region somehow directs nutrient flow toward itself. Another view is that hormonal substances are produced in shoot apices, which after translocation inhibit the growth of lateral buds below.

Early opinion favored an undefined secretion, hormone, or inhibitor as the effective agent (Errera 1904; Dostál 1909, 1926), but some opposition to this idea developed. Loeb began a study of the subject with a hormone hypothesis in mind, but he abandoned it after very systematically investigating the nutrition effects. In a summarizing book Loeb (1924) maintained that inhibited buds are not inherently dormant and can grow if sufficient nutrients are available to them. Subsequently the work of Snow (1925, 1929, 1937) again strengthened the case for hormonal control. Snow suggested the existence of a nonauxin, lateral bud growth inhibitor and relegated auxin itself to a minor role.

The observation that apical buds usually have a higher auxin content than lateral buds, and that removal of apical buds is followed by growth of laterals, led Thimann and Skoog (1933, 1934) to the discovery that application of sufficient auxin to the stumps of decapitated shoots can prevent growth of lateral buds as effectively as intact apical buds. This poses the enigma that auxin appears to inhibit lateral bud growth and yet has no apparent effect upon apical buds in which it is present in even higher concentrations.

Went (1936) attempted to allay the confusion by combining hormonal and nutritional control in the suggestion that the apical bud, by virtue of its high auxin content, is somehow able to divert to itself essential nutrients and hormones, including caulocaline. This, however, does not satisfy the objection that direct application of auxin to lateral buds may also result in inhibition.

Ferman (1938) modified Went's hypothesis by suggesting the active agent to be an auxin precursor rather than auxin itself (see also Libbert 1955). Thimann (1937) proposed that lateral buds have much lower auxin concentration maxima for growth than have apical buds. But this necessitates explaining why buds should so differ because of their position. The possibility that sensitivity differences to growth substances between lateral and apical buds may exist was demonstrated by Naylor (1950) in experiments with maleic hydrazide.

The auxin theory of correlated inhibition and apical dominance is still supported by some workers (Wickson and Thimann 1960), though others have been quite critical of it. Champagnat (1955), for example, studied the problem in woody plants and found that lateral buds in *Syringa* are inhibited by mature leaves poor in auxin.

Apical buds rich in auxin have little effect. Jacobs et al. (1959) stated definitely that apical dominance in *Coleus* is not controlled by auxin from the apex.

The nutritional aspects of the problem were again brought to the fore by Gregory and Veale (1957). Their position differs from that of Loeb (1924) in that auxins too are given a role, specifically that of controlling development of the vascular strands. High auxin levels in the stem are envisioned as preventing formation of functional vascular elements leading to lateral buds, thus indirectly depriving them of nutrients.

Booth et al. (1962) have interpreted experimental data as indicating auxin-directed transport of nutrient materials to young growing regions and suggested that such directed transport may be a factor in apical dominance and correlated inhibition of buds. Another approach is that of Libbert (1962) who believes correlated inhibition to be maintained by an inhibitor produced in green leaves and roots. Hydrolysis products of the inhibitor may include auxin (Libbert 1955).

Loeb's (1924) position that correlatively inhibited buds could grow if sufficient nutrients were available to them has been given new significance by some recent findings. Kinetin has been successfully used in breaking correlative inhibition of buds (Chvojka et al. 1961; see also Engelbrecht and Mothes 1962). According to Mothes (1961) this effect of kinetin is related to its ability to promote accumulation of solutes, including auxin, by cells. If this is so, kinetin can be a most important agent in the control of correlated inhibition by virtue of a role other than its supposedly classical one of regulating cell division (*p. 146 ff.*).

It must be emphasized that the physiology of correlated inhibition is still largely obscure. More detailed discussions are given by Söding (1952, 1956), Gregory and Veale (1957), Audus (1959), Jacobs et al. (1959), Libbert (1961), and Mothes (1961).

Correlated inhibition is a kind of dormancy. Its induction and subsequent breaking may be responsible for episodic growth when unfavorable environment is not a direct factor. Correlated inhibition is different from the more profound dormancy, here called rest, which prevails in fall and early winter in many species. Yet there is no sharp demarcation between the two types, only a gradual transition in time. One way in which this transition is illustrated is in differing response to experimental defoliation as the season progresses.

Commonly defoliation early in the season results in rapid opening of buds which would otherwise have remained dormant until the next growth flush. Late in the season such buds are much less responsive to defoliation (Molisch 1908–1909; Jesenko 1912, Späth 1912). The dormancy prevailing in late summer and fall seems to be of a different type. It is not dependent upon the presence of leaves, nor is it necessarily induced by lack of water or available nutrients. Some other factor controls rest induction, and that factor is not the low temperatures of fall and early winter (Coville 1920; Weber 1921). The photoperiod, a long unappreciated environmental variable, in many instances seems to be the missing factor.

Experimental Control of Growth and Dormancy in Various Species

An Introduction to Photoperiodism in Woody Plants

Many woody plants are able to perceive the progressively longer nights and shorter days of late summer and autumn as an environmental condition different from that prevailing earlier. They respond in ways which indirectly result in a kind of dormancy more profound than correlated inhibition. Species differ widely in their response to various photoperiodic conditions. Ecotypic and individual differences within species are also noticeable.

The importance of the length of the daily light and dark periods in controlling growth characteristics and time of flowering of many herbaceous species has been generally recognized since publication of the classical work of Garner and Allard (1920, 1923, 1925).[15] However, the idea that decreasing day length or increasing night length in late summer might be an important factor in inducing rest in woody plants was slow in gaining wide recognition.

The work of Klebs (1914), which showed that the usual winter dormant period of *Fagus*, *Quercus*, and *Fraxinus* could be prevented by continuous electric illumination, was not extended to include the effects of dark periods in dormancy induction. Garner and Allard (1920, 1923) were aware of earlier work showing the effects of continuous light or darkness upon plant development, but they did not directly follow the lead opened by Klebs (1914) with regard to dormancy in woody plants.

Garner and Allard (1923) quite independently discovered that *Liriodendron tulipfera* when greenhouse grown throughout the winter under extended photoperiod conditions does not enter rest. Continuous light is not necessary to maintain growth. On the basis of this, and of more thorough knowledge of photoperiodic effects upon herbaceous plants, Garner and Allard (1923, p. 905) stated the following:

> In general, exposure of annuals to the optimal illumination period for flowering tends to induce rapid senescence and death. In the same way exposure to certain definite day lengths causes perennials to enter into a state of dormancy. Deciduous trees and shrubs, in which the laying down of resting buds on the stem precedes leaf fall, enter into a form of dormancy involving a temporary weakening, but not complete loss, of capacity for apogeotropic functioning. Herbaceous perennials enter into a form of dormancy in which there is more complete loss of apogeotropic function. In both cases there is loss of leaves and photosynthetic activity is mostly suspended. That the first-named type of dormancy may be prevented by maintenance of a relatively long illumination period is shown by experiments with tulip poplar (*Liriodendron tulipifera*) described in the preceding discussion of abscission and leaf fall. That the second type of dormancy also may be prevented by maintaining a long illumination period has been shown in experiments with *Aster linariifolius*.

The great interest in photoperiodic control of development and flowering in herbaceous plants, however, overshadowed the above mention of dormancy prevention by long photoperiods and it received little attention. Summers (1924) in his detailed analysis

[15] For general accounts of the discovery and development of photoperiodism see Kellerman (1926) and Murneek (1948). Later developments have been reviewed by Parker and Borthwick (1950) and, with special reference to woody plants, by Wareing (1956) and Nitsch (1957b).

of factors governing bud formation did not mention photoperiodic effects, but concluded that the rest period was not necessarily correlated with mean temperature relations or variations in food reserves. He recognized that some other responsible factor might have been left out of consideration. Gradually a few workers in the field of woody plant growth recognized the significance of photoperiodic effects and provided a foundation of experimental work.[16]

The literature concerning photoperiodism in woody plants has been reviewed by Wareing (1949, 1956) and by Nitsch (1957b). Vaartaja (1962) has discussed ecotypic variation of photoperiodism in trees and suggested that photoperiodic control may be more significant in northern than in southern trees.

From available experimental evidence it is possible to draw the generalization that a regimen of long photoperiods and short nyctoperiods promotes vegetative growth whereas the reciprocal condition tends to inhibit growth and induce dormancy. However, there are many exceptions to this generalization. Nitsch (1957b), following a proposal by Chouard (1946), grouped woody plants as follows:

	Class
I. Long days prevent the onset of dormancy:	
1. Short days cause dormancy—	*Example*
a. Long days cause continous growth	*Weigela*
b. Long days cause periodic growth	*Quercus*
2. Short days do not cause dormancy	*Juniperus*
II. Long days do not prevent the onset of dormancy	*Syringa*

Nitsch (1957b) also collected information from many sources and published a table in which over a hundred species of trees and shrubs were classified according to the above scheme. Because of the various methods and criteria used by different authors, Nitsch considered many of the classifications tentative. It must also be noted that no universally accepted nomenclature of dormancy exists, and that many authors have not specified the type or localization of dormancy they induced or postponed by photoperiodic treatments. Nevertheless, the fact that some species fall in each of Nitsch's classes makes it impossible to predict the behavior of the many species which have not yet been studied.

Schemes such as the above can be criticized for their distraction from "natural" classification of woody species first of all into groups on the basis of the growth potential inherent in the embryonic shoot within the bud. In many species the number of leaves and internodes to be expanded during the vernal growth flush is predetermined by the number existing in the winter bud. Scales for the next bud may already be present (*p. 49 ff.*). In other species, seeming potential for continued growth is cut short by apical abortion early in summer (*p. 62 ff.*). In these two groups, long photoperiods do not generally prevent induction of dormancy following the first growth flush. Long photoperiodic treatments of seedlings of some of these species may, however, greatly prolong the vernal growth flush and delay apical abortion (*pp. 92–94*).

Long photoperiods may also sometimes induce newly formed buds to open and produce a second growth flush. In still other species

[16] Bogdanov 1931; Moshkov 1930, 1932, 1935; Gevorkiantz and Roe 1935; Kramer 1936; Bulgakova 1937; Phillips 1941.

growth of shoots is basically indeterminate, being neither limited to primordia and internodes present in the bud, nor abruptly terminated by apical abortion. In the latter group opportunities for demonstration of photoperiodic control over shoot elongation and bud formation are greater. Generalizations must be cognizant of such species differences if they are to be realistic and useful.

Experimental inhibition of stem elongation and promotion of bud formation by short photoperiods does not imply that photoperiodic conditions necessarily have such controlling influence in natural environments. Whereas formation of buds or induction of winter rest can be hastened by subjecting trees to short days and long nights, in many species elongation ceases and buds are formed while photoperiods are still near their summer maximum (Wareing 1949, 1956). The natural role of photoperiodism in control of elongation growth and terminal bud formation remains to be determined. Evidence for its involvement in rest induction is perhaps somewhat stronger.

Demonstration of photoperiodic responses in experimental environments does not prove their importance in natural environments. Likewise, lack of response to photoperiod in an experimental system does not imply a similar lack under natural conditions. In some species photoperiodic responses are limited to a specific temperature range. High temperatures (Vegis 1953, 1955) as well as low temperatures may prevent expression of the responses. In spite of all the exceptions and uncertainties it is probably safe to assume that the mechanism allowing detection of changes in photoperiod-nyctoperiod relationships is widely distributed among woody plants. Certainly such a mechanism is present in members of 34 genera listed by Wareing (1956) as showing photoperiodic sensitivity with respect to extension growth.

Further generalization is not profitable at this time. An appreciation of the complex physiology involved and the variability of responses can best be gained by considering the behavior of several species which have been investigated in some detail. Such considerations follow.

Pinus sylvestris

In the development of the *Pinus sylvestris* seedling, emergence and elongation of the hypocotyl is followed by a rosette stage. This is the result of an initial lag in internodal elongation between cataphylls (primary leaves). After perhaps 2 months internodes between basal leaves of the rosette do begin to elongate, keeping pace with, but not overtaking, development of new leaves at the apex. Thus an apical rosette is maintained. This manner of growth is finally ended by formation of a terminal bud in the center of the rosette.

Bud formation implies a change in developmental pattern of primordia and inhibition of internode elongation between them. This first post-embryonic bud is formed de novo. It was not predetermined in the embryo. However, growth during each subsequent season is largely predetermined by the number and type of primordia present in the buds which open that season (Wight 1933). It should be noted that the seedling leaves are chlorophyllous cata-

phylls of determinate growth, whereas the paired needle leaves characteristic of older shoots are borne on the lateral short shoots in the axils of cataphylls (which may or may not be green). The short shoot needles have a relatively much longer growth period than the cataphylls.

First year seedlings of *Pinus sylvestris* respond to photoperiodic stimuli (Wareing 1949, 1950a; Karschon 1949; Downs and Borthwick 1956a). Short photoperiods alternating with long nyctoperiods [17] induce early cessation of extension growth and formation of a terminal bud. Long nyctoperiods exaggerate the rosette type of development by inhibiting stem elongation while allowing formation of additional primordia. When seedlings are grown under a range of photoperiod-nyctoperiod regimens, maximum stem elongation and leaf number are attained when 20 hours of light alternate with 4 hours of darkness. *Salix babylonica* and *Pyrus ussuriensis* also require nyctoperiods of at least 4 hours for maximum growth (Moshkov 1932).

In *Pinus sylvestris* nyctoperiods longer or shorter than 4 hours cause reduced leaf number and reduced stem growth. The introduction of a daily 4 hour nyctoperiod into a continuous light regimen results in increased stem elongation and leaf number. The effects of a second similar nyctoperiod, separated from the first by 8 hours of light, are additive. Conversely, the inhibitory effects of long nyctoperiods are greatly reduced by median interruption with a short photoperiod (Wareing 1950a).

Pinus sylvestris seedlings have a terminal rosette during active growth. Under long nyctoperiod treatment appearance of new leaves and internode extension seem to stop simultaneously. Under 4-hour nyctoperiods elongation of internodes at the base of the rosette continues for a time after new primary leaf formation has stopped and a terminal bud is obvious; thus fewer leaves remain in the rosette. In general, longer nyctoperiods result in more leaves remaining in the rosette. Wareing (1950a) interpreted this to mean that the apical meristem (initiation of primordia) and subapical meristem (stem elongation) have independent responses to photoperiodic conditions. This should not be surprising. Numerous microphenological studies referred to earlier revealed that dormancy and activity of apical and subapical meristems are not necessarily synchronous (*pp. 43–53*).

New leaf formation appears to cease prior to terminal bud formation, but it is only development of primordia which is altered. The apical meristem continues to initiate primordia. A series of these develops into bud scales. Later cataphylls bearing primordial short shoots in their axils are initiated within the bud. The problem of localizing and characterizing dormancy is again evident. Under natural conditions in summer and early fall it is mainly stem

[17] Most of the literature on photoperiodism is concerned with growth and flowering of herbaceous species which have been classified as long-day and short-day plants. Consequently the term "day length" has been used very extensively. However, it is now well known that it is not the day length but the uninterrupted dark period length which is the more influential factor both in the control of flowering and vegetative growth. Thus the term "long day" really implies "short night" and "photoperiod" implies a complementary "nyctoperiod." The latter term is used herein wherever accuracy demands.

and leaf elongation which is inhibited within the new bud. Other types of growth and development may continue.

Downs and Borthwick (1956a), using *Pinus sylvestris* from a Swedish seed source, obtained most nearly continuous growth (implying approximate synchrony between apical and subapical meristems) under 14-hour photoperiods. Plants under 16-hour photoperiods grew just as tall but less steadily. Of course, provenance differences are to be expected (Wassink and Wiersma 1955; Langlet 1944), as are differences due to temperature conditions during the experimental period.

When *Pinus sylvestris* seedlings are grown under natural photoperiods, but with 25-foot candles of light applied from sunset to sunrise, induction of dormancy is actually hastened. Length of stem under this regimen is greater than normal because internodes are much longer. Leaf number is less because initiation of primordia is not accelerated and bud formation begins sooner. The continuous light promotes stem elongation so much that the terminal rosette is eliminated. The presence of elongating leaves and internodes very close to the apex may be related to induction of bud scale formation (*p. 76*). Terminal buds formed under such continuous light conditions are much smaller than normal (Wareing 1951a). If the continuous light regimen is maintained, these small buds may soon open and produce a second growth flush. However, according to Balut and Żelawski (1955) the characteristics of the second flush are definitely abnormal. Detailed information on the anatomy of the buds is not available.

Pinus sylvestris in the second and subsequent seasons of growth is likewise responsive to photoperiodic conditions. But the number of leaf and stem units appearing in a growth flush is already predetermined in the bud. There is usually no elongation of newly formed internodes after all the bud-borne internodes have been expanded. Thus, lack of same-season photoperiodic effect upon number of leaves and internodes expanded by second-year seedlings is understandable. Long nyctoperiods are again effective in reducing internode extension and leaf length. Wareing (1950b) published evidence that the effect of photoperiodic conditions upon stem growth is a direct one and is not mediated through the leaves. This is consistent with Karschon's (1949) observation that *Pinus sylvestris* hypocotyls are responsive to the photoperiod while the cotyledons are still within the seed.

Wareing (1950b) also found that photoperiodic treatment of older leaves alone has little effect upon the growth of new shoots. Nonetheless, presence of older leaves seems essential to normal bud break and shoot development. The basis of this is not clear, particularly since it is not necessary that the older leaves be illuminated. Long nyctoperiods inhibit stem elongation and may induce dormancy within a few weeks. It does not necessarily follow that continuous darkness would have the same effect. Active extension of the shoot can occur in the dark if the older leaves are intact. The meristematic condition of the apical region after several weeks of continuous darkness has not been studied.

Activity of *Pinus sylvestris* cambium also may be under indirect photoperiodic control. In the second and later years of their

growth, the trees complete elongation of new shoots in June, whereas cambial activity continues until late October (in England). By means of a 15-hour photoperiod regimen Wareing (1951a) was able to extend cambial growth considerably, but not to prolong it indefinitely. Induction of cambial dormancy was also somewhat hastened by long nyctoperiod treatment, but the effect was slow to develop.

Initiation of cambial activity in spring is preceded by some shoot growth, but completion of extension growth in June is not accompanied by cessation of growth in the cambium (Wareing 1951a). It is not certain whether development within the new buds actually continues as long as cambial activity. Wareing concluded that photoperiodic control over cambial growth is exerted independently of other growth phenomena. Perception presumably occurs in the needles.

The normal winter dormancy of *Pinus sylvestris* includes a rest phase which is broken by exposure to cold. Trees kept in a heated greenhouse all winter usually show delayed sprouting in spring. Cold treatment during the winter facilitates early spring growth. Continuous light may overcome the correlated inhibition of newly formed buds in summer before rest has been induced by the long nights of late summer and fall. It may also help in overcoming inhibition of sprouting in spring due to unsatisfied chilling requirements. Continuous light, however, is only slightly effective in breaking dormancy in fall after development has ceased and rest has set in (Wareing 1951a).

In simplified summary, extension growth, leaf growth, and cambial activity are prolonged by long photoperiod and short nyctoperiod conditions, whereas growth cessation, bud formation, and dormancy are promoted by reciprocal conditions.

Fagus sylvatica

Jost (1894), in a series of experiments with *Fagus sylvatica* saplings, found that bud break can be delayed by withholding light. Individual branches can be kept dormant throughout the summer by enclosing them in lightproof boxes. When returned to normal photoperiods in August they remain dormant until the following spring. When whole trees are kept in darkness only a few buds break dormancy. These expand a few short internodes, then form new buds. Some may exhibit second and third flushes of the same type.

On the basis of such observations Jost concluded that light is a major factor in controlling bud dormancy of *Fagus sylvatica*, but that correlated inhibition is also involved. By means of a glass-walled CO_2-free cabinet, Jost showed that inhibition of normal bud opening by darkness is not due to lack of CO_2 assimilation. He concluded that photoreactions other than those of photosynthesis are of importance in controlling *F. sylvatica* bud dormancy. Jost (1894) noted the behavior of this species to be atypical. Most other species he tested developed long etiolated shoots in darkness, although only a few formed viable buds on the shoots. MacDougal (1903) in his studies on etiolation found that *F. americana* buds also fail to open in complete darkness.

Klebs (1914, 1917), as part of a very extensive study of the growth habits of *Fagus sylvatica*, grew young trees under continuous light for several months and observed almost continuous stem elongation and production of new leaves. When trees were transferred from continuous light to greenhouse conditions in winter, growth ceased and dormant buds were formed. When returned to continuous light the trees again began to grow, even if they were leafless at the time of transfer. Klebs also found that removal from continuous light to outdoors in May was not followed by formation of dormant buds.

These results can now be interpreted in terms of seasonal differences in duration of the daily dark period. Klebs believed dormancy and episodic growth to be controlled by environmental factors as well as by nutrient and water supply. In interpreting these experiments he was on the verge of discovering photoperiodic control of dormancy. Nevertheless, he failed to make the critical deductions and attached importance to total length of illumination and its intensity rather than to the daily light-dark cycle.

Dostál (1927) confirmed and extended the results reported by Klebs. He grew *Fagus sylvatica* and *Quercus pedunculata* under continuous light at 21° C. Dostál reported that if competing buds were excised and young leaves removed from the leader as they appeared, then growth at the apex was so nearly continuous that no bud scales were formed between successive flushes. Dostál (1909, 1927) realized the importance of correlated inhibition in inducing bud formation and dormancy in spite of constant external environment.

Kramer (1936) first provided evidence of photoperiodic control over maintenance and breaking of dormancy in beech, using *Fagus grandifolia*. Resumption of growth in spring was hastened by long photoperiods and retarded by long nyctoperiods. Wassink and Wiersma (1955) prolonged the growth of *F. sylvatica* in fall by using 16-hour photoperiods. Under their conditions onset of dormancy was postponed, but not prevented.

Wareing (1953, 1954) made a detailed study of the photoperiodic responses of *Fagus sylvatica*. He could find no evidence to support Klebs' idea that total duration of light exposure was the operative factor in bud break. Instead, his results indicate that the response of dormant buds is controlled by length of the nyctoperiod rather than the photoperiod or the total illumination time. It is not long days which are important, but short nights. Dormancy can be broken under a regimen including short days if the accompanying long nyctoperiods are nullified by dividing them into two with short-light periods. Bud break of *F. sylvatica* will also occur if growth promotive cycles are alternated with dormancy-inducing cycles. In contrast, photoperiodic induction of flowering in herbaceous species does not usually occur under such alternation of cycles.

Response of leafless plants to photoperiodic conditions raises the question of locus of perception. Experiments involving scale removal led Wareing (1953) to believe that the locus of perception is in the tissue of the primordial shoot within the bud. Even if only one percent of the incident light of a normal day penetrates the scales, intensities within will still be within the range of photoperiodic effectiveness. Similar perception and response may occur

in buds of *Betula pubescens*, but probably not in *Acer pseudoplatanus* (*p. 96 ff.*) or *Robinia pseudoacacia* (*p. 92 ff.*).

The type of dormancy characteristic of *Fagus sylvatica* in winter is different from that of many other woody species. The buds have no chilling requirement. Chilled buds have no inherent advantage over unchilled buds in spring (Klebs 1914; Wareing 1953). In fact, greenhouse plants may grow faster than outdoor plants if temperatures are low after natural photoperiodic conditions have become favorable for growth. Though there is no chilling requirement, the early winter dormancy seems to be a type of rest. It is more profound than mere quiescence induced by low temperature. Klebs (1914) found that potted plants put under continuous light in early September sprouted in 10 days. Those transferred in mid-November required 36 to 38 days, but those brought in in late February again sprouted in 10 days.

The dormancy of winter buds of *Fagus sylvatica* can readily be broken by continuous light, but according to Klebs (1914) they do not respond readily to warm water, ethylene, or similar agents which are effective with many other species. Howard (1910), who did not use continuous light, also found *F. sylvatica* very difficult to force. Weber (1916a) claimed to have broken dormancy in December with acetylene and concluded that light was not the limiting factor. Gassner (1926) reported success with hydrocyanic acid. More recently Thorup (1957) broke bud dormancy even in early autumn with a mixture of ethylene chlorhydrin, ethylene dichloride, and carbon tetrachloride.

The fact that dormancy can be broken by unphysiological chemical treatment does not detract from the evidence that photoperiodic conditions are very important in the natural regulation of dormancy in the species. A possible clue to the kinds of mechanisms lying between perception of photoperiodic stimuli and control of growth arises from the work of Lona and Borghi (1957). They were able to induce sprouting of dormant *Fagus sylvatica* buds in spite of short photoperiods by treating with gibberellic acid (*pp. 140–145*).

Redington (1929a, b) reported that *Fagus sylvatica* seedlings grown in continuous artificial light for 5 months were much larger and better developed than those grown under 16-hour photoperiods. In contrast some of the other species studied made almost no growth after the first few months under continuous light. After the first season, however, seedlings may require some darkness for normal growth.

Balut (1956) grew *Fagus sylvatica* seedlings under continuous light and constant environmental conditions for many months. Seedling growth was much prolonged but terminal buds were finally formed. If uniform conditions were maintained the plants were soon again forced into growth, but development was abnormal and death followed. Similar results were obtained with *Abies alba*. Balut concluded that periodic changes in the environment are necessary for some species, because the dormant condition they induce is essential to important steps in plant ontogenesis (*p. 77*).

As an experimental plant *Fagus sylvatica* offers several interesting features. Both induction and breaking of dormancy are to a large extent photoperiodically controlled. Rest is not related to

TB 1293 (1963) USDA TECHNICAL BULLETINS UFDAIA
MERISTEMS, GROWTH, AND DEVELOPMENT IN WOODY PLANTS: AN ANALYTICAL
ROMBERGER, J.A. 2 OF 2

a chilling requirement and is not readily broken except by long photoperiod or continuous light regimens or by chemical treatment. Bud dormancy may be maintained for long periods by withholding light. The species deserves continued study.

Robinia pseudoacacia

Robinia pseudoacacia is one of a considerable number of hardwood tree species which have a sympodial growth habit. Shoot apices are aborted each season and true terminal buds are not formed (*pp. 62–65*). Klebs (1917), however, was able to maintain growth and prevent apical abortion of well-fertilized seedlings for as long as 10 months by giving continuous artificial light during the winter.

The effect of photoperiodic conditions upon the growth of *Robinia* was first clearly illustrated by the work of Moshkov (1930, 1932, 1935), who studied *R. pseudoacacia* and other trees planted north of their normal ranges near Leningrad. Maximum summer day length there is 20 hours. Under field conditions *R. pseudoacacia* did not abort its apices and did not become dormant, but continued to grow until killed by frost in autumn. If photoperiods were shortened artificially by covering the trees with boxes, growth ceased earlier, plants became dormant, and survived the winter.

Bogdanov (1931) and Kramer (1936) confirmed photoperiodic effects upon dormancy induction in *Robinia pseudoacacia*. The work of Phillips (1941) demonstrated not only a response to artificially extended days but also to wave lengths of supplementary light used. Red light was almost twice as effective as blue light. This effect is now understandable in terms of the reactions of the photoperiodic receptor pigment, phytochrome (*p. 106 ff.*).

Beginnings of understanding of the photoperiodic responses of *Robinia pseudoacacia* came with the work of Wareing (1954) and Wareing and Roberts (1956). They found that after seedlings had been made dormant by exposure to 9- to 10-hour photoperiods for a month, subsequent treatment of the leafy plants with continuous illumination for 59 days failed to break bud dormancy. Similar behavior was observed with *Acer pseudoplatanus*, although *Betula pubescens* and *Fagus sylvatica* responded with renewed growth.

A plausible explanation is that photoperiodic perception by the dormant bud tissues of *Robinia pseudoacacia* is very slight. There are no terminal buds, and the lateral buds are hidden beneath the petiole bases. Correlated inhibition of lateral buds by leaves is maintained even in continuous light. Photoperiodic perception by leaves seems to be overriding in growing plants also. Growth is halted and apical abortion induced when leaves are given long nyctoperiods even if the apex itself is continuously illuminated. Conversely, when the apex is under long nyctoperiods and the leaves in continuous light, perception by the leaves is again overriding and dormancy is averted. Thus in *R. pseudoacacia* photoperiodic response is mediated primarily through mature leaves (Wareing 1954).

The extension growth of mature *Robinia pseudoacacia* trees is frequently completed before midsummer. Seedlings, however, may grow for much longer periods (Klebs 1917; Wareing 1949). In

older trees correlated inhibition of shoot growth by leaves may induce apical abortion before photoperiodic conditions become limiting as judged by the behavior of young trees. Whatever the reason for different responses of young and old individuals to the same photoperiodic conditions, it is important to remember that most of the literature is concerned with the behavior of seedlings or young transplants. Deductions made on this basis are not necessarily applicable to mature trees.

Cambial activity in seedlings of *Robinia pseudoacacia* is also influenced by photoperiodic conditions, but there is no direct synchrony between responses of apical and cambial meristems. Cambial activity depends upon exposure of leaves to long photoperiod conditions. By placing plants under long nyctoperiod conditions for several weeks extension growth may be stopped and apical abortion induced. Upon return to long photoperiods cambial growth is often maintained or resumed without renewed extension growth. Meristematic activity at shoot apices is not essential to cambial growth in the stem (Wareing and Roberts 1956). This must also be true of such species as *Tilia americana* which abort their apices very early in the season (*p. 65*).

Catalpa bignonioides

Catalpa bignonioides, like *Robinia pseudoacacia*, has a sympodial growth habit. The end of a growth flush is marked by apical abortion and is thus easy to recognize. The species is very responsive to photoperiodic treatment and appears to be a good experimental plant though it has not been widely used.

Downs and Borthwick (1956a) kept seedlings of *Catalpa bignonioides* growing continuously for a year under 16-hour photoperiods. The plants were 3 m. tall at the year's end. Others grown for a year under 8-hour photoperiods were only 5 cm. tall. The intensity of the artificial light used to extend the natural photoperiod need not be high. The effect is definitely a photoperiodic one and not related to total available light. Results, however, are quite different depending upon whether incandescent or fluorescent lamps are used.

Stem elongation is much less with fluorescent lamps although the number of nodes is not reduced. This indicates differences in response between apical and subapical meristems. Downs and Borthwick (1956a) attributed morphogenic differences elicited by fluorescent versus incandescent lamps to the far-red component of the spectrum which is much stronger in light from incandescent sources.

A few weeks under a regimen of 8-hour photoperiods and 16-hour nyctoperiods will cause *Catalpa bignonioides* to cease stem elongation and abort its apices. If the plants are then immediately transferred to a reciprocal regimen, growth is quickly resumed from axillary buds. However, continuation of the long nyctoperiod treatment for several weeks more than necessary to induce apical abortion increases the difficulty of breaking axillary bud dormancy after return to long photoperiod conditions. The buds are apparently in a state of rest, not merely one of correlated inhibition.

Several weeks of cold treatment will break this rest, after which long photoperiods are again effective in promoting growth. Plants beyond the seedling stage seem to enter rest more readily and are less responsive to long photoperiods afterward (Downs and Borthwick 1956a).

Catalpa bignonioides plants made dormant by long nyctoperiod treatment often retain their leaves for several months. Removal of leaves, however, does not change the requirement for chilling and long photoperiods to break dormancy of lateral buds (Downs and Borthwick 1956a).

Weigela florida

Weigela florida var. *variegata* is very sensitive to photoperiodic conditions. Internode length is greatly reduced by short photoperiod regimens. For example, Downs and Borthwick (1956b) found that under photoperiods of 8, 12, 14, and 16 hours mean internode lengths were 3.7, 9.3, 24.4, and 29.8 mm., respectively. When plants are transferred from long to short photoperiod conditions, reduced growth rates are noticeable within 2 weeks. Nevertheless, the apical meristem continues to initiate additional primordia. Under short photoperiods, however, primordial development is altered so that several pairs of primordia develop into bud scales rather than leaves.

Within a sheath of scales, apical meristem activity continues and a terminal bud complete with embryonic shoot is formed (*p. 45*). Such buds, produced in response to long nyctoperiods, can be maintained in a dormant condition by long nyctoperiods. But this dormancy is quickly and easily broken by long photoperiods. There is no need for cold treatment. In this respect *Weigela florida* differs from *Pinus sylvestris* and *Catalpa bignonioides* and is like *Fagus sylvatica*.

The dormancy maintained in *Weigela* by long nyctoperiods appears to be an inhibition imposed upon the subapical meristem by the leaves. Plants with dormant buds, which have been treated with 8-hour photoperiods for as long as 3 months will show renewed growth within a few days if completely defoliated. This occurs even if the dormancy-inducing treatment is continued. Growth, however, is quite limited because the new leaves soon become large enough to act as photoperiodic receptors, whereupon they somehow inhibit internodal elongation and induce formation of a new terminal bud.

If dormant, leafless plants are put under a regimen of photoperiods of 14 hours or longer, growth begins and will continue for long periods. Under natural conditions *Weigela* leaves are abscised in fall after terminal buds have formed and abscission is not followed by renewed growth. Presumably winter dormancy in *Weigela* is quiescence imposed by low temperature and is not due to physiological conditions within the buds (Downs and Borthwick 1956b).

Photoperiodic control of vegetative growth in *Weigela florida* is thus mediated by foliar mechanisms which perceive the stimuli and produce hormonal or other factors which, in turn, control stem

elongation and development (but not initiation) of primordia. It is noteworthy that Bukovac and Wittwer (1961) were partially successful in breaking bud dormancy in *Weigela* with gibberellins A_1 and A_3 after inducing it with 9-hour photoperiods.

Waxman (1957), working with *Weigela florida* clone Eva Rathke, obtained results (discussed by Nitsch and Nitsch 1959) which fully confirm those of Downs and Borthwick (1956b) and further indicate that growing leaves ½ to ¾ their mature size are most effective as receptor organs in the photoperiodic control of vegetative growth and dormancy.

Cornus florida

Cornus florida responds rapidly to photoperiodic stimulation. Stem elongation of rapidly growing plants may be halted completely by 2 weeks of photoperiods shorter than 12 hours (Waxman 1957; Nitsch and Nitsch 1959). In addition, primordial development is altered so that bud scales instead of leaves are produced (*p. 45*). Apical meristem activity within the enclosing scales is not inhibited and a terminal bud is formed.

In experiments with decapitated plants of *Cornus florida* Waxman (1957) found that the uppermost pair of leaves alone, when exposed to short photoperiods, could strongly inhibit development of axillary buds. But under long photoperiods there was no such inhibition. This behavior could be explained by the production of varying types or amounts of growth regulators under long and short photoperiods.

Waxman (1957) grew *Cornus florida rubra* plants under 9-, 12-, 15-, and 18-hour photoperiods for almost a year, then (beginning in November) exposed them to the natural photoperiods of winter (Ithaca, N. Y.) at 5° C. minimum temperature. Growth, if any, ceased and leaf abscission followed. Buds opened in May. Those on the plants which had previously been grown under short days opened first. But, though they started earliest, the 9-hour photoperiod plants of the previous year produced only about one-sixth as much growth as the 18-hour photoperiod plants.

Nitsch and Nitsch (1959) have interpreted Waxman's results as indicating overwinter storage of growth promoting substances produced by leaves under long photoperiods during the previous season. It was assumed that the leaf produced growth regulators involved in the induction of dormancy by short photoperiods were destroyed by a low-temperature-mediated mechanism during fulfillment of the chilling requirement. This interpretation implies synthesis of both stem growth promoters and inhibitors in leaves.

Waxman (1957) reported some preliminary attempts to learn what differences in growth substance production might exist in tips of *Cornus florida* grown under different photoperiods. Extraction, chromatography, and assay revealed striking differences. Long photoperiods induced formation of several substances promoting growth of *Avena* coleoptiles. Extracts of tips from plants under short photoperiods were lower in promoters and higher in inhibitors.

Rhus typhina

Two weeks of short photoperiods are sufficient to halt stem elongation of *Rhus typhina*. Application of gibberellic acid to stem tips of plants under short photoperiod treatment is effective in preventing drastic reduction of elongation. The same treatment, however, also increases growth of plants under long photoperiods.

Extraction and assay of *Rhus typhina* stem tips has revealed greatly reduced levels of endogenous auxins after 2 weeks of short photoperiods. But tips treated with gibberellic acid showed high endogenous auxin levels in spite of short-day treatment. Thus whenever active growth is maintained by long photoperiods or by gibberellic acid treatment, rather high levels of endogenous auxin can be found.

Growth cessation is accompanied by a decline in growth promoter and a rise in growth inhibitor content (Nitsch 1957a; Nitsch and Nitsch 1959). Such behavior is consistent with the hypothesis that a photoperiodic receptor mechanism in the leaves influences production of regulators which, in turn, control growth at the stem tip. Cotyledons of *Rhus typhina* lack some of the photoperiodically controlled mechanisms of true leaves. Growth of seedlings is not inhibited by short photoperiods until the first pair of true leaves has been expanded (Nitsch 1957a).

Acer pseudoplatanus

The photoperiodic conditions to which mature leaves of *Acer pseudoplatanus* are exposed have a great influence upon the behavior of the apex. If the apices are given long photoperiods and the leaves short ones, dormancy is induced almost as rapidly as if both are given short photoperiods. Conversely, exposure of apices to short photoperiods and mature leaves to long ones induces some reduction in internode length, but dormancy does not result. Likewise, exposure of apices of defoliated plants to short photoperiods does not induce dormancy (Phillips and Wareing 1958, 1959).

The behavior of *Acer pseudoplatanus* is in contrast to that of *Betula pubescens*, in which dormancy can be induced by short photoperiodic treatment of the apices even when the leaves are receiving long photoperiods (Wareing 1954). *Betula*, of course, differs from *Acer* in that *Betula* aborts its apices upon induction of dormancy and does not form terminal buds (*p. 62 ff.*). However, *Robinia pseudoacacia* also enters dormancy by apical abortion, but its response to photoperiodic treatment of leaves is probably more like that of *Acer* than that of *Betula*. This points out the futility of attempting to generalize about mechanisms of photoperiodic response of woody plants on the basis of present information.

Photoperiodic conditions to which leaves of *Acer pseudoplatanus* are subjected determine whether they have greater or lesser inhibitory effects upon growth of shoot apices. An important effect of exposing leaves to long photoperiods or continuous light may be suppression of growth inhibitor synthesis which presumably otherwise occurs during long nyctoperiods. This is in agreement

with some interpretations of photoperiodic responses in *Pinus sylvestris* (Wareing 1951a).

Phillips and Wareing (1958) were able to demonstrate presence of a growth inhibitor in *Acer pseudoplatanus* apices throughout the year. The inhibitor is presumably synthesized in the leaves and translocated to the apices where it accumulates, especially during the late summer and autumn. During winter there is some decrease in inhibitor content of buds, which may be the result of chilling. The assay method used did not reveal the involvement of auxin.

Further work (Phillips and Wareing 1959) demonstrated that inhibitor level is influenced by photoperiodic conditions. Plants under short photoperiods contain more inhibitor in mature leaves and shoot apices than do similar plants under long photoperiods. After transfer from long to short photoperiod regimens, increases in inhibitor level can be detected after only 2 to 5 days, before any marked effect upon elongation rate is evident. This lends weight to the suggestion that the high growth-inhibitor level accompanying short photoperiod treatment is a cause of reduced elongation growth and not a result of it.

Further studies of *Acer pseudoplatanus* are to be encouraged, particularly in view of the background of anatomical information already available. Especially noteworthy is the detailed work of Schüepp (1929) on the developmental anatomy and morphogenic cycle of the species.

The Significance of Photoperiodism

Are Photoperiodic Receptor and Response Mechanisms General?

On the basis of the behavior of the species discussed above there can be no denial that photoperiodic conditions, particularly the length of the uninterrupted dark period, can be a major environmental factor in control of elongation growth and induction of dormancy. Furthermore, results obtained with these species suggest that such control may be remote and mediated through more direct control over synthesis, activity, or transport of growth regulators or essential metabolites. It must be remembered, however, that the species most studied and discussed were not randomly selected. There has been some tendency to concentrate effort on those species known to be responsive. It does not follow that all other species are similarly responsive.

Photoperiodic conditions do not necessarily always control the inception of dormancy even in those species demonstratedly capable of photoperiodic response. For example, a photoperiodic regimen may be effective in inducing dormancy within a limited temperature range, but not outside of it (Moshkov 1935; van der Veen 1951; *p. 163*). After detailed study, Olmstead (1951) concluded that the role of photoperiodism in controlling bud dormancy in *Acer saccharum* is frequently less than a dominant one. Unfavorable temperature or light intensity may also induce dormancy in *A. rubrum* in spite of photoperiodic conditions which, in themselves, favor continued growth (Perry 1962).

Some species continue growth in spite of prolonged exposure to short photoperiod regimens and may be capable of growth in late fall or winter if temperature permits. The following are examples:

Species	*Reference*
Abelia grandiflora	Kramer 1957; Waxman 1957
Juniperus horizontalis	Waxman 1957
Pyracantha coccinea	Nitsch 1957a
Spiraea sorbifolia	Howard 1910

Some temperate zone species may be very slow to become dormant under the influence of 8-hour photoperiods, though they show a definitely reduced growth rate. An example is *Ulmus americana* (Downs and Borthwick 1956a).

Some of the few tropical and subtropical woody species studied show distinct growth responses when subjected to photoperiodic treatments more extreme than those prevailing in their natural ranges. Prolonged short photoperiod treatment of *Rauwolfia vomitoria* (Piringer et al. 1958) and *Coffea arabica* (Piringer and Borthwick 1955) greatly inhibits growth, but does not induce dormancy. Somewhat similar results have been obtained with several species of *Citrus* (Piringer et al. 1961).

Whereas short photoperiods do not induce dormancy in all species, long photoperiods are ineffective in preventing its induction in others. The following species have been reported to be but slightly responsive to extended photoperiod treatment:

Species	*Reference*
Aesculus hippocastanum	Downs and Borthwick 1956a
Buxus sempervirens	Waxman 1957
Cerasus avium	Chouard 1946
Paulownia tomentosa	Downs and Borthwick 1956a
Syringa vulgaris	Waxman 1957
Tilia americana	Ashby 1962
Viburnum prunifolium	Waxman 1957

The authors cited above do not claim that the species mentioned are also insensitive to photoperiodic conditions when growing in their natural habitats. Under greenhouse conditions when day and night temperatures are not rigorously controlled, or are controlled at arbitrary levels, photoperiodic responses are not necessarily identical to those of the same species under natural conditions. Yet, the possibility exists that some species lack the photoreceptor or other mechanisms needed to regulate growth by detection of seasonal photoperiodic changes.

Present information supports the idea that mechanisms capable of modulating growth and development in response to photoperiodic conditions are widespread, but perhaps not universal. However, the availability of such mechanisms does not mean that they do, in fact, control. Redundant control systems are found expedient in complex, man-made devices. It is logical to suppose that redundancy of growth control systems developed during the long evolution of higher plants, because it is not difficult to envision instances in which such redundancy would have survival value. An example of a redundant photoperiodic control system often subordinated by others may be that of *Tilia americana*, in which the photoperiodic receptor mechanism is present although growth responses

to photoperiodic treatment are slight (Ashby 1962), and the vernal growth flush is normally ended by apical abortion (*p. 65*).

Questions of function and possible redundancy are also raised by photoperiodic growth responses in *Rauwolfia vomitoria*. This species is native to Central Africa between 10° and 20° N. latitude. Even at 20° N. seasonal limits of day length are only 10.9 to 13.3 hours. Growth of *R. vomitoria* is markedly accelerated or retarded when subjected to photoperiods longer or shorter, respectively, than these natural limits (Piringer et al. 1958). Some tropical plants apparently do have photoperiodic receptors. Perhaps this is linked to the presence of nonperiodic photomorphogenic mechanisms such as those suppressing etiolation or mediating light intensity responses. Detailed studies of photoperiodic responses of trees native to equatorial regions would be of considerable theoretical interest.

Even if photoperiodic receptor and response mechanisms were known to exist in all species it would not follow that the photoperiod is the only environmental factor which can control inception of dormancy. I am inclined toward the view that any factor which retards elongation growth can be involved in the induction of dormancy. Mechanisms may be quite indirect, involving, for example, arrival at the apex of increased amounts of substances produced by maturing leaves (*p. 76.*)

The photoperiod may be an important factor in determining what substances actually are produced in leaves and in regulating the distance between maturing leaves and the apex. However, in my opinion, present evidence does not indicate that photoperiodism is the only or necessarily the most important factor controlling dormancy of woody plants in their natural habitats. Photoperiodism is a valuable experimental tool. Its study may provide considerable additional insight into regulation of growth and morphogenesis, but we should not expect any one regulating system to be omnipotent in all situations.

Mechanistic Implications of Photoperiodic Responses

The study of photoperiodic responses of plants is a specialized aspect of the broader and more senior subject of photomorphogenesis. In general, photomorphogenesis implies perception of light according to its spectral quality and intensity, and responses to it which ultimately result in changes in plant form and structure. A photoperiodic response implies, in addition, a response to a regularly repeated pattern of light and dark phases in which the periodicity, not the total duration of light or darkness, is the significant factor. It implies the existence of a time measuring device within the plant.

The seemingly inherent unlikelihood of a clocklike system within the plant was probably a factor in the failure of men such as Jost, Molisch, Klebs, and Howard to deduce the existence of photoperiodic responses from their data on seasonal differences in growth responses to various treatments. But once the fact of time measuring by the plant is granted, many barriers to the understanding of photoperiodic growth control remain.

A major barrier is inadequate understanding of growth control at the cellular level. What are the functions of auxins, gibberellins, and kinins? How are these and other regulators synthesized,

translocated, activated and inactivated? How do leaf influences prevent elongation of cells in subapical meristems of buds? Until such questions can be answered, understanding of photoperiodic control of vegetative growth will remain very incomplete.

A safe assumption is that the photoperiodic receptor is mechanistically remote from the immediate control of growth and development at the cellular level. Intervening mechanisms may include various types of metabolic regulators which can also be parts of other systems of growth control. Observation of photoperiodic responses of additional tree species will enrich in variety and detail the knowledge already available, but study of cellular growth and growth regulating substances may be more helpful in the long view.

Photoperiodism is not an isolated subject. It must be viewed along with the broader subjects of photomorphogenesis and endogenous rhythms in plants. It must be considered at least partially, and perhaps wholly, dependent upon metabolic regulators and other intermediates in the exertion of its ultimate effects. The following sections are attempts at such broad views and considerations.

POSSIBLE MECHANISMS OF GROWTH AND DORMANCY CONTROL

Photomorphogenesis

Early Work on Light Intensity and Spectral Quality

During the 19th century many plant scientists recognized morphogenic effects of light upon plants aside from those directly related to photosynthesis. The advent of electric lamps made meaningful experimental work possible, and by 1900 a large literature on the subject had accumulated. The separate effects of light intensity, quality, and duration were all studied. MacDougal (1903) and Wiesner (1907) reviewed the early literature.

The early work established that optimum light intensity for growth of many species is less than that of full sunlight.[18] Some reduction in intensity from full sun will often promote increases in stem elongation and foliage area, though it may restrict root growth (Gourley 1920). Lower light intensities also favor less compact cell arrangement and more succulent tissues.

Some species, particularly coniferous forest trees, are able to survive and grow at intensities much less than full sunlight. *Sequoia sempervirens* is outstanding in this respect. It can grow rapidly when receiving artificial light of total radiant energy equivalent to only 10 percent of full sunlight. It can put on appreciable growth even at the 1-percent level. *Pinus edulis*, which can barely survive at 6 percent, is at the other end of the scale. Various other pines and *Picea engelmannii* are intermediate (Bates and Roeser

[18] Responses to reduced light intensity vary widely with species. Young *Acer saccharum* transplants may grow taller and accumulate twice as much dry matter under 80 percent shade as in full sun. In contrast, even a 50 percent light intensity reduction greatly reduces height growth and dry matter accumulation by *Tilia americana* (Ashby 1961).

1928). These values may be considerably in error, but are of comparative value.

Though a tree may survive and grow throughout a wide range of light intensities, gross morphology and anatomy vary with light intensity as well as light quality (Korstian 1925; Shirley 1929, 1936). Prolonged exposure of plants to very low light intensity or to darkness produces more profound morphogenic effects grouped under the term "etiolation" (*p. 104 ff.*).

Work concerning comparative effects of light of different spectral quality produced no immediate clear-cut results. Some species exhibited abnormally rapid stem elongation under red light and greatly reduced elongation under green and, especially, blue light. Other species reportedly grew best under white light. Some of the confusion undoubtedly arose from disagreement on measurement of growth. Height growth may be greatest under red light, but dry-weight increase is greatest under blue or white light.

Flammarion (1899) reported on extensive experimental work with a large variety of species. In general, red light produced much taller plants than white light, but with thinner stems and lesser dry weight. Plants grown under blue light were poorly developed, probably at least partly because the intensity was low.

Commercial motives prompted much research into use of colored glass in greenhouses. In most of this work resulting differences in intensity and temperature were disregarded. Even the extensive work of Schanz (1918, 1919) included no control over light intensity in the different spectral regions or over temperature under the various colored glasses. Schanz concluded that short wavelength light inhibited plant growth, because the more short rays were filtered out the taller the plants became. He recommended use of yellow glass in greenhouses. The importance of determining exact transmission spectra was not generally recognized in this early work, and glass was generally referred to by its apparent color. Some differences in results may be traceable to such lack of specificity.

Popp (1926) studied the effects of different spectral regions under approximately equal intensities and with a fair degree of temperature control. Popp's more refined methods produced results largely in agreement with earlier work. The promotion of stem elongation by red light was obvious, as was its retardation by blue-violet. However, dry matter production by the shorter stemmed blue-violet grown plants was actually greater than that by the taller red light grown plants. This trend had been noted by Flammarion, though he could not validate it because of intensity differences. Popp's results were generally confirmed by Shirley (1929) and Funke (1931).

Anatomical differences resulting from growth under light of different spectral qualities were studied by Pfeiffer (1928). By 1930 the long suspected existence of morphogenic effects of specific regions of the spectrum was established. Further work on spectral effects eventually led to the discovery of photomorphogenic receptor pigment now called phytochrome.

Duration of Light

The effects of light duration, as distinct from intensity or quality, are complex. They include responses to continuous light as well as those resulting from regimens of regularly repeated cycles of photo- and nyctoperiods. The latter type of response has already been discussed. Continuous light effects are emphasized here.

Experimenters have achieved continuous illumination by three methods: (1) use of continuous natural light in summer at high latitudes, (2) natural light by day supplemented by artificial light at night, and (3) continuous artificial light. Results varied somewhat according to the method used.

The extremely rapid growth and development of plants in the continuous light of the Arctic summer was observed and discussed by Linnaeus and numerous others. Furthermore, several botanists reported that temperate zone plants taken north in summer were not injured by 6 to 8 weeks of continuous light. This literature was reviewed by Smith (1933).

A few extended day and interrupted night experiments with carbon arc lamps convinced Siemens (1880) that artificially prolonged days or continuous artificial light could promote plant growth. This was soon confirmed by others and the era of electrohorticulture began (Bailey 1892, 1893).

Bonnier (1895) published results of experiments, designed to separate effects of light intensity from those of duration. He used arc lamps producing light roughly similar to sunlight in spectral quality. Bonnier grew a variety of plants under high- and low-intensity artificial light given both continuously and as 12-hour photoperiods. He concluded that morphogenic effects of continuous artificial light were due to the continuity itself, not to intensity or quality. We now know that intensity and quality factors also have morphogenic significance.

Bonnier's work is still of interest and value because it clearly indicates anatomical and morphological differences between continuous and intermittent light grown individuals of *Pinus austriaca*, *Fagus sylvatica*, *Picea excelsa*, and many other species. Results with *Fagus sylvatica* are interesting in that normal cutinized stem epidermis did not develop in continuous light, nor did the usual fibers develop external to the primary vascular tissue. Bonnier's plants were exposed to continuous light for only a few months and serious injury was not evident.

Though Bonnier was a careful observer, his results have been confirmed only in part (Ramaley 1931). Maximov (1925) grew several herbaceous species in continuous artificial light and 12-hour photoperiods and could not validate the marked anatomical differences reported by Bonnier. Maximov, however, used incandescent filament lamps whereas Bonnier had used arc lamps. Spectral differences may explain lack of agreement.

Whatever changes in plant form and structure are induced by continuous light, they do not seem to interfere with completion of the normal life cycle in some herbaceous species. Harvey (1922) grew a variety of plants under continuous electric light. Many blossomed and set viable seed. The experiments, however, included no intermittent-light control plants. Castor bean (Adams 1925)

and wheat (Sande-Bakhuyzen 1928) have also been grown to maturity under continuous light without evidence of injury.

But with the work of Pfeiffer (1926, 1928), Redington (1929a, b), and Arthur et al. (1930) evidence began to accumulate that, though artificially extended photoperiods of up to 18 or 20 hours may be beneficial, continuous light may lead to injury in some species if treatment is long maintained. Arthur (1936), summing up the then available information, suggested that young plants are attuned to continuous light but that with aging a progressive decrease in optimum photoperiod occurs and a daily nyctoperiod becomes essential to optimum growth and development. Some experimental evidence is compatible with this idea.

Redington (1929a, b) compared growth of plants under continuous artificial light with that of control plants under similar light for 8- or 16-hour photoperiods. In the early stages of the experiments practically all species grew more rapidly in continuous light, but the growth rate generally declined after a few weeks or months. Finally, in all species except *Fagus sylvatica*, the plants under 16-hour photoperiods were larger. Some herbaceous plants grew very little after 2 months in continuous light. In contrast, *F. sylvatica* grew more under continuous light than under 16-hour photoperiods and was still growing vigorously after 5 months. Redington interpreted the behavior of herbaceous plants as resulting from increased transpiration and water stress accompanying continuous light.

Continuous light may promote vigorous growth of *Fagus sylvatica* for some months (Klebs 1914; Redington 1929a, b), but it will not necessarily do so indefinitely. Bałut (1956) found that continuous light, constant temperature conditions maintain growth in *F. sylvatica* and *Abies alba* seedlings for perhaps 18 months, but once terminal buds have been formed and broken, further continuation of constant environment treatment results in death (*p. 77*). Bałut and Żelawski (1955) also found abnormal development in *Pinus sylvestris* after several months under continuous light and constant temperature. However, harmful action of constant temperature itself must be ruled out before detrimental or eventually lethal action of continuous light can be established by such experiments.

Work of Moshkov (1932), Wareing (1950a), Nitsch (1957a), and others also supports the suggestion (Arthur 1936) that complete elimination of the daily dark period results in growth reduction even though photoperiods of 18 to 20 hours are highly favorable to growth. Leman (1955), however, reported that most woody species he tested grew best with 24-hour illumination. Only *Syringa* and some *Pinus* species grew better with a 22-hour photoperiod. Leman (1955, 1958) also emphasized that the effects of continuous light treatment may persist for many years in the form of increased vigor and precocious development after transplantation to natural conditions.

The efficacy of continuous light in delaying dormancy has already been mentioned. In some species, e.g. *Cornus florida* (Downs and Borthwick 1956a), continuous light treatment can be substituted for cold in overcoming the chilling requirement and breaking rest.

Etiolation

The effects of prolonged darkness upon plant growth cannot strictly be considered as photomorphogenic because theoretically no light is involved. However, study of growth and development in darkness emphasizes by contrast the very great, and otherwise unnoticed, role of photomorphogenesis in *normal* development. Plants grown in darkness are etiolated, but very small amounts of light are effective in decreasing the etiolation effects. Etiolation suppression is of theoretical interest in relation to photoreceptors and growth regulator mechanisms (*p. 156*).

According to MacDougal (1903), the great English botanist John Ray had already described the characteristic features of etiolation in 1686, and in Switzerland Bonnet published results of experimental work as early as 1754. MacDougal (1903) wrote a monograph including a comprehensive review of the literature on etiolation and related subjects published prior to 1900. He also contributed extensive original work on the etiolation of woody plants. MacDougal's monograph is still of interest because it remains the most comprehensive study available, particularly with reference to woody plants.

The extraordinary sensitivity of etiolated plants to light was not at first realized, and many experimenters were led astray because they took inadequate precautions to assure complete darkness or tacitly assumed that brief exposure of plants to light for daily observation would have no effect. This, along with inherent behavioral differences between species, resulted in confusion and controversy. A particular point of controversy concerned leaf development in darkness (see Priestley and Ewing 1923).

The work of Trumpf (1924) and Priestley (1925) called attention to the great departure from the effects of total darkness caused by a few minutes of light per day, as during daily observation. *Vicia faba* and *Pisum sativum* grown in total darkness show no signs of leaf development and have a distinct plumular hook. When grown with 2 minutes of light per day the plants have a much less pronounced hook and small leaves are present (Priestley 1925). Such behavior implies the presence of an exceedingly sensitive photoreceptor mechanism which can change the course of growth and development.

Brief daily exposure of etiolated plants to white light typically results in reduced stem elongation, but increased leaf growth. Red light and blue light, often in markedly different ways, also counteract the effects of etiolation (Trumpf 1924). The mechanisms involved are still not understood, but may include more than one primary photoreceptor or photoreaction (Mohr 1957, 1959, 1961, 1962; Borthwick and Hendricks 1961).

Most of the gross morphological features characteristic of etiolated dicotyledonous plants result from increased stem elongation and inhibition of growth and development in leaf primordia. There are, of course, diverse exceptions, particularly among the monocots. Etiolated *Calla* leaves expand almost normally and those of *Narcissus* may be longer than normal (MacDougal 1903). Whether growth of a leaf is inhibited or promoted by etiolation may be a

function of its physiological-morphological relation to the stem (Williams 1956). Certainly there is something stemlike about some petioles and midribs. According to MacDougal (1903) some etiolated woody seedlings actually expand more internodes than normal and hence increased stem growth may result from either longer internodes or more of them.

The internal anatomy of etiolated stems is reportedly different from that of normal stems. Not only are cell walls thinner, but normal differentiation is retarded and altered. Stems become more rootlike. Whereas stems of most higher plants lack a well differentiated endodermis,[10] such a layer may, as a result of etiolation, appear in stems where it is not normally found. This was already noted by Costantin (1883) in his study of subterranean and aerial stems. MacDougal (1903) reported a deep-seated periderm in etiolated *Castanea*, *Carya*, *Quercus*, and other seedlings whereas normal stems had more superficial periderms. However, MacDougal's histological work was quite limited and the structures he observed in etiolated stems may have been of endodermal origin.

Priestley and Ewing (1923) postulated that development of an endodermis in etiolated stems results in limitation of growth activity to regions enclosed by it. Such limitation would account for lack of leaf development and for the frequently observed development of adventitious roots by etiolated stems. This postulate was supported by anatomical work with etiolated *Vicia* and *Pisum* plants (Priestley 1926) which showed acropetal development of a typical endodermis coupled with the disappearance of the endodermoid starch sheath.

Priestley believed that stored carbohydrates were partly converted to fatty materials some of which were later deposited as the Casparian strips. Light presumably inhibited these reactions. Histological work gave some support to these ideas. Priestley also believed that lack of light resulted in increased lipid content in cell walls of the subapical region and that deviation from normal development was related to decreased facility of translocation from vascular tissue to the organogenic region of the apical meristem because of these lipids. Such conditions were thought to favor internodal elongation over leaf initiation and development.

Priestley's ideas, largely based upon the probably atypical etiolation responses of *Vicia* and *Pisum* stems, were found to be inapplicable to the behavior of etiolated stems in general (Bond 1935). The nature and mechanism of etiolation effects upon stem anatomy must still be regarded as an open question.

Leaves and stems of dicotyledonous plants respond differently to etiolation. Stem elongation in darkness is usually greater than in light, but leaf development in total darkness is minimal or even nil. As light is increased leaf growth is promoted and stem elongation is inhibited. This does not necessarily imply different photoreceptors, although more than one may exist. Parker et al. (1949) found the action spectrum for increase in leaf size of etiolated

[10] Lack of agreement as to exactly what constitutes an endodermis or an endodermoid layer makes the distribution of these structures difficult to ascertain (see Guttenberg 1943; Zeigenspeck 1952; Esau 1953; Van Fleet 1961).

Pisum sativum to have a peak in the red region like that of photoperiodic flower induction.

Borthwick et al. (1951) obtained a similar spectrum for suppression of elongation of the second internode of *Hordeum vulgare* in etiolated normal and etiolated albino plants. Goodwin and Owens (1951) also found the red region of the spectrum to be most effective in inhibiting internode elongation in *Avena sativa*. Such results clearly indicated the presence of a red-absorbing photoreceptor.

The photoreceptor pigment, however, is present in very low concentrations and cannot be detected by ordinary spectroscopic techniques even in etiolated plants. The amount of energy which can be absorbed by a mere trace of pigment during a short light period must be exceedingly small. Yet a macroscopic effect is produced. This suggests that the primary reaction is a photochemical one involving a minute amount of substance. Subsequent changes in more ponderous systems must be responsible for actual control of growth and development. If this is so, response of etiolated plants to irradiation should not be a function of temperature during the irradiation period. Such temperature independence was reported by Trumpf (1924) and Biebel (1942).

Further work by Downs (1955) on the action spectrum of photocontrol of stem elongation and leaf development in etiolated kidney beans established the reversibility of effects of red irradiation (peak at about 640 mμ) by subsequent far-red irradiation (peak at about 730 mμ). Separation of red and far-red treatments in time showed that decay of products of red irradiation was much slower than in the case of flower induction in *Xanthium*, but otherwise the basic mechanism appeared similar. Liverman et al. (1955) also demonstrated a reversible photoreaction in bean leaf discs.

Thus it appears that etiolation, in part at least, is another manifestation of response to the state of a photomorphogenic pigment system which may be the same as that implicated in photoperiodic control of flowering and vegetative growth. Spectroscopic demonstration of such a pigment followed by its partial purification has recently given reality to this postulated substance (see review by Borthwick and Hendricks 1960). Some of the properties and the possible mode of action of this pigment, now called phytochrome, are discussed below.

Detailed studies of the physiological effects of etiolation upon woody plants have not been made. Study of metabolic changes induced in etiolated plants by brief exposure to light may offer an approach to unraveling some of the mechanism of photomorphogenesis. Seedlings of large-seeded woody species may be suitable experimental material because at least some of them can be grown for considerable periods in darkness (MacDougal 1903).

Phytochrome—A Photomorphogenic Receptor

Morphogenic effects produced by the abnormal conditions of continuous light, continuous darkness, or light of narrow spectral distribution were widely recognized by physiologists before the possible morphogenic effects of seasonal changes in relative length of

night and day were seriously considered. Perhaps this was so because response to photoperiod or nyctoperiod length requires a time measuring system in the plant—a requirement which, until recently, could not be satisfied by any known biological mechanism.

The work of Garner and Allard (1920, 1923, 1925) clearly established the fact of photoperiodic control over numerous aspects of plant morphogenesis in a variety of species. Though emphasizing herbaceous plants, they included sufficient tests of woody species to demonstrate photoperiodic responses in these also. Garner and Allard left the question of the nature of the photoreceptors and time-measuring devices unanswered. Insight into these problems came gradually. It was accelerated by work on another kind of photomorphogenic control, that over germination of light-sensitive seeds.

Meanwhile evidence accumulated for applicability of the concept of photoperiodic control over growth and development in many woody species. The reviews of Gevorkiantz and Roe (1935), Phillips (1941), Wareing (1949, 1956), and Nitsch (1957b) document the accumulation of observations and gradual progress toward understanding their implications.

In their first paper on photoperiodism Garner and Allard (1920) remarked about the relatively low-intensity incandescent light which was effective in extending the natural photoperiod. Tincker (1925) found .5 ft.-c. to be effective in extending daylength. Further experiments by Tincker (1928), Ramaley (1934), and others made it clear that similar photoperiodic effects can be obtained by shortening natural long photoperiods of summer or artificially extending winter photoperiods. As long as photosynthetic needs are met, the response is largely governed by light duration, not intensity. This was further emphasized by Withrow and Benedict (1936) who got definite responses at less than 1 ft.-c. of incandescent light used to extend the day, but little increase in response when intensity was increased from 10 to 100 ft.-c. This means that saturating light intensities for photoperiodic control of morphogenesis are much lower than those needed for any significant amount of photosynthesis. Intensity is above saturation, even in the shade on a cloudy day, until after sundown, when it suddenly drops below saturation almost to zero. Natural daytime variations in intensity are of little importance to the photoperiodic receptor mechanism (Withrow 1959).

Withrow and Benedict (1936) obtained increases in dry weight when the intensity of light used to extend the day was as low as 0.3 ft.-c. It is hardly conceivable that such a small amount of radiant energy could have any direct effect upon synthetic processes. The primary photoreaction is probably mechanistically remote from reactions directly involved in growth and development.

Withrow and Benedict (1936) also made crude spectra of the effectiveness of various wavelength ranges in extending natural photoperiods. The orange-red region was found most effective and the near infrared region quite ineffective. White incandescent light had about the same effect as red, but green was inactive in extending the day (Withrow and Biebel 1936). Withrow and Withrow (1940), on the basis of additional work, postulated that the photo-

688-803 O—63——8

receptor absorbs strongly in the red and probably weakly in the blue and green regions.

Further work on photoperiodic induction of flowering led to the realization that opposite responses of so-called short-day and long-day plants, and their reversal by radiation between 700 and 800 mμ, probably arises from the same controlling photoreaction involving a pigment strongly absorbing in the red region (Parker et al. 1950). A very similar action spectrum was found for photoinhibition of stem elongation in dark-grown *Pisum* and *Hordeum*, even in six albino types of the latter (Borthwick et al. 1951).

Cieslar (1883) already had reported that germination of some seeds was promoted by yellow light and inhibited by violet light. In the following decades numerous investigators studied light as a quantitative factor in germination, but wide appreciation of the special significance of certain limited spectral regions did not come until much later.

Flint and McAlister (1935) discovered that, in contrast to the promotive effect of red light, radiation in the far-red region is a potent inhibitor of germination of light-sensitive lettuce seeds. Borthwick et al. (1952b) verified these effects and more precisely determined the action spectrum. They found the germination response to be readily and repeatedly reversible by irradiation with red or far-red light. Red, with a maximum near 650 mμ, promotes germination; far-red, with a maximum near 730 mμ, inhibits it. This behavior was taken as evidence for the existence of a photoreceptor pigment in two forms, red absorbing and far-red absorbing, each form convertible into the other by irradiation in the wavelength range of its apsorption peak.

Borthwick, Hendricks, and Parker (1952a) extended the study of the reversible photoreaction to the control of flowering. The results of this and earlier work led them to propose the following scheme (*for later modifications see pp. 110–111*):

$$\underset{650\ m\mu\ max.}{\text{Pigment}} + RX \underset{\text{dark}}{\overset{\text{red}}{\rightleftharpoons}} \underset{730\ m\mu\ max.}{\text{Pigment X}} + R$$
$$\text{far-red}$$

The above reaction was supposedly displaced to the right by daylight because sunlight at the earth's surface is richer in red (650 mμ) than in far-red (730 mμ) light (Moon 1940). In darkness the reaction was presumed to go spontaneously to the left at a rate which would determine the effectiveness of the dark period. Reversion of the pigment from the far-red to red absorbing form is accordingly the time measuring part of the system. Because sunlight contains both red and far-red light, the pigment balance is at neither extreme during daylight, but it favors the right.

Red light given just prior to the nyctoperiod would displace the reaction farther to the right and additional time would be required for the reversion of P_{730} to P_{650}. This explained the increased dark requirement following such treatment and also the opposite effect of treatment with far-red light just prior to the nyctoperiod. Implicit in the scheme was the assumption that some significant dark

reactions concerning morphogenesis are not activated until a threshold amount of reversion from P_{730} to P_{650} has occurred. Involvement of the additional reactants R and RX was hypothetical.

Thus by 1952 the special importance of red and far-red light in controlling a variety of photomorphogenic reactions was recognized, a possible receptor and timing mechanism had been postulated, and actual isolation of the photomorphogenic pigment became an objective. Direct approaches were not successful. The proposed receptor pigment was not detectable even in etiolated and albino tissues by the usual spectrophotometric techniques. However, indirect methods yielded valuable information in spite of the pigment's elusive qualities.

The reversible photoreaction reportedly follows first-order kinetics with respect to energy in both directions and, as followed by the *Lactuca* seed germination response, has a temperature coefficient of unity between 6° and 26° C. Temperature independence is not totally incompatible with involvement of reactants other than the pigment itself, but it does seem to make it less likely.

The actual photoreaction may merely involve two forms of the same substance, interconvertible through a common excited state. In addition to being driven by far-red irradiation, reversion of P_{730} to P_{650} occurs thermally in the dark, presumably because P_{650}[20] is in a lower energy state than P_{730}. The dark equilibrium is far to the left (Borthwick et al. 1954).

Because the photoreaction is reversible, follows first-order kinetics, and is coupled to measurable physiological responses, the method of Warburg and Negelein (1928, 1929) can be used to calculate the fraction of the total pigment converted from one form to the other by irradiation with a known amount of energy in a specific absorption region. Determination of the fraction allows calculation of absorption coefficients and quantum efficiencies for pigment conversion.

This approach was successfully used by Hendricks et al. (1956). Absorption coefficients of both forms of the pigment were estimated to be greater than 10^{-7} cm.^{2} per mole. An additional result of this approach was tentative evidence favoring P_{730} as the biologically active form. The evidence came mostly from measurements in a few objects in which half-maximal responses were obtained with only 10 percent conversion from P_{660} to P_{730} and physiological saturation at about 75 percent conversion. This, and additional nonrigorous evidence led Hendricks et al. (1956) to conclude that P_{730} has enzymatic properties (see also Borthwick and Hendricks, 1960, 1961).

Pronounced biological responses can be achieved by low-intensity irradiation with red or far-red light, and such effects can be explained as resulting from interconversion of the two forms of the photomorphogenic pigment. However, high-intensity irradiation does not necessarily produce the same results even if the wave-

[20] The red absorbing form of the pigment, first abbreviated as P_{650}, is referred to as P_{660} in later work. The abbreviation P_{735} rather than P_{730} has also sometimes been used with reference to the far-red absorbing form. The subscript numbers represent the approximate wavelengths in $m\mu$ of the absorption peaks.

lengths employed are the same as before. This phenomenon was analyzed by Hendricks and Borthwick (1959a, b) who, at the time, assumed the basic reversible reaction to be:

$$P_{660}H_2 + A \underset{\text{far-red}}{\overset{\text{red}}{\rightleftharpoons}} P_{735} + AH_2$$
$$\text{dark}$$

In this postulated scheme $P_{660}H_2$ represents the reduced form of the pigment (red absorbing). P_{735} represents the oxidized form (far-red absorbing). These tentative identifications were based upon general arguments (Hendricks and Borthwick 1959b). A and AH_2 represent the oxidized and reduced forms, respectively, of a hypothetical additional reactant.

Though the two forms of the pigment have apparently well-separated absorption maxima, their absorption bands nevertheless overlap considerably. High-intensity irradiation, particularly with light of appreciable band width, will continuously excite both forms of the pigment even when the wavelength peak of the applied radiation coincides with the absorption maximum of one form. Consequently high-irradiance action spectra may be quite different from those obtained at low-intensity irradiance.

Anthocyanin synthesis in some species behaves as though it were controlled by just such simultaneous excitation of both forms of the pigment (Hendricks and Borthwick 1959a, b). This finding made possible, in theory, estimates of the concentration of the photoreceptor pigment in anthocyanin synthesizing cells. The method involved irradiation with a known amount of energy and assay of the anthocyanin produced. Estimated concentrations of photoreceptor pigment were in the range of 10^{-6} to 10^{-7} *M* (Hendricks and Borthwick 1959b; Butler et al. 1959). In this work it was assumed that interconversion of the two pigment forms involved oxidation and reduction reactions with the postulated substances A and AH_2. Later work has not supported this assumption, but results obtained through its use nevertheless contributed to progress because they encouraged attempts to isolate the actual photoreceptor pigment.

A group of workers at Beltsville, Md., cooperating with Drs. S. B. Hendricks and H. A. Borthwick, were successful in adapting a sensitive differential spectrophotometer to assay of the photomorphogenic pigment in living tissue and in solution (Butler et al. 1959). They called the pigment *phytochrome*. The spectrophotometric assay along with conventional methods of protein chemistry permitted extraction and partial purification of phytochrome from dark grown *Zea mays* seedling shoots and other plant materials. The photoreversible pigment was retained in solution after dialysis, but reversibility was lost by heating to 50° C. Earlier speculation that the pigment might be a protein was thereby greatly reenforced.

The phytochrome obtained in solution by the Beltsville workers exhibited photoreversibility in vitro, but it did not undergo spontaneous reversion from P_{730} to P_{660} in darkness. It is possible that the reversion is not merely thermal, but enzymatic, and that the extracts lack factors essential to the process (Borthwick and Hendricks 1960). The exact nature of the reversion process and its

degree of temperature dependence in vivo is of great theoretical interest. It is the key to many puzzling problems.

Bonner (1960, 1961), working independently, succeeded in extracting and partially purifying phytochrome from *Pisum sativum*. His results are in general in agreement with those of the Beltsville group. He found, however, that mild oxidizing and reducing agents had no effect upon the light-induced interconversion of the pigment forms. Normal involvement of redox reactions in interconversion therefore seemed doubtful.

The Beltsville workers also found photoreversibility to be uninfluenced by oxidants and reductants, as well as by dialysis. Photoreversibility at –70° C. still continues at one-tenth of the rate at 0° (Hendricks 1960b). Such results are seemingly incompatible with earlier concepts of a photoreversible reaction involving oxidation and reduction. The reaction postulated earlier (Hendricks and Borthwick 1959a, b) was therefore simplified to (Bothwick and Hendricks 1961, p. 325):

$$P_{660} \underset{\text{far-red}\atop\text{dark}}{\overset{\text{red}}{\rightleftharpoons}} P_{730}$$

The above simple scheme should be regarded only as a working hypothesis to be abandoned if a better one becomes available. In some systems the product of red-light irradiation may react with another substance before far-red reversal is possible. Klein et al. (1957b) found that maximum far-red reversal of red-light promoted straightening of bean seedling hypocotyl hooks did not occur until about an hour after red treatment. The indicated secondary reaction was reported to be temperature dependent (Withrow and Klein 1957). Related observations have been discussed by Liverman (1960).

Instances of failure of far-red reversibility of flower induction by red irradiation have also been a matter of concern to the Beltsville workers (Hendricks and Borthwick 1959b; Nakayama et al. 1960). This means that under some conditions P_{730} is not converted to P_{660} by far-red irradiation, or that such conversion is divorced from measurable responses. So little is yet known about the nature of phytochrome and about the mode of action of growth regulating hormones that explanations of such observations can only be speculative (see Hendricks 1960b).

Valuable literature reviews and discussions on the more theoretical aspects of photomorphogenesis and photoperiodism have been provided by Borthwick and Hendricks (1961) and Naylor (1961), respectively.

Responses to Light of Limited Spectral Regions

While the Beltsville group was employed in the work which led to spectrophotometric demonstration and partial characterization of phytochrome, much work on photomorphogenesis was also being done in The Netherlands. Publications resulting from this work are rich in experimental detail and in data on responses obtained after subjecting plants to regimens in which time schedules, inten-

sity and spectral quality of light, and frequently temperature also, were closely controlled.

Although some of the Dutch work was concerned specifically with control of flowering, results of most of it have some bearing upon the overall problem of photomorphogenesis. The earlier Dutch work has been reviewed by Stolwijk (1954), Wassink and Stolwijk (1956), and Meijer (1959a). It is instructive to examine some of the more recent work and test compatibility with the concept of a photoreversible photomorphogenic pigment proposed by the Beltsville workers.

If photomorphogenic control were mediated by a reversible pigment system, the spectral quality of light as well as the duration of the photoperiod should, under suitable conditions, affect responses. The relative amounts of P_{660} and P_{730} at the beginning of the nyctoperiod would logically depend largely upon light quality during the latter part of the photoperiod. In turn, the actual photomorphogenic value of the dark period should be a function not only of the time dependent reversion of P_{730} to P_{660}, but also of the initial P_{730}/P_{660} ratio. Furthermore, the morphogenic value of lightbreaks during nyctoperiods should be somewhat dependent upon the spectral quality of the interrupting light and of the light of the main photoperiod. Other interrelations and dependencies become obvious upon detailed examination of the problem.

For the induction of long-day responses in *Hyoscyamus niger* (stem elongation and flowering) the long photoperiod irradiation must include some violet, blue, or far-red light. Green light is ineffective and red almost so (Stolwijk and Zeevaart 1955; Wassink et al. 1959).[21] *Salvia occidentalis* (short days required for flowering) does not flower under long photoperiods in daylight, in blue light, or in red light, but green light is again ineffective in eliciting the long-day response of continued vegetative growth, and flowering is not prevented (Meijer 1957; Meijer and van der Veen 1957; Meijer 1959a).

Other plants have also been found to require blue or far-red for expression of long-day responses (Meijer 1959b). Interestingly, the ineffectiveness of long photoperiods of red or green light in promoting stem elongation and flowering of *Hyoscyamus niger* can be partly overcome by applications of gibberellic acid. Some evidence suggests that the requirement for blue or far-red (Stolwijk and Zeevaart 1955) is really only a far-red requirement which can also be satisfied by gibberellic acid (Wassink et al. 1959). Can these observations be interpreted in terms of the P_{730}/P_{660} ratio prevailing during photoperiods and at the beginning of nyctoperiods?

Long photoperiods imply short nyctoperiods, and according to the phytochrome hypothesis short nyctoperiods have their effect because there is insufficient dark time for reversion of P_{730} to P_{660} beyond a threshold. Low- to moderate-intensity green light apparently has little effect upon pigment balance although both forms absorb green to a slight extent. Thermal or enzymatic conversion of P_{730} to P_{660} can continue and the effect is that of a long dark period. The inability of low- or moderate-intensity green light to elicit long day responses is, therefore, not surprising.

[21] Ineffectiveness of green light in extending photoperiods had already been reported by Withrow and Biebel (1936).

The reasons for red light ineffectiveness may be quite different. After a long photoperiod in red light the plant would be expected to enter the nyctoperiod with its phytochrome very largely in the P_{730} form. Reversion during the short nyctoperiod would be incomplete and long-day effects favored. This is compatible with lack of flowering of *Salvia occidentalis* (short-day plant) after long photoperiods in red light (Meijer 1959a) but not with lack of flowering in *Hyoscyamus niger* (long-day plant) after similar treatment (Stolwijk and Zeevaart 1955).

The ineffectiveness of red light in eliciting long-day effects in some species may be a consequence of displaced pigment balance during red-light photoperiods. During red irradiation P_{660} would be almost totally converted to P_{730}, a condition which does not occur in normal daylight. The abnormally high P_{730} level may inhibit or alter essential processes so that long-day responses are not induced. If this explanation is valid, it also follows that red-light ineffectiveness may be limited to low and moderate intensities. At high intensities simultaneous excitation of both forms of phytochrome might be expected to relieve abnormalities resulting from conversion of practically all of the pigment to P_{730}. This is compatible with results of experiments with *Larix leptolepis* discussed later.

The effectiveness of blue or the combination of far-red and red, or far-red and blue, in inducing long-day responses (Meijer 1959a) seems to indicate need for an intermediate pigment balance in which both forms are present in appreciable amounts. However, a combination of green and far-red is effective in eliciting long-day responses in *Salvia occidentalis* whereas pure green is not (Meijer 1959a). Both treatments should result in almost complete conversion to P_{660}. Do such results mean that interconversion between P_{660} and P_{730}, with only P_{730} being physiologically active, is an oversimplification of the reactions of the photomorphogenic receptor? It is difficult to design experiments which can give unequivocal answers to these questions.

The Dutch work also revealed that morphogenic responses of some plants, of which *Larix leptolepis* is an example, appear not to show wavelength dependence. *L. leptolepis* is very sensitive to short days and can be forced into dormancy by a week of short photoperiod treatment. Dormancy is prevented by long photoperiods, and blue, red, and green are all effective at high intensity (Meijer and van der Veen 1957; Meijer 1959b). The effectiveness of green in this instance could be ascribed to high irradiance, simultaneous excitation of the two pigment forms, or perhaps the admitted slight contamination of the light source with red (Meijer 1957).

Meijer (1959a, b) also recognized intensity effects as a possible explanation for the variable effectiveness of green. The high-intensity argument can likewise be applied to blue and red. Another source of variability and confusion when a green plant is irradiated with blue light is excitation of fluorescence by chlorophyll. Chlorophyll fluorescence in the red and far-red regions could possibly be the basis of some of the blue-light effects reported by the Dutch workers and also the basis of the reported blue-red antagonism (Meijer, 1958b, 1959a; Wassink et al. 1959).

Attempts to prevent dormancy induction in *Larix leptolepis* by interjecting a short light period into the long nyctoperiod accompanying short days revealed a dependence upon spectral quality of the main light period, and perhaps upon that of the interrupting light—this even though no spectral dependence was evident when long photoperiods were actually given. The observations have been summarized by Meijer and van der Veen (1957) and by Meijer (1959b) who believe that long-day effects can be induced by short photoperiods under blue light (or possibly far-red) combined with a red or green nightbreak, or alternately by actually giving long photoperiods of natural, or blue (and possibly far-red) light. These interpretations are largely, but not entirely, compatible with the concepts expressed by the Beltsville group.

The technique of interrupting long nyctoperiods with a short period of illumination (nightbreak) has been widely used in experimental work to induce long-day effects despite short main photoperiods. The method has been generally effective and has been explained in terms of reversion of P_{730} to P_{660} as the time measuring dark raction. On this basis red light might be expected to be particularly effective, and it is. However, in *Salvia occidentalis* the quality of light during the main photoperiod determines whether or not a red nightbreak causes a long-day effect.

Under some conditions red nightbreaks in combination with phototreatment that by itself causes long-day effects can prevent appearance of those effects. Red-light nightbreaks can actually induce short-day effects, but this tendency is nullified by increasing the length of the red nightbreak or by following it with a period of far-red irradiation (Meijer and van der Veen 1960). Such behavior is difficult to understand in terms of P_{730} interconversion with P_{660} as the only receptor level reaction in photoperiodism.

Meijer (1959a) suggested that two different photoperiodic reactions are involved in induction of long-day effects. His concepts include a nightbreak reaction, particularly sensitive to red light, and a main light period reaction most sensitive to far-red and blue light. This contradicts the idea that the main light period has no direct photoperiodic function other than regulating the length of the nyctoperiod.

De Lint (1960) made a very detailed analysis of the effect of light on elongation and flowering in *Hyoscyamus niger*. He suggests that short-day inhibition of development is a consequence of production of an inhibitor precursor during the main light period. The precursor is largely inactive during light periods but is converted into active inhibitor in darkness. The presumed inhibitor, however, is not persistent and becomes innocuous during long periods of continuous darkness.

De Lint proposed inhibitor precursor synthesis to be controlled by a photomorphogenic pigment absorbing in the red and, weakly, in the blue regions. Far-red irradiation is assumed to antagonize inhibition by inactivating the inhibitor precursor. In some cases, as in high-intensity irradiance with red light, high precursor concentration accumulating during a long photoperiod may result in appearance of significant amounts of inhibitor even in the light. This would result in the observed inhibition of long-day effects by long photoperiods in red light. Differences between this hypotheti-

cal mechanism and that suggested by the Beltsville workers are considerable. However, at this stage of our understanding of photomorphogenesis, consideration should not be denied any well-formulated hypothesis.

Diverse views regarding spectral dependence of internodal elongation have long existed both among the Dutch workers and elsewhere. Some have proclaimed blue light to be more inhibitory than red while others reported the reverse. Meijer (1959a) reviewed the literature on this subject. Many of the disagreements in early results could easily have been due to spectral impurity of light, still a source of some difficulty.

Another source of variable results is pretreatment of plants. Both light- and dark-grown plants have been used in experimental work. The effect of light, almost irrespective of spectral quality, upon dark-grown (etiolated) plants is one of inhibition of elongation (*p. 104 ff.*). Elongation of plants grown under light has already been restricted by that light and further phototreatment can only modify an existing inhibition. Galston and Kaur (1961) discussed this point with respect to different photoresponses of green and etiolated *Pisum* stem sections.

Far-red irradiation has an inhibitory effect upon elongation of dark-grown seedlings (Withrow 1941; de Lint 1957) which is not reversible by red, but similar irradiation promotes an elongation of light-grown plants which is reversible by red (Downs et al. 1957; de Lint 1957). Wassink and Stolwijk (1956) made the generalization that radiation in the red region most effectively inhibits elongation of dark-grown plants whereas blue-violent maximally inhibits elongation of light-grown plants. But there are exceptions in which the opposite appears true (Meijer 1958a, 1959b). Lack of agreement may be related to intensity differences.

Meijer (1959a) found the same species to respond differently at different light intensities. Another source of confusion is difference in response of various parts of the plant axis (hypocotyl, epicotyl, first internode, later internodes). Effects of light upon overall axis elongation are obviously somewhat dependent upon the locus of active elongation at the time of treatment.

A Second Photomorphogenic Receptor?

The Dutch work discussed above emphasized the difficulty of explaining all observed photomorphogenic effects on the basis of a single photoreaction—the photoreversible reaction of phytochrome. Many of the results would be more readily interpretable if a second photoreaction existed. Indeed, a second reaction requiring high-intensity light for its activation had already been proposed by Siegelman and Hendricks (1957) and by Mohr (1957) with regard to nonperiodic photocontrol of anthocyanin synthesis.

Hendricks and Borthwick (1959a, b) believed the second reaction to be dependent upon simultaneous excitation of both forms of phytochrome at high light intensity. But the reasoning at the basis of this belief included an assumption that the reversible photoreaction was bimolecular (involving redox or other reactants). That assumption has, however, become untenable (Borthwick and Hen-

dricks 1961, p. 325), and earlier arguments based upon it thus lost their force.

Detailed arguments against the proposals of Hendricks and Borthwick (1959a, b) were published by Mohr and Wehrung (1960). Mohr (1959) and Kandeler (1960) believe the high-energy photoreaction to be mediated through a blue and far-red absorbing pigment system independent of the low-energy reactions of phytochrome. The blue, far-red pigment is thought to control activation of some important, but still unidentified enzyme.

Despite the lack of a generally accepted theory on the nature of the high-energy photoreaction, progress has been made in discriminating physiologically between it and the reactions of phytochrome. This was possible because in *Sinapis alba* the two systems are synergistic. The same photoresponses can be elicited by either photoreceptor system, and other conditions allow approximate determination of the action spectrum of the high-energy reaction corrected for that of phytochrome (Mohr 1959).

The two pigments may not have the same relation to one another in all species, and the separate existence of a blue, far-red pigment is still somewhat hypothetical. Nevertheless, the scheme proposed by Mohr (1959) for light-induced expansion of cotyledons of dark-grown *Sinapis alba* seedlings is an aid in organizing ideas and can serve as a point of departure for further studies.

According to Mohr's scheme (fig. 6) the blue, far-red absorbing pigment promotes the reaction A → B whenever it is absorbing sufficient radiant energy. The far-red absorbing form of phytochrome (P_{730}) may itself be an enzyme, as has also been postulated by the Beltsville group. The hypothetical product B and enzymatic action of P_{730} elicit metabolic changes which in turn control photomorphogenesis.

It would, of course, be very interesting to know the nature of the metabolic changes induced by the reactions of the pigments, but only a little progress has been made in that direction. The work of Sisler and Klein (1961) does not encourage the supposition that

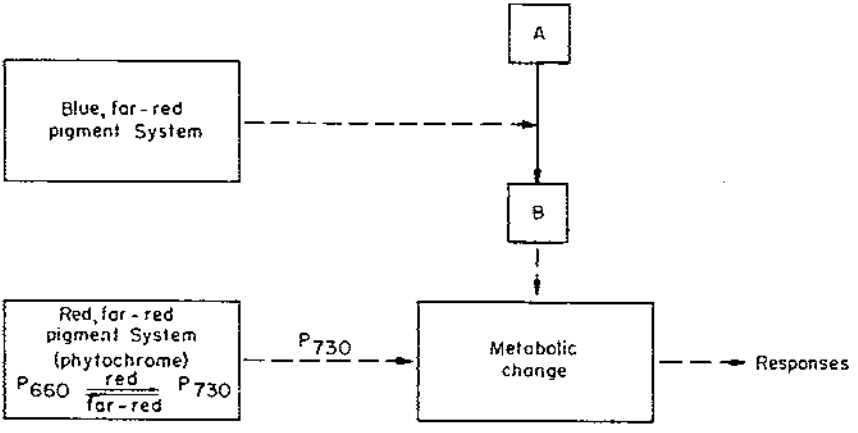

FIGURE 6.—Schematic representation of synergistic control over photomorphogenesis in *Sinapis alba* by two pigment systems. (Adapted from Mohr 1959.)

adenosine triphosphate metabolism is directly affected. There are a few reports of photocontrolled enzyme formation or activation (Hillman and Galston 1957; Marcus 1960; Hageman and Flesher 1960).

The possible existence of two rather than one photomorphogenic pigment calls for extreme caution in interpreting results of experiments involving treatment with light of limited spectral composition. Further theoretical work in this area may suggest experimental approaches to separate study of responses to high and low energy, periodic and nonperiodic photostimulation. Woody plants should furnish suitable material for some of this work.

Some Kinetic Aspects of Photomorphogenesis and Photoperiodism

While intensive research on action spectra, monochromatic light effects, and photoreceptors was under way at Beltsville and in Europe, a sustained effort by the late Dr. R. B. Withrow and his collaborators and successors at the Smithsonian Institution resulted in data and concepts of importance to the general theory of photomorphogenesis.

According to Withrow (1959), regulatory photochemical reactions fall into two classes: (a) those in which yellow pigments are activated by blue light, and (b) those mediated by red or far-red absorbing pigments. Photoreactions of these two classes may produce similar effects upon growth, but possibly by different mechanisms. When plants are irradiated with monochromatic blue light one cannot distinguish between direct activation of yellow (blue absorbing) pigments, and weak, indirect activation of red or far-red absorbing pigments. Thus, according to Withrow's ideas, the blue light effects later reported by Meijer (1959a, b), de Lint (1960), and others could partly be due to yellow pigment activation. Indeed, Withrow's ideas were not altogether foreign to those now held by Mohr (discussed above).

The extreme variety of known red, far-red responses, and the wide range in energy needed to induce them, suggests to some workers that they are not all of the same type. Furthermore, opposite effects may be produced in different tissues of the same plant. For example, in dicotyledonous plants red light may inhibit hypocotyl elongation, stimulate that of the epicotyl, and accelerate leaf expansion. In monocotyledonous plants it may accelerate coleoptile elongation, but inhibit that of the first internode.

Even in the seemingly simple response of anthocyanin synthesis different types of control by red light appear possible. The time lag and very low-energy requirements found by Withrow et al. (1953) and Klein et al. (1957a) for anthocyanin synthesis in *Zea* and *Phaseolus* seedlings bespeak a mechanism different from the high irradiance precursor conversion in apple fruit skin and other tissue postulated by Siegelman and Hendricks (1957, 1958a, b) and by Hendricks and Borthwick (1959b). Another variation is exhibited by *Sorghum vulgare* in which anthocyanin synthesis potentiated by high-energy irradiance in the blue region (absorption by Mohr's blue, far-red pigment system?) is actually modulated by the red, far-red reaction (Hendricks 1960a).

The work cited above raises the possibility of different kinds of photoreactions being involved in photomorphogenesis. An alternate possibility is that photosensitivity of cells having common photoreceptor mechanisms might be quite different. These possibilities are not, in my opinion, mutually exclusive. Different tissues could conceivably have widely different sensitivity ranges and have either the same or different photoreceptors. It might be expected, too, that the location of the receptor pigment within the plant and the light filtering qualities of intervening tissues would have some effect upon intensity of incident light needed to elicit specific effects. Whether such differences could be large enough to account for all observed effects is an open question.

The great intensity range over which red or far-red light responses can be induced poses problems. *Phaseolus* hypocotyls and *Avena* first internodes have a photomorphogenic threshold for continuous irradiance thousands of times less than the intensity of full moonlight (Klein et al. 1956), but actual photoperiodic control of plant development may require irradiation at energy levels 10^{10} higher than this threshold (Withrow 1959). Such an enormous range is difficult to reconcile with the idea of a single type of link between the photoreceptor and plant development.

Withrow (1959) divided red, far-red photoresponses into two kinetic classes: (a) nonperiodic photomorphogenic responses characterized by a rate which is a continuous graded function of energy and is not closely related to any time phasing of the light, and (b) time phase controlled (photoperiodic) reactions which characteristically result in threshold type all-or-none responses.

On this basis altered growth rates of stems and leaves upon transfer from darkness to some continuous light regimen would be a nonperiodic photomorphogenic response, and its intensity would have some discernible relation to light intensity. Responses elicited by periodic light treatment, such as dormancy induction, are different. They bear little relation to light intensity and much more to its duration and periodicity.

A significant difference is that the responses elicited by nonperiodic light treatment characteristically begin to develop at very low incident energy and increase (not necessarily linearly) with increasing energy to a saturation level. In the threshold type of ungraded responses which are characteristically induced by periodic light treatment, no response is evident until a threshold of stimulus intensity is attained. The response then rapidly becomes maximal and further increase in intensity has little effect.

The amount of energy involved in eliciting minimal nonperiodic photomorphogenic responses is exceedingly small, probably only a few quanta per cell per hour (Withrow 1959). Responses result from many hours of continuous exposure. The intensity of radiant energy needed to induce photoperiodic threshold type responses is hundreds or thousands of times greater, but still very much less than full sunlight. Withrow believed that the various differences between graded (photomorphogenic, but nonperiodic) and nongraded (photoperiodic) responses were not correlated with primary reactions of photoreceptors, but depended upon physiological and biochemical conditions in the tissues involved (Withrow 1959).

In general, both periodic and nonperiodic red, far-red photomorphogenic systems fail to obey the Bunsen-Roscoe reciprocity law. This means that equal responses are not obtained when the product of the intensity of light and the time for which it acts is held constant. In nonperiodic systems particularly, continuous irradiation is more efficient than any regimen of intermittent irradiation (see Withrow and Withrow 1944).[22] This implies that continuous renewal of a photochemical product is required to overcome decomposition or ineffective utilization.

Reciprocity failure in photoperiodic systems can result from time phase requirements as well as initial photoproduct losses. For example, the effectiveness of a light flash during a long night depends not only upon its intensity and duration, but also upon the position of the flash within the time span of the dark period (Salisbury and Bonner 1956). Lack of reciprocity is not incompatible with the phytochrome concept. With P_{730} to P_{660} reversion as the "clock," the phytochrome mechanism could explain time phase requirements within any one cycle. But the usual requirement is for several weeks of repetitive photoperiod-nyctoperiod cycles before morphogenic changes are induced. There are few data on biochemical or physiological changes occurring within the plant during this period.

In the minds of some physiologists time phase requirements imply existence of some rhythmic process within the plant which determines responsiveness to photostimulation at any particular time. Such endogenous rhythmic processes (circadian rhythms or biological clocks) can be demonstrated. Their possible interaction with photomorphogenic stimuli is discussed in the following section.

Circadian Rhythms in Relation to Photo- and Thermoperiodism

Endogenous Circadian Rhythms

Some physiologists have concentrated upon those aspects of growth control encompassing light quality, light intensity, action spectra, photoreceptors, and mode of action of the latter. In studying photoperiodism they tended to emphasize photo- and neglect periodism. At the same time other physiologists regarded photoperiodism merely as another manifestation of the endogenous rhythms known to exist in a great number of plants and animals. These latter physiologists stressed periodism and paid less attention to purely photo- aspects. This dichotomy is understandable in the light of the historical development of this branch of plant physiology.

The existence of endogenous, approximately diurnal rhythms in plants was known for almost 200 years before Garner and Allard (1920) published the first paper on photoperiodism. Such illustrious names as Duhamel, de Candolle, Dutrochet, Sachs, Hofmeister, Pfeffer, and Darwin occur frequently in the early literature (for references see Bünning 1960a). Because of the long tradition of descriptive and speculative approach to the study of plant rhythms, the subject, especially in the minds of biochemical physiologists, has

[22] The universal validity of this statement has been brought into question, however, by some recent work (Borthwick and Cathey 1962) concerning prevention of flowering in *Chrysanthemum* by providing intermittent light during long nights which would otherwise induce flowering.

acquired an aura of mysticism which is only slowly being dispelled by results of experimental work.

Although the early literature refers to the endogenous rhythms made manifest by such phenomena as leaf movements, as being diurnal, this is not strictly correct. Under constant environmental conditions[23] deviations of several hours from the theoretical 24-hour period are common. Furthermore, by means of crosses between strains of *Phaseolus* with varying endogenous periods Bünning (1935) found that such deviations are inheritable characteristics. Under natural conditions the endogenous periodicity (the so-called physiological clock) is corrected to approximately 24 hours by a recurrent event such as dawn or dusk.

Because the rhythms are not inherently diurnal, the term "circadian" (*circa* L. = about + *dies* L. = day) was proposed by Halberg et al. (1959) and has gained wide acceptance. Implicit in the term "circadian rhythm," as it is currently used, is the concept of an endogenous rhythm. The periodic features of the natural environment are presumed to act mainly as modifying or entraining agents, but sometimes as initiating agents. In general, a circadian rhythm becomes diurnal when it is modulated by the natural environment.

Professor Erwin Bünning has long been a leader in research in circadian rhythms in plants, and any discussion of the subject must rely heavily upon data provided by Bünning and his collaborators. Yet Bünning's ideas have not been universally accepted. In turn, significant developments arising from work at Beltsville, in the Netherlands, and at the Smithsonian Institution, for the most part have not been incorporated into, or reconciled with, Bünning's ideas. Bünning (1958) has discussed his ideas in a book entitled "Die physiologische Uhr" (the physiological clock), but important modifications of his concepts have appeared more recently (Bünning 1959a, b, c, 1960a, b, 1961). The significance of Bünning's modified ideas in relation to other recent developments is discussed below.

Circadian rhythms or oscillations are often lacking in plants that have long been maintained under constant environmental conditions, but frequently a single stimulus can induce oscillation (Bünning 1931; Ball and Dyke 1954). Such a stimulus might be a short light period within otherwise continuous darkness, or a transfer from continuous light to continuous darkness. Induction of oscillation under these conditions could possibly be only a synchronization of preexisting nonsynchronized rhythms within individual cells.

Rhythmic fluctuations of nuclear volume have been suggested as being a manifestation of a basic cellular periodicity (Bünning and Schöne-Schneiderhöhn 1957). Wassermann's (1959) data, however, do not support the synchronization hypothesis of whole plant rhythm induction. He found that in *Vicia faba* rhythmic changes in nuclear volume cannot be detected in cells prior to induction of whole plant rhythms.

[23] Truly constant environmental conditions may, however, be an unattainable ideal. It is probable that variable pervasive geophysical factors are always present in even the most rigorously controlled experimental systems (Brown 1960).

According to some reports the induction and maintenance of organism level rhythms is most effectively accomplished by red light and is antagonized by far-red (Bünning and Lörcher 1957; Lörcher 1958). This action of far-red is not often mentioned in discussions of photo-effects upon plants.

Once an endogenous rhythm has been induced, its period under natural conditions is automatically adjusted to 24 hours. Under artificially regulated photoperiods the period of oscillation may be shortened to as little as 16 hours, but more extreme conditions may cause the endogenous rhythm to override the stimulus of the imposed photoperiod and revert to its natural ciracadian period (Kleinhoonte 1929). Awareness of the possibility of such behavior is essential to interpretation of some experiments in photoperiodism.

In Bünning's opinion, endogenous circadian rhythms are of special importance to time measurement in photoperiodism. Under natural environmental conditions the rhythm takes the form of a diurnal oscillation between extreme physiological states. One cycle of the oscillation consists of two phases,[24] each lasting 11 to 13 hours. The extreme physiological states are highly significant because certain essential reactions can presumably occur only when a specific extreme state prevails. If the oscillations are damped out by long exposure to constant conditions, the extreme states are no longer reached and the reactions dependent upon them are inhibited.

The above is a theoretical explanation for physiological injury of plants grown under continuous light when temperature is also held constant. Such injuries have been observed in tomato plants by Hillman (1956) and in *Fagus sylvatica* and *Abies alba* by Bałut (1956). Suppression of the endogenous rhythm may result in inhibition of discrete steps in development. For example, *Oenothera* forms buds in continuous light but the buds fail to open (Arnold 1959). Such developmental inhibitions may be removed by treatments which re-induce endogenous oscillation. Return to natural environmental cycles is effective, but so is a single dark period of 6 to 10 hours, or even a low-temperature treatment of that duration in the light (Wassermann 1959). In some algae a single dark or cold period given once a week will prevent inhibition of development (Ruddat 1961).

Circadian Rhythms and Photoperiodism

Bünning (1936, 1958, 1960a, b) believes that the endogenous oscillation is the basis of photoperiodic response because it alternately activates different cellular processes which in turn cause rhythmic changes in light sensitivity or responsiveness of cells. Also involved, however, is the inherited time scale which can be demonstrated in many organisms. Its most common manifestation is critical day or night length for a particular response.

In effect, actual day or night length is compared to the critical length, and when the latter is exceeded certain processes are favored while others are inhibited. The result is a photoperiodic response. The presumed relation between the endogenous oscillation and the

[24] Until recently Bünning referred to the two phases as "photophil" (light loving) and "scotophil" (dark loving). "Tension phase" and "relaxation phase" have now replaced the older terms.

inherited time scale is that the former determines light sensitivity at any time, whereas the latter operates as a stimulus threshold which must be exceeded before some types of response are possible.

The question of the mechanism by which plants measure time is older than the modern concept of photoperiodism and was already implicit in 19th century work on endogenous rhythms. Most hypotheses have involved reactions beginning at the beginning or end of light or dark periods. Such reactions were supposed to promote gradual synthesis or decay of a substance to some threshold level. A difficulty with these hypotheses has always been that critical day or night lengths are only slightly temperature dependent (Lang and Melchers 1943). The latter fact favors physical rather than chemical time-measuring systems.

Bünning, as early as 1936, suggested that time-measuring reactions in photoperiodism are dependent upon endogenous rhythms (for arguments see Bünning 1958). Went (1959) has, in part, supported these ideas. It appears to me that this concept really implies two time-measuring systems. One controls or maintains the period of the basic endogenous rhythm and measures elapsed time within a cycle. The other is a system able to recognize photoperiods or nyctoperiods as longer or shorter than the critical length and measures time only in a comparative sense. Saying that time measurement depends upon endogenous rhythms only transfers the problem because maintenance of endogenous rhythms itself must depend upon time measurement if cycle length is to be uniform and relatively independent of temperature. Bünning does not discuss these difficulties.

According to Bünning (1960b), diurnal oscillations control photoreactions by inducing quantitative differences in sensitivity to light and qualitative differences in response. An example of the former is cyclic behavior of chlorophyll synthesis in *Hyoscyamus* exposed to light breaks at various times during a long dark period (Clauss and Rau 1956). The obvious argument is that if chlorophyll synthesis is so distinctly cyclic in its response to light, why cannot the synthesis or activity of photoreceptor pigments also be cyclic?

The effect of a break in a long dark period need not be maximal in the middle of the dark period (Harder and Bode 1943). The maximum occurs at a definite time with respect to the beginning of the preceding light period, or, in some species, the beginning of the dark period (Clauss and Schwemmle 1959; Claes and Lang 1947). Bünning (1960b) has interpreted other work employing 48-hour cycles (Claes and Lang 1947; Bünsow 1953) as supporting the hypothesis of a qualitative change in light sensitivity about every 12 hours. Reportedly circadian changes of this type may persist for 3 days or more in constant darkness (Melchers 1956).

Bünning (1960b) does not deny that a photomorphogenic pigment is involved in photoperiodism, but he does not believe that the pigment reactions are in themselves the basic time-measuring elements. His hypothesis is that some other physiological clock causes cyclical changes in conditions controlling pigment-linked processes. How does the clock do this? No explanations are available.

As for actual operation of the physiological clock, Bünning (1960a, b) has postulated that it is characterized by regular alternations of tension and relaxation phases (*p. 121*). Presumably the tension phase is endergonic and the relaxation phase is exergonic. Interruption of energy supply, by treatment with respiratory inhibitors or low temperature, during the tension phase causes reversion to the relaxed state and hence a shift in phase. The magnitude and direction of the shift depends upon when the energy supply is restored. If energy supply is maintained at a low level, tension phases are shortened and consequently relaxation phases also. Thus, under low-temperature conditions periods of endogenous rhythms become shorter and of lesser amplitude. Near 0° C. they may be damped out completely.

Red light increases period length and periods under continuous red light may be 3 to 5 hours longer than in darkness (as measured by leaf movements in *Phaseolus*). Far-red has the expected antagonistic effect (Lörcher 1958). Bünning believes that red light effects are a consequence of its increasing the "driving force of the oscillator" so that the tension phase lasts longer. Various surfactants and some alkaloids (Keller 1960) also lengthen periods.

These ideas on the nature of the physiological clock mechanism have not yet won wide acceptance. It is difficult to equate tension and relaxation phases with presently known cellular processes. While there is an interaction between photoreceptors and the physiological clock, the latter is not directly dependent upon photochemical energy. Obviously, much remains to be discovered in this field.

The work of F. W. Went and his collaborators has also contributed greatly to our understanding of the significance to plant development of periodic changes in the environment. Emphasis was put upon photoperiodism and thermoperiodism, and there was a constant awareness of Bünning's work on endogenous rhythms. Highkin and Hanson (1954) reported that continuous light or abnormal cycles of alternate light and dark periods 6 or 24 hours long are all injurious to tomato plants. Under constant environmental conditions the apical meristem produces fewer primordia and becomes smaller (Went 1959). Hillman (1956) found that injury caused by continuous light could be relieved either by normal light-dark cycles or by 24-hour cyclic temperature changes over a sufficient range. Dark periods at 48- or 72-hour intervals also were partially effective.

Reports such as those cited above led Went (1960, 1961) to postulate that for normal development at least some plants require a diurnal rhythm in the environment, and that this may be either a light or a temperature cycle. Thus Went and Bünning agree that for a part of the plant growth process to proceed normally the endogenous circadian rhythm must persist and be synchronized with a circadian environmental cycle. Like Bünning, Went believes that the physiological clock can be reset or synchronized by light, but that there is no direct link with the photomorphogenic pigment system because temperature cycles are also effective, even in constant light.

688-803 O—63——9

Went (1960) disagrees with Bünning on temperature independence of period length of endogenous cycles. Bünning accepts Leinweber's (1956) results showing no temperature effect upon cycle length, although his own early work (Bünning 1931) did indicate an effect. Went (1959, 1960) ascribes the negative results of Leinweber and others to diurnal changes in the redox level of the atmosphere (due to air pollution) and maintains that if growth chamber air is purified cycle length does vary with temperature. The Q_{10} for cycle length, however, is only 1.2 to 1.3. To account for this low Q_{10} Went (1960) suggests that a diffusion of macromolecules is involved and that it, rather than a chemical process, controls cycle length.

Circadian Rhythms and Thermoperiodism

Even under favorable photoperiodic conditions some plants are adversely affected by constant temperature and respond markedly to a day-night temperature differential. *Pyrus ussuriensis* gives such a response but *Malus baccata* does not (Potapenko and Zakharova 1940). *Pinus taeda* grows much better with a warm day and a cool night than at a uniform temperature (Kramer 1957, 1958). Hellmers (1962) obtained similar results with *Pseudotsuga menziesii* but found that growth of *Pinus sabiniana* seedlings was not inhibited by 8 months exposure to a constant temperature of 17° C. Hellmers and Sundahl (1959) also reported that growth of *Sequoia sempervirens* seedlings was not significantly inhibited by lack of a day-night temperature differential. The physiological significance of such results is not clear.

When a thermoperiod is superimposed upon a photoperiod it is difficult to say which effects are mediated primarily via the thermoperiod and which arise through temperature effects upon the photoperiodic mechanism. Even if reversion of P_{730} to P_{660} is the nyctoperiod measuring reaction and is not particularly temperature sensitive, subsequent enzymatic reactions in the linkage between the photoreceptor pigment and growth control are presumably temperature sensitive. A night temperature change would affect these reactions and, consequently, affect plant development even though no endogenous cycle or thermoperiodic requirement were involved.

It does not yet seem necessary to postulate the existence of a thermoperiodic receptor. The effects of thermoperiods separate from those of nonperiodic temperature conditions and photoperiods in woody plants remain to be studied. Hillman (1956) and others (see Went 1959) have obtained definite responses to thermoperiods per se in tomato.

In summary, the interrelation between photoperiodism, circadian rhythms, and thermoperiodism is real, but still nebulous. This is true not only because of the extraordinary complexity of the physiology concerned, but also partly because various groups of physiologists have not made maximum use of each other's results and ideas. However imperfect current ideas on circadian rhythms may be, there is, in my opinion, scant justification for interpretation of results of experiments involving photoperiodic responses entirely without reference to the possible existence of such rhythms.

Endogenous Growth Regulators

Introduction

Interrelations between photoperiodism, thermoperiodism, and circadian rhythms are still poorly defined. A second area of confusion exists with respect to the relationships between photomorphogenic pigments, endogenous growth regulators, and the ultimate biochemical mechanisms which control growth and development. There is at present little understanding of the relationship between rhythmicity in any of its aspects and the more concrete realm of pigments, hormones, and enzymatic reactions.

The dearth of basic information, particularly with respect to mode of action of the so-called growth regulators, makes an intelligent and balanced discussion of the entire subject impossible at this time. An attempt will be made here to survey the confused situation, to define some of the gaps in our knowledge, and hopefully to aid the reader in locating areas in which additional research would be most helpful.

Before so-called growth regulators can have any effect upon metabolism they must first be synthesized by metabolic reactions. These reactions involve enzymes, the synthesis and activity of which is also regulated by some means. Ultimately there must be some first stage of regulation determining which segments of the total complement of information encoded in the genetic material of the cell shall be operative and which enzymes shall be synthesized. This first stage control may be exercised by the cellular environment. But the indirect consequences of a particular set of environmental conditions may persist long after those conditions have changed (*pp. 19, 21*). Because of this lag the total environmental effect is an integrated resultant of past and present environmental stimuli.

In some instances the persisting effects of past environmental (whole plant) conditions may actually be mediated through persisting growth regulators (*pp. 95, 152*). There is, however, no need to assume that this is always true. The responses to more immediate environmental conditions could also be mediated by the action of hormonal or other regulators upon metabolism. But it should not be assumed that the regulators are necessarily directly involved in the reactions of intermediary metabolism. There is no inherent reason why they could not act upon enzyme synthesis or activation. Gibberellin, for example, can reportedly enhance amylase activity (*p. 145*).

Are plant growth regulators necessarily hormonal? Do they exert control by participating in the reactions of intermediary metabolism; by activating or inactivating enzymes; by controlling enzyme synthesis; or by controlling availability of genetic information (*p. 163*)? Are they agents by which receptors of environmental stimuli, such as phytochrome, influence the course of growth and morphogenesis? The reader can, no doubt, formulate additional meaningful questions which cannot yet be answered. I ask him to maintain this questioning frame of mind while reading what follows.

In the following pages recognized growth substances, about which a massive literature has accumulated, are considered by classes. The generic terms "auxin," "gibberellin," and "kinin" are employed in keeping with generally accepted current usage, but it is recognized that these terms may have little general significance when applied beyond the limits of a specifically defined test situation. It is recognized also that similar physiological responses in test objects may result from substances which are chemically quite different. Ability to produce a similar ultimate response in bioassays may be the only obvious region of similarity between the various compounds now considered as auxins by some workers. In fact, the term "auxin" as it is used here and in other contemporary literature is little more than a convenient figure of speech.

The Auxin Concept

The word "auxin" immediately brings to mind 3-indoleacetic acid (IAA). Although this compound is commonly accepted as being a (or even *the*) major growth regulator and as having wide distribution in the plant kingdom, proof of this is not nearly as rigorous as might be supposed. IAA has been isolated and crystallized from maize kernels (Haagen-Smit et al. 1946) and from the vegetative parts of cabbage (Post 1959, cited by Fawcett 1961). However, its supposed widespread occurrence is based almost entirely upon chromatographic and other nonrigorous evidence (Bentley 1958; Fawcett 1961). In addition, results of IAA assays have been negative for numerous tissues (references given by Bentley 1958; Crosby and Vlitos 1961).

Many papers dealing with the manifold effects of auxin on growth and metabolism have been based upon the view that IAA is the major auxin. Recently, however, there has been a rapidly increasing awareness that several, or many, naturally occurring auxins may exist, that IAA may have a special position only because of its prior discovery, and that attention should be given to other auxins also.

There is now considerable justification for Bentley's (1958) opinion that IAA, as such, is probably not the auxin which is physiologically active in normal growth, and that numerous other indolic and nonindolic auxins do occur. Bentley also suggested that the relatively ether-insoluble auxins might be the physiologically active forms, even though research has been almost exclusively devoted to ether-soluble auxins. Literature concerning indole auxins has been reviewed by Fawcett (1961) whereas the possible interconversion of ether-soluble and ether-insoluble auxins has been treated by Bentley (1961).

The widespread use of simple extraction techniques combined with one-dimensional paper chromatography has resulted in publication of a large number of histograms, in which areas on papers are demonstrated to contain compounds which accelerate or retard normal events in various test systems. The resolving power of such methods is inherently poor, particularly when relatively crude extracts are used. In addition the superposition of growth inhibitors and promoters may result in both being undetected.

Extraction techniques also have sometimes been at fault in not preventing enzymatic conversion of tryptophan to IAA during extraction (Wildman and Muir 1949). More refined techniques are now needed. Greater efforts toward obtaining separation of specific classes of compounds in extraction and fractionation prior to chromatography should be rewarding. The necessary task of isolation and identification of the compounds responsible for activity on chromatograms would thus be lightened.

The ultimate utility of work leading to estimates of free or diffusible IAA in various tissues or in organs at various stages of development is now also open to question. IAA may be only a transport form of the active auxin and the amount of free, diffusible, or extractable IAA may, therefore, not be the important physiological parameter that was once supposed.

An additional difficulty is the apparent widespread occurrence of enzymatic systems capable of inactivating IAA. These so-called auxin oxidases are frequently so active at cut surfaces and in homogenates that they greatly reduce the amount of free IAA obtainable. There is no proof that the oxidases are equally active in intact tissue. Naturally occurring auxin oxidase inhibitors are, in fact, quite well known (Ray 1958; Sacher 1961, 1962). The activity of such inhibitors may sometimes be influenced by thermoperiodic, photoperiodic, or light intensity conditions (Garay et al. 1959; Watanabe and Stutz 1960). Auxin oxidase inhibitors have also been assigned a role in the auxin-sparing hypothesis of gibberellin action (Vlitos and Meudt 1957; Brian and Hemming 1958; Galston and Warburg 1959; Garay et al. 1959).

Steeves et al. (1953) found that cyanide could be used to inhibit auxin destruction at cut surfaces and that yields in agar block diffusion tests could thus be greatly increased. It is important to note that no cause and effect relationship between auxin destruction and growth control has yet been unequivocally demonstrated (but see Pilet and Dubouchet 1962). Because of auxin-destroying enzymes, inhibitors of such enzymes, auxin-complexing agents, and other aforementioned difficulties, measurements of auxin activity diffusing from cut surfaces or assays of activity in homogenates are of doubtful physiological significance.

A fundamental block to progress is, of course, our lack of knowledge about the mode and mechanism of action of auxins within the cell. This subject has recently been reviewed by Galston and Purves (1960) who concluded that none of the multitude of chemical and physical changes observable in responsible cells after treatment with auxin has yet been causally related to subsequent growth of the cell, and that the mechanism of action of auxin remains unknown. These authors analyzed the problem and formulated it as a series of questions approachable by present techniques.

It is now of great importance to determine the intracellular locus of the primary auxin reaction and to determine the form of auxin mediating this reaction. There is a possibility that there is no one primary locus of action, but that regulatory reactions in different parts of the cell are controlled by several auxins and that the type of growth and development resulting depends upon coordination of these by other auxin or nonauxin regulators. A similar argu-

ment could be made on the tissue level (Sachs 1961). The study of auxin complexes such as auxin-protein, ascorbigen, and indoleacetylaspartate (for reference see Fawcett 1961) may be fruitful because such complexes could be related to the active forms.

Slow progress in elucidation of the mechanism of auxin may be partly attributed to the tacit assumption during past decades that IAA and closely related compounds assayable by the various *Avena* tests were *the* growth regulators to be studied. However, recognition of gibberellins and kinins as naturally occurring growth regulators has gradually forced a reevaluation of the old auxin concept (Kefford and Goldacre 1961). Present open-mindedness and improved techniques may lead to advances.

Auxins in Buds and Shoots

Went's (1927, 1928) demonstration of a quantitative relationship between auxin and elongation in the *Avena* coleoptile and his development of auxin assaying methods opened a new era of research on growth control. An obvious point to be investigated was whether dormancy could be the result of a deficiency of growth substances.

Boysen-Jensen (1936) attacked this problem directly by injecting auxin solutions into the internodal pith cavities of resting shoots of *Forsythia*. He also decapitated resting shoots of *Salix*, *Syringa*, and *Aesculus* and put their basal ends in solutions of growth substances. Neither treatment had any dormancy breaking effect. Other investigators, however, found that under some conditions auxins applied to the cut top surface of woody shoots were effective in activating dormant cambium (Gouwentak 1936; Söding 1936; Brown and Cormack 1937). Gouwentak and Maas (1940) pointed out the nonequivalence of applying hormones to the basal and apical ends of cut twigs.

Further work by Gouwentak (1941) revealed that auxin applied to apices of *Fraxinus ornus* can activate the cambium to produce earlywood, but only if rest has already been broken by normal chilling or by chemical or warm bath treatment. Amlong and Naundorf (1938) also found the work of Boysen-Jensen (1936) unconvincing because Le Fanu (1936) and Snow (1936) had reported that passage of auxin upwards through stems has an inhibitory effect on growth. They painted auxin solutions on *Syringa* buds which had not received normal winter chilling. In most instances treated flower buds opened somewhat earlier than controls, but there was little effect upon leaf buds (Amlong and Naundorf 1938). Gouwentak (1941) used these results to strengthen her contention that auxins are not rest-breaking agents and are effective only as activators after rest is already broken.

While some physiologists were studying the effects of treating dormant twigs with growth substances, others approached the problem by investigating the auxin content of twigs.

Huber (1931) used the *Avena* coleoptile curvature test to assay dormant buds of *Fagus*, *Picea*, *Quercus* and other genera for growth substance content. Results were negative. A few years later, however, Czaja (1934) had no difficulty in obtaining diffusible auxin from *Fagus sylvatica*, *Pinus sylvestris*, *Picea pungens*, *Quercus*

rubra, and numerous other species. The significant difference was that Czaja used swollen or unfolding buds rather than dormant buds. This is illustrated by the results of Zimmermann (1936).

Zimmermann found that resting or quiescent buds generally yield no diffusible auxin, but that content increases rapidly as buds open and soon again declines. In *Faxinus* and *Acer* he obtained larger yields from elongating internodes than from the buds above them. Avery et al. (1937) could obtain no diffusible auxin from winter buds of *Aesculus hyppocastanum* or *Malus molus*. Like Zimmermann they found a peak yield just prior to the period of most rapid shoot elongation. They considered the locus of auxin production to include the terminal bud and young internodes (*pp. 130–133*).

Bennett and Skoog (1938) correlated the appearance of diffusible auxin in fruit tree buds with the end of rest. They applied solutions of growth substances to the cut surfaces of decapitated dormant shoots and got some positive results. IAA was moderately effective in inducing bud break, but yeast extract was much more so. Mirov (1941) measured diffusible auxin in shoots of *Pinus ponderosa* and *P. torreyana*. In developing shoots, the uppermost 5 mm. yielded the least auxin. The yield increased with distance from the apex to reach a maximum near the base of the new shoot. Thus the region of maximum diffusible auxin is not necessarily coincident with the region of most rapid growth, a point also noted by Zimmermann (1936).

Changing levels of growth-regulating substances accompanying bud swelling and unfolding is not confined to diffusible auxins and may not always include the latter. An increase in water soluble extractives of the bios group (vitamins of the B complex) has also been reported (Dagys 1935, 1936; *see also pp. 136, 150*). In *Syringa vulgaris* a seven- to twentyfold increase in bios-type substances may follow breaking of rest by warm bath treatment (Jarkovaja 1939). Substances assayable by the *Avena* coleoptile bending test reportedly also increase, but to a much lesser extent. From the work of Guttenberg and Leike (1958), likewise with *Syringa*, it appears that growth after artificial rest breaking is not always accompanied by appearance of demonstrable auxin.

When total extractable auxin is measured the results may be quite different from those obtained by measuring only diffusible auxin. Kassem (1944)[25] found a much larger amount of total auxin in *Pyrus* shoots early in rest than later. There was a continuous decline in yield as the end of rest approached. Eggert (1951) obtained somewhat similar results with *Malus*. Kassem, however, also found that diffusible auxin increased in spring.

Observations made by Allary (1957, 1958) indicate that the relations between total and diffusible auxin fractions are probably different in different species. Disagreement in the literature is therefore to be expected. In *Syringa vulgaris*, *Ginkgo biloba*, *Sambucus nigra*, and *Viburnum opulus* expanding leaves liberate, not diffusible auxin, but a precursor which is converted to such auxin in the internodes. In *Faxinus excelsior* and *Acer pseudoplatanus* diffu-

[25] Kassem, Mohamed Mahmoud. The seasonal variation of hormones in pear buds in relation to dormancy. 1944. (Doctoral Diss., Univ. California, Berkeley.)

sible auxin may be obtained from all organs of the growing shoots. In *Quercus pedunculata* diffusible auxin cannot be detected in any of the organs during the growth period, but assayable auxin can be extracted with ether. In view of such variability results of diffusible and extractable auxin assays must be interpreted with caution.

The work cited above justified the view that, whatever its significance, diffusible auxin yield in many resting or quiescent buds is negligible, but that within about a month in spring it may rise to a high value and again decline almost to zero as the new shoots elongate. This work also made it obvious that relations between auxin and growth in developing woody shoots are far more complex than they appear to be in the case of the classic experimental material, the *Avena* coleoptile. Studies of the development of long and short shoots of *Ginkgo biloba* and other species have provided additional observational data on the relations between auxin and growth.

In considering the early work on auxins the reader should note that full appreciation of the uncertainties introduced into auxin assays by the existence of endogenous growth inhibitors (*pp. 150–154*) has only recently been attained. Such inhibitors may or may not have their effects via auxin-regulated systems, but it is easy to see that their presence in extracts and diffusates could interfere with auxin assay by the usual curvature or straight growth tests. The question to be asked is whether assay results represent total activity of a certain type or fraction of auxin or are instead a resultant of the opposite effects of growth promoters and growth inhibitors.

Auxins in Developing Long Shoots Versus Short Shoots

In the early stages of bud development in *Ginkgo biloba* there is no morphological difference between potential long and short shoots. All active buds begin development as short shoots. Bud-borne embryonic leaves initiated during the preceding season expand rapidly, but the internodes between them elongate little. Within a few weeks apical meristems of some shoots initiate additional primordia which rapidly develop into leaves. Internodes between these elongate and long shoots are formed. The subapical meristems of other shoots are not activated, and additional primordia initiated by their apices usually develop into bud scales (Sprecher 1907; Foster 1938; Gunckel and Wetmore 1946a, b).

Morphological differences between long shoot and short shoot leaves have been reported (Sprecher 1907; *see discussion of leaf dimorphism pp. 43–44*). Available data do not allow one to be certain that all long shoot *Ginkgo* leaves are derived from primordia initiated in spring or that the internodes between leaves present in the winter bud never elongate.

The pattern is different in *Larix decidua* in that the putative long shoot and short shoot buds are morphologically dissimilar, their anatomy and mode of development having been determined by events of the preceding season (Frampton 1960; *p. 52*). The control of subapical meristem activity and consequent internodal elongation is at the crux of the dormancy problem; therefore, the manner in which subapical meristem activity is promoted in some buds of *Ginkgo* and inhibited in other similar buds is of great interest.

Gunckel and Thimann (1949) and Gunckel et al. (1949) studied *Ginkgo* shoot development with respect to diffusible auxin yield. In agreement with results from other species, they found no appreciable auxin in dormant buds collected in March (Cambridge, Mass.) A transient phase of high auxin yield accompanies swelling in all buds and a decline begins prior to scale opening. When petioles begin elongating (which precedes axial elongation in *Ginkgo*) some buds show a secondary increase in auxin yield. These buds develop subapical meristem activity and become long shoots. The auxin yield of other buds continues to decline. These fail to develop subapical meristem activity and remain short shoots.

As the long shoots begin to expand internodes, auxin yield from the apical regions (including the three youngest visible nodes) declines greatly. The region of maximum auxin yield shifts to the second or third node above the base of the new shoot. This means that maximum yield is obtained from internodes which have already passed their peak growth rate and that growth declines *before* diffusible auxin yield.

Gunckel and Thimann (1949) suggested that growth is limited by a factor other than auxin and that diffusible auxin may merely be a surplus which was not used in elongation. The locus of maximal auxin production in the elongating shoot was not determined. It may be in internodes higher than those giving maximal yields. Although there is plentiful evidence that auxin is produced by young leaves in many angiosperms, the auxin in young *Ginkgo* shoots is probably produced in the internodal tissue itself.

Young *Ginkgo* leaves yield insignificant amounts of diffusible auxin. Nonetheless they seem essential to normal auxin production because shoots yield much less a few days after defoliation (Gunckel and Thimann 1949). The work of Hatcher (1959) with *Malus* and *Prunus* also indicates that the free auxin content of the shoot apex may be less than that of the expanded internodes below and that internodal tissue may synthesize its own auxin.

Decapitation studies in *Ginkgo* have shown that development of most lateral buds into short rather than long shoots is due to apical dominance. After decapitation, one or two upper lateral buds, which would otherwise have produced short shoots, become long shoots. Gunckel et al. (1949) were able to prevent this response by application of a suitable concentration of naphthaleneacetic acid to the stump after decapitation. Interestingly, IAA was only slightly effective, but this may have been because of unsuitable concentrations. These results were interpreted as showing that hormones from developing terminal long shoot buds and young internodes could inhibit activation of subapical meristems in lateral buds.

The factors initiating subapical meristem activity in putative long shoot buds are still unknown. The characteristic secondary rise of auxin yield in developing long shoot buds precedes visible internodal elongation and may be correlated with subapical meristem activity. However, this does not mean that such auxin is the cause of the activity, for the appearance of large amounts of diffusible auxin in some buds and not in others is itself a manifestation of a more basic control mechanism.

According to the work of Titman and Wetmore (1955), in *Cercidiphyllum japonicum*, as in *Ginkgo biloba*, long or short shoots arise from buds which are morphologically indistinguishable. Leaves are dimorphic (*pp. 43–44*) in *Cercidiphyllum* a single precociously expanding leaf appears from each opening bud. In the short shoot this is the only leaf expanded. Enlargement of the remaining primordia and activation of the subapical meristem is completely inhibited. After a brief spurt, accompanying expansion of the precocious leaf, diffusible auxin yield declines to zero.

In some buds expansion of the precocious leaf is followed by activation of the subapical meristem, internodal elongation, expansion of remaining leaf primordia, and, thus, long shoot formation. As the shoot elongates, diffusible auxin rises rapidly to a peak about threefold higher than during precocious leaf expansion. Attainment of the peak is followed abruptly by rapid decline in auxin yield, cessation of elongation, and apical abortion.

As in long shoots of *Ginkgo*, the center of auxin production in *Cercidiphyllum* long shoots is probably in the subapical region of elongating internodes rather than in the apical meristem or leaves. Again it is not possible to decide whether increased auxin production is a consequence of internodal elongation or vice versa, and a more remote control mechanism is indicated. And again, as in *Ginkgo*, it is evident that the more significant control is that exercised on the subapical rather than on the apical meristem.

Seedlings of a few *Pinus* species, notably *P. palustris*, normally undergo a so-called grass stage of from 2 to 15 years or longer before active height growth begins. Such dwarf seedlings lack subapical meristem activity (*p. 34*). They are in some ways similar to the short shoots of *Ginkgo* but are more complex. Because the needles of *Pinus* are themselves borne on short shoots, a dwarf (grass stage) *P. palustris* seedling consists of a short shoot axis bearing numerous short shoot branches. When elongation growth begins, subapical meristem activity is initiated in the axis and it develops into a long shoot.

Normally, as in other pines, the needle-bearing dwarf shoots of *Pinus palustris* always remain short. In *Ginkgo*, occurrence of lateral short shoots can often be ascribed to dominance exercised by an apical long shoot. In *P. palustris* seedlings, apical dominance is not a factor in maintaining the grass stage. The developing terminal bud itself lacks significant subapical meristem activity.

Brown (1958) studied the auxin relations of grass stage *Pinus palustris* seedlings using *Avena* coleoptile curvature tests as an assay. He found no diffusible auxin at any stage of development. Ether extractable auxin could be obtained only during the 2- or 3-week period including bud swelling and opening. The yield of extractable auxin dropped to zero as needles began to elongate. Yields from long and short shoots were similar.

Brown also reported that apices of grass stage seedlings will take up IAA from agar blocks and transport it upward without utilizing or destroying it at any appreciable rate. He interpreted this as evidence that the usual basipetal transport mechanism is inoperative in these buds. However, the importance of this observation cannot yet be evaluated because IAA may not be a naturally occurring growth

substance in *Pinus*, and the polar transport of those auxins which do occur has not been tested.

Allen (1960), using elongation of *Pinus elliottii* hypocotyl sections as an assay, was able to demonstrate extractable growth substances in long shoot buds of *P. palustris* saplings even in January. With the approach of spring, content of growth-promoting substances increased and that of growth inhibitors decreased. Allen suggested that seasonal changes in these compounds are correlated with regulation of the rest period. Although one promoter behaved similarly to IAA on paper chromatograms, no positive identifications were made.

Growth substances in buds and shoots of *Pinus* are of special physiological interest because of the occurrence of short shoots on even the most vigorously growing long shoots and because of the latent capacity of short-shoot meristems to give rise to long shoots. The study of *Pinus* growth substances, however, has advanced only enough to reveal that a variety of compounds may occur. Fransson (1953, 1959) obtained a substance from *P. sylvestris* seedling shoots which stimulated *Avena* coleoptile growth but was not identical with IAA. He called it Pinus I.

Ogasawara (1961a) and Ogasawara and Kondo (1962) studied the growth substances of *Pinus thunbergii* buds and needles by extraction, chromatography, and *Avena* straight growth tests. They found three growth promoters and two inhibitors which gave positive tests with Ehrlich reagent. An additional promoter giving color reactions and R_f values similar to those of IAA was found after treatment of buds and leaves with tryptophane. Ogasawara (1961b) obtained similar results with *P. strobus*. He tentatively identified one of the growth promoters as IAA.

Thus there is accumulating evidence that IAA does occur in some woody shoots and buds. However, recent work makes it seem likely to me that most species have a multifarious complement of growth promoters and inhibitors of which the classical auxin, IAA, is a frequent but not necessarily ubiquitous component.

Auxins and Cambial Activity

The nature of the stimulus which causes the dormant cambium to become active in spring has long been a subject of speculation and research (for references see Jost 1891; André 1920; Ladefoged 1952; Larson 1962a). Before the widespread acceptance of the plant growth-hormone concept, investigations were chiefly directed toward determining the time and locus of reactivation of the cambium in spring, the rate of propagation of meristematic activity, and the time relations between shoot elongation and cambial growth. These aspects are still important because they define the operational characteristics of the control mechanisms which must be present.

It is easy to suppose that a product of renewed development and growth of buds in spring provides the stimulus initiating cambial activity in the twigs beneath and that, therefore, cambial growth should be first observable in the twigs each season. Evidence of this idea is present in the work of Thomas Hartig (1853) and Mer (1892) both of whom also noted, however, that in some trees cam-

bial activity appears to begin almost simultaneously throughout branches and trunk.

Robert Hartig (1892) reported that in isolated trees initiation of cambial activity was almost coincidental throughout branches and trunk but that under forest conditions initiation proceeded from the small twigs downward. The suggestion that initiation of cambial activity occurs in larger branches and the middle trunk has also been made for *Pinus rigida*, *P. strobus* (Brown 1912, 1915), and *Larix laricina* (Knudson 1913).

Such reports illustrate the diversity of opinion concerning initiation and propagation of cambial activity (for details see Grossenbacher 1915). Priestley (1930) analyzed the then available information and, in agreement with T. Hartig (1853), concluded that in dicotyledonous trees cambial activity invariably commences in bud bases and is propagated downward. The rate of propagation, however, may be much greater in ring-porous than in diffuse-porous species (Priestley et al. 1933; Priestley and Scott 1936; Wareing 1951b), which explains some early reports of simultaneous initiation throughout hardwood trees.

The situation in conifers is less clear cut. Priestley (1930) conceded the possibility that reactivation of the cambial meristems might sometimes occur without benefit of bud influences. This may be related to the persistence of slight cambial activity in the trunk or needles throughout the winter (Münch 1938; Oppenheimer 1945). However, even in conifers normally differentiated vascular elements are produced only if developing buds are present (Münch 1938; Jost 1893).

Reports that cambial activity may be initiated in the trunk and propagated in both directions have never been substantiated. Accumulated evidence indicates that, in general, the initiating stimulus arises in the bud and is propagated basipetally throughout the aerial part of the plant. In roots some acropetal propagation may occur as a continuation of the initiation wave down the stem (Brown 1935).

Concomitant with efforts to determine the origin of the cambial stimulus were attempts to characterize the stimulus itself. The suggestion that cambial growth depends on some influence coming from the leaves, particularly growing leaves, was made by Jost (1891, 1893). This was based upon experiments involving ringing disbudding, defoliating, and withholding light from twigs. He conceived the influence to be translocated morphologically downward, but not upward, and to be distinct from the nutrient supply. At about the same time R. Hartig (1892) proposed that renewed cambial activity results from increased food supply from new leaves. Subsequent advances supported Jost's idea of a nonnutritive initiating agent moving basipetally (Kastens 1924; Coster 1927–1928; see also Jost 1940).

In work with herbaceous plants Snow (1933) demonstrated that the cambium activating influence can pass a protoplasmic discontinuity by diffusion and is likely to be a soluble hormone. Subsequently he showed that pure synthetic auxins at very low concentrations can induce cambial activity in *Helianthus* stems (Snow 1935). Demonstration of the presence of auxins in woody plants eliciting reactions

similar to those in herbs (Czaja 1934; Zimmermann 1936) prompted the testing of Snow's ideas with trees. It is noteworthy that the subsequent widespread use of IAA (then called "heteroauxin") in experimentation with trees resulted from its ready commercial availability and not from proof that IAA is the most important native auxin in twigs and buds. Such proof is not yet available.

IAA was shown to be effective in inducing undifferentiated cell proliferation in the cambium of decapitated twigs of *Tilia* sp. which had been held dormant for over a year (Gouwentak and Hellinga 1935). Excised and decapitated *Fraxinus ornus* and *F. excelsior* twigs treated with low concentrations of IAA in February and March (Wageningen, The Netherlands) sometimes responded with normal wood production a short distance below the point of application (Gouwentak 1936).

Repetition of such experiments with disbudded shoots of *Populus nigra* and *Salix fragilis* after a very severe winter resulted in normal wood production throughout the length of the test shoots. However, similar shoots treated in autumn produced only a little new wood in the first few millimeters below the point of IAA application (Gouwentak and Maas 1940). Others also reported only localized wood production following synthetic auxin application to twigs in winter or early spring (Söding 1937a; Brown and Cormack 1937).

These collective results led Gouwentak (1941) to suggest that IAA can activate the cambium to produce new wood along the whole shoot length only if rest has already been broken by cold treatment or by other agents. She maintained that auxin itself may elicit cambial activity in resting stems, but only in local areas where rest was broken as a result of wounding (Brown 1937). Gouwentak (1941) also showed that treatment of resting *Fraxinus ornus* twigs with the chemical rest-breaking agent, ethylene chlorhydrin, prior to auxin application, greatly increases the extent of new wood production. This can be used as an argument that cambial as well as subapical meristems do pass through a resting phase and that auxin is not a primary rest-breaking agent.

Auxin is often effective in inducing cell division in cambia of resting and nonresting stems, but it does not follow that it is normally the only, or the primary, agent participating in cambial control. Extracts of scrapings from *Acer circinatum* cambium are much more effective in promoting cell division in *Helianthus* cambia than would be expected on the basis of their measurable auxin content alone (Söding 1940).

The presence of other active agents is suggested. Using *Phaseolus multiflorus* as a test plant Künning (1950) found that thyamine and ascorbic acid were just as effective as auxin in stimulating cambial activity. Furthermore, extracts of both resting and active *Tilia ulmifolia* cambium were effective in promoting cell division, the action being similar to that of yeast extract. This agrees with the report of Dagys (1936) that extracts of dormant and active *Salix fragilis* cambium scrapings are about equally effective in promoting cell division of yeast.

Tissue culture of cambial explants has revealed that growth factors other than IAA influence development and are needed for long-

term survival. However, the explants, even when taken from a dormant parent tree, often contain sufficient growth substances, vitamins, and cofactors to maintain proliferation for 6 to 8 weeks (Gautheret 1948; Jacquiot 1950). Dormant buds and twigs also contain readily assayable amounts of thiamine, riboflavin, pyridoxine, niacin, inositol, pantothenate, and biotin.

Riboflavin, niacin, and inositol undergo considerable increases as the buds swell and burst in spring (Burkholder and McVeigh 1945). In view of such results it is likely that cambial control is actually achieved by the interaction of several regulators, a concept which has been discussed by Söding (1952) and by Wareing (1958b).

There is some evidence that the auxin obtainable from twigs and branches is actually localized in the cambium (Söding 1937b, 1940). Indeed, Kramer and Silberschmidt (1946) found more auxin in the cambium of a variety of woody species than in any other tissue. Measurements of the distribution of growth substances between wood and bark after peeling are of little interest because it can be shown by scraping that almost all of it is derived from the cambial layers and their immediate derivatives.

Though sieve tubes of some trees reportedly contain large amounts of growth substances (Huber et al. 1937; Huber 1939), these may actually be derived from the cambium by diffusion. Whether the cambium itself can translocate growth substances is of interest because many species have no functional phloem present during the period of cambial initiation (Söding 1952). This is because secondary sieve tubes commonly become functionless during the later part of the season in which they are initiated (Esau 1950). However, in some species functional phloem may be present in spring (Gill 1932; Elliott 1935; Huber 1939; Esau 1950; Bannan 1955) and could serve to transport hormones.

Present evidence strongly supports the concept that cambial activity is normally initiated in the bud bases and is propagated basipetally from there (see Priestley 1930; Ladefoged 1952; Fraser 1952). Nonetheless there have been reports of bark slippage or actual wood formation in spite of removal of buds or rings of bark above the stem sections examined (Münch 1938; Wareing 1951b; Reines 1959; Dvořák 1961). Among the hardwoods there is considerable difference between diffuse-porous and ring-porous species in this respect.

Both diffuse-porous and ring-porous hardwoods develop adventitious [26] buds after the original buds are removed. However, in ring-porous species the stimulus from adventitious buds in very early stages of development is sufficient to initiate cambial activity which is then autocatalytic. In diffuse-porous species even strong adventitious buds elicit only weak cambial development immediately below. Wareing (1951b) has ascribed these differences to the presence of a reserve of auxin precursors in the cambium of ring-porous species and to the lack of such reserves in diffuse-porous species.

In conifers, as in ring-porous hardwoods, cambial activity can spread rapidly after being initiated by buds in early stages of de-

[26] The term "adventitious" is used loosely here. It is likely that many supposedly adventitious buds are really supplemental axillary buds (*p. 59*) of the type discussed by Sandt (1925).

velopment (Priestley et al. 1933). In some pines even the old short shoots may produce growth substances which in association with wound responses are sufficient to maintain or initiate wood formation below debarked rings (Münch 1938; Onaka 1950). Logs from trees felled and topped in mid- or late winter often become peelable in spring, though later than standing trunks (Huber 1948).

Wounding, of course, always accompanies cutting and ringing operations. Because of this, cambial activity after disbudding, below bark rings, or in isolated stem segments is not a strong argument for the cambial meristem being able to initiate its own activity in an intact plant. Even so the possibility of exceptions to basipetal propagation cannot be excluded entirely. Stewart (1957) believes that high bark temperatures may lead to conversion of stored precursors to active auxin and induce some activity before a stimulus has arrived from more apical regions. According to Dvořák (1961) new xylem can be found at the base of the stem in *Prunus armeniaca* independently of activity in the twigs from which all normal and adventitious buds had been removed.

If cambial activity is initiated by a flow of growth regulators from developing tissues in the bud into subjacent tissues, then the transition from production of the large diameter cells of the earlywood to the narrow summerwood cells may be a result of a decline or change of composition of the regulator flux after the initial burst of spring growth. It has been reported that appearance of new leaves, after defoliation by insects or other agents, is often accompanied by renewed earlywood formation (Kny 1882; Jost 1891; Studhalter 1955). Similar results have been obtained by experimental defoliation (Kühns 1910). However, it is possible that if starvation becomes a factor because of repeated or late-season defoliation, failure of normal cell wall thickening of latewood may give the false appearance of earlywood (Harper 1913).

Fraser (1949, 1952) found that reversion from latewood to earlywood formation can be induced experimentally by application of suitable concentrations of IAA. Priestley (1935) proposed that the same conditions which inhibit further stem elongation and induce winter bud formation also cause transition from earlywood to latewood production. Wareing (1958a) elaborated this hypothesis to the extent of pointing out the photoperiod as an important external factor and plant growth substances as the mediating agents (see also Wareing 1951a; Wareing and Roberts 1956). Experimental testing of this hypothesis was undertaken by Larson (1960a, b; 1962a, b). Results support the hypothesis.

The literature contains a considerable number of reports on the time relations between cambial activity, bud break, and elongation growth.[27] In most of this work, girth increase or bark peelability was taken as a criterion of cambial activity. Actually neither of these criteria is indicative of actual meristematic activity.

Swelling is the first step in reactivation, and the bark may be peelable as much as a month before cell divisions begin (Huber

[27] Christison 1889; Mischke 1890; Reuss 1893; Walter 1898; Wieler 1898; Buckhout 1907; MacDougal 1921; Lodewick 1928; Chalk 1930; Cockerham 1930; Priestley et al. 1933; Kienholz 1934; Fowells 1941; Friesner 1942; Reimer 1949; additional references are given by Ladefoged 1952.

1948; Wilcox et al. 1956; Wilcox 1962a). It is, then, not surprising that various authors reported xylem formation to begin anywhere from several weeks before to several weeks after bud break. There is general agreement that rapid xylem production continues until the main flush of elongation growth has passed, and then declines. In young shoots, at least, this period coincides with the period of high diffusible auxin yield (Zimmermann 1936; Hatcher 1959), but evidence that the same is true in older branches and the main trunk is still lacking.

It is generally accepted that auxin is produced in actively meristematic tissues. If so, there is no need to postulate a mass flow of auxin down the stem as the cambium is reactivated. Perhaps only a minute amount of growth regulator need diffuse in turn from each cambial cell into its subjacent neighbor. It is also possible that an auxin precursor is already present in the dormant cambium (Wareing 1951b) and that most of the cambial auxin at any time is present in a bound form (Hatcher 1959). Failure to demonstrate diffusible auxin in the cambium of older stems is, therefore, not particularly strong evidence against auxin involvement in control of cambial development. Of course, even if it were proven that auxin is always involved in initiating cambial cell division, the possibility would remain that auxin is only a mediating agent in turn controlled by other regulators.

The fact that phloem is also a product of cambial cell divisions and that the distribution in time of its production is different from that of xylem implies operation of a complex regulating system. In many tree species there is little phloem production during the vernal surge of xylem formation. Generally phloem development is lesser in amount, begins later, but continues longer and at a slower, more steady rate than xylem development.[28]

Perhaps the diffusible growth regulator supply associated with bud swelling and rapid shoot elongation is required, not for cambial activity per se, but for xylem differentiation, whereas continued cambial cell division and differentiation of phloem may have other requirements (*p. 40*). There is some evidence that the regulator supply required for normal wood production includes both auxin and gibberellin components (Wareing 1958b). The involvement of additional regulators would not be surprising. The role of mutual mechanical pressures and spatial relationships must also not be overlooked (*p. 67*).

The Significance of Auxins in Dormancy Control

The accumulated evidence concerning the role of auxin in dormancy control in buds and in the cambium is quite inconclusive. A factor more significant than total auxin content may be the relative efficacy of growth promoters and growth inhibitors. Experimental treatments may change these relations. Furthermore, certain treatments may change the content of a specific fraction of the auxin complement more than the total. The occasional successes of

[28] Strasburger 1891; Raatz 1892; Rees 1929; Cockerham 1930; Elliott 1935; Fraser 1952; Grillos and Smith 1959; Bannan 1962.

some workers in breaking rest with auxins or auxin precursors (Bennett and Skoog 1938) can thus be understood.

The occurrence of supraoptimal and inhibitory concentrations of auxin has long been invoked in attempts to explain lateral bud dormancy associated with apical dominance (Thimann and Skoog 1933, 1934; Thimann 1937) (*pp. 82–83*). Although these explanations are not necessarily correct (Jacobs et al. 1959; Libbert 1961), empirical attempts have been made to prolong, as well as to break, dormancy of woody plants by applications of growth substances.

Marth (1942, 1943) tested the ability of a variety of growth substances to prolong dormancy of rose bushes in nonrefrigerated storage. Naphthaleneacetic acid and some of its derivatives prevented bud growth for as long as 60 days, but the effective concentration range was narrow. Injury resulted from too high concentrations, whereas too low concentrations promoted rather than inhibited growth.

Ostrom (1945) tested similar methods on forest tree seedlings and successfully used naphthaleneacetic acid and mixtures of other synthetic auxins to prevent formation of etiolated shoots during nonrefrigerated storage of *Fraxinus americana* seedlings. Results with other species were variable and did not indicate that auxin treatment could be substituted for the usual cold storage. Way and Maki (1946) and Maki et al. (1946) reported similar variable results.

The possibility of delaying bud break in spring by use of synthetic auxins has received some attention because it appears to offer a means of reducing late spring frost damage. Results of winter or spring applications have not been promising because of associated toxic effects.

Spring applications of IAA and naphthaleneacetic acid derivatives in lanolin emulsion to dormant tung tree (*Aleurites* spp.) buds delayed bud break, but also killed many buds. Lanolin alone, to a lesser extent, also prolonged dormancy and was not as toxic. A commercial vegetable-based shortening was slightly effective and still less toxic (Sell et al. 1944). The observed effects may have been partly due to interference with gas exchange. Another possibility is that some constituents of lanolin or vegetable-based shortening have growth substance activity (see Crosby and Vlitos 1961).

Hitchcock and Zimmerman (1943) used a different approach. They reported that summer or autumn spraying of fruit trees with potassium naphthaleneacetate retarded opening of buds the following spring. Light dosages in July were as effective and much less injurious than considerably heavier ones in September. Others, however, found similar treatments rather ineffective (Batjer 1954).

Readers specifically interested in applications of auxins to horticultural problems, such as control of flower bud opening, control of blossom and fruit drop, or induction of parthenocarpic fruit development, may wish to consult the monograph by Audus (1959) for discussion and references.

It is, I believe, unlikely that anything definite about the true role of auxin in dormancy control can be established until the mode of action of auxin within the cell becomes known.

688–803 O—63——10

Gibberellins

The generic term "gibberellin" refers to all substances having a carbon skeleton similar to, or identical with, that of gibberellic acid, and which promote cell elongation, cell division, or both, in plants (Phinney and West 1960a). Of the nine chemically distinct gibberellins which have been isolated and characterized, only giberellic acid (also known as gibberellin A_3 and abbreviated as GA or GA_3) has been readily available in sufficient amounts to allow testing on a variety of plants. In most of the literature dealing with responses of woody plants the term "gibberellin" refers to gibberellic acid. In this discussion gibberellic acid and the abbreviation GA refer to the specific compound otherwise known as gibberellin A_3.

Although the first gibberellins to be isolated were metabolites of the fungus *Gibberella fujikuroi*, many plant extracts have since yielded substances with properties similar to the fungal gibberellins (Mitchell et al. 1951; Radley 1956). The natural occurrence of gibberellins in plants has now been established,[20] and these compounds are recognized as being functional in the control of plant growth and development, along with auxins and other regulators (Stodola 1958; Phinney and West 1960a.)

There is some evidence that gibberellins may occur in arborescent species as well as in herbaceous plants. Sumiki and Kawarada (1961) isolated crystalline gibberellin A_1 from abnormal, witches'-broom type apical bud sprouts of *Citrus unshiu*. Unidentified gibberellin-like substances have been found in stem callus tissue cultures of *Ilex aquifolium* (Nickell 1958) and in immature seeds of woody legumes including *Robinia pseudoacacia*, *Sophora angustifolia*, and *Cercis chinensis* (Murakami 1959). Recently Kato et al. (1962) reported finding gibberellin-like substances in *Juniperus chinensis torulosa* fruits and in the immature fronds of the tree fern *Alsophila cooperi*. Extracts from the *Juniperus* fruits had a specificity pattern on dwarf *Zea mays* mutants characteristic of gibberellins A_5 and A_9 rather than A_3.

More evidence is needed to establish the occurrence of gibberellin-like hormones in the vegetative parts of normal trees. Westing (1959) found no indication of such hormones in *Pinus radiata* shoots, but other methods may yet succeed in demonstrating their presence. Naturally occurring inhibitors of gibberellin-induced growth are also known. Corcoran et al. (1961) found such substances in immature seeds of *Ceratonia siliqua* (the carob tree).

The ready availability of synthetic GA has resulted in a large amount of empirical testing of responses of a variety of trees to exogenous application of this particular gibberellin. Because in most instances no special attention was paid to environmental factors, state of plant development, or type and amount of endogenous gibberellins present at the time of treatment, this approach has not yielded much information of fundamental value. It has, however, shown that responses to exogenous GA can be extremely varied and that much more information about the mode of action of gibberellin

[20] Stowe and Yamaki 1959; Brian 1959; Phinney and West 1960a; Adler et al. 1961; MacMillan et al. 1961.

and other hormones is needed before the varied responses can be understood.

Gibberellin has been shown to promote sprouting of hardwood cuttings collected in winter (Larson 1960b), to shorten the dormant period of certain trees and shrubs, but also to prolong dormancy in some instances. Growth of *Carya illinoensis* seedlings is promoted by soaking seeds in GA solutions and by spraying such solutions on the plants (Wiggans and Martin 1961). Weekly spray application of GA from August to November resulted in autumn elongation of new shoots in *Acer pseudoplatanus*, *Betula verrucosa*, and *Liriodendron tulipifera*, but not in numerous other species. In addition, bud break of *A. pseudoplatanus* and *B. verrucosa* was delayed the following spring, whereas in *L. tulipifera* bud break was unaffected but leaf expansion was slowed. *Fagus sylvatica* showed no autumn response, but nonetheless exhibited prolonged dormancy in spring (Brian et al. 1959a, b).

Direct introduction of GA potassium salt solutions into the xylem of *Populus* hybrids in spring had no effect upon growth during the normal growing season but elicited renewed growth in September (Hacskaylo and Murphey 1958). GA has also been applied to dormant trees in attempts to circumvent normal requirements for cold treatment or specific photoperiodic conditions. The chilling requirement for normal elongation of peach seedlings can reportedly be circumvented by GA treatment (Donoho and Walker 1957; Nitsch 1957b). It should be noted, however, that the effect of GA upon the epicotyl dormancy of dwarf peach seedlings from unchilled seeds may be temporary. The dwarf syndrome may reappear after the supply of exogenous GA has been exhausted (Flemion 1959; *see also p. 162*). According to Barton and Chandler (1957), GA applied to the hypocotl of the germinated seed replaces cold treatment in breaking the epicotyl dormancy of *Paeonia suffruticosa*. After the cold requirement has been satisfied, GA treatment hastens leaf and shoot development in some woody species, but not in others (Guzhev 1961).

The dormancy of *Fagus sylvatica* induced by short photoperiods can be overcome by GA treatment (Lona and Borghi 1957). The same is true in *Camellia japonica* (Lockhart and Bonner 1957), and in *Weigela florida* (Bukovac and Wittwer 1961). Some specificity of gibberellin type was found in the latter species in that gibberellins A_1 and A_3 (gibberellic acid) were effective in overcoming photoperiodically induced dormancy, whereas A_2 and A_4 were not. In contrast, dormant *Pinus coulteri* and *Pseudotsuga macrocarpa* held under short photoperiods under greenhouse conditions, did not respond to GA treatment even though these species are not known to require cold treatment (Lockhart and Bonner 1957).

The ability of GA in some species to counteract dormancy induction by short photoperiod treatment could mean that growth inhibition under short days results from a deficiency of a gibberellin-like growth regulator. Such a simple explanation is not favored by Nitsch's (1957a) results with *Acer palmatum*, *Quercus borealis*, *Rhus typhina*, and *Picea pungens*, all of which respond to GA under long as well as short photoperiods. Similar results were obtained with *Weigela* (Bukovac and Davidson 1959). However, *Pinus elliottii*

reportedly responds to GA only under short photoperiods (Bourdeau 1958), the contrary results of Nitsch (1957a) and Lockhart and Bonner (1957) with other conifers notwithstanding. The detached bits of information now available are sufficient to justify including gibberellin-type regulators, along with photoperiodism, temperature, and auxins, among the complex of factors controlling shoot dormancy.

Some cambial activity in disbudded cuttings of *Populus nigra* and *Fraxinus excelsior*, and in disbudded, potted *Acer pseudoplatanus* plants may be induced by IAA or by GA, but significant amounts of normal wood are produced only if both substances are supplied (Wareing 1958b). It is, therefore, probable that normal xylem development requires endogenous supplies of both auxin and gibberellin and that an abnormal ratio between the two leads to abnormal development of cambial derivatives (*p. 138*).

Aside from effects on dormancy, significant height growth increases resulting from GA treatment have been reported for a number of broad-leaved species,[30] although such increases were not always accompanied by increases in dry weight (Scurfield and Moore 1958). Effects on growth of conifers have been generally unspectacular (Westing 1959), sometimes nil (Knight 1958) or even detrimental (Kraus and Johansen 1960), yet some significant growth increases have been reported (Bourdeau 1958; Yatazawa et al. 1960; Melchior and Knapp 1962).

Bourdeau's point that most tests have been made under the naturally long photoperiods of summer and that different results may be obtained under short photoperiods is valid. However, the varied results obtained thus far indicate the need for more research on the natural occurrence of gibberellins and similar regulators in trees with particular emphasis upon species specificity. Lack of response to GA (one of nine known gibberellins) does not obligate the plant to behave similarly toward other gibberellins and does not eliminate gibberellin deficiency as a factor in growth control.

The mechanism of action of gibberellins, like that of auxins, is not yet known, though considerable effort has been expended on the problem. No general discussion of the subject can be included here (see Hillman and Purves 1961; Kato 1961; Galston and McCune 1961; Brian and Hemming 1961; and other papers in the same volume as those cited). However, a limited line of evidence concerning a possible mode of action of gibberellins will be treated because of its direct bearing upon control of shoot growth and dormancy (*see also p. 156*).

Some evidence suggests that gibberellins may be important regulators of subapical meristem activity. Rosette plants, like short shoots, or dormant buds, lack subapical meristem activity. In addition there is evidence that dwarf habit of peach seedlings grown from insufficiently chilled seed results from suppression of cell division in the subapical meristem (Holmsen 1960). Alleviation of the dwarf conditions by GA treatment (Nitsch 1957b;

[30] Marth et al. 1956; Nelson 1957; Seth and Mathauda 1959; Koverga and Koverga 1961; Nekrasova 1961; Wiggans and Martin 1961; Melchior and Knapp 1962.

Donoho and Walker 1957) implies reactivation of cell division (*but see pp. 141, 161–163*). In general, activation of the subapical meristem converts the rosette plant to a caulescent plant, the short shoot to a long shoot, and the bud to an elongating axis. GA can definitely activate the subapical meristems of some rosette plants and overcome inhibition of subapical meristem activity in some caulescent plants.

Lang (1956) found that *Samolus parviflorus* rosettes show a great increase in mitotic figures in the subapical region within 24 hours after GA treatment. The zone of cell division gradually increases in length, but cell elongation does not begin for about 72 hours, during which time two or three generations of cells have divided. It was also found that diffusion of GA to its site of action within the subapical meristem requires only about 2 hours and is not a factor in the delayed reactions (Sachs et al. 1959a). The initial observable effect is upon cell division, not upon elongation.

The GA-induced cell divisions in subapical meristems of rosette plants are mostly transversely oriented (Sachs et al. 1959b; Sachs and Lang 1957), and subsequent cell enlargement contributes mostly to elongation growth. Work by Negbi and Lang (1961) indicates that control of orientation of planes of GA-induced divisions depends upon supply of some substance from developing leaves. In defoliated apices that substance can be replaced by IAA. Thus GA is able to initiate cell divisions in the subapical meristem and also to promote subsequent cell elongation. Yet, normal cell orientation and stem tissue organization may require auxin.

Cytological studies have made it clear that stem elongation, the characteristic response to GA treatment in numerous rosette plants, is a consequence of greatly enhanced subapical meristem activity. By comparison, the contribution of cells to internodes by the more apical regions of the meristem region is so small that it may be disregarded (Sachs et al. 1959a, b). Sachs and Lang (1961) reported that rosette plants, in which stem elongation was induced by environmental manipulation, exhibit a subapical meristem development similar to that of GA-treated plants.

The importance of the subapical meristem to stem development is not limited to bolting of rosette plants. Cytological examination of apices of several normally growing caulescent plants have revealed a subapical zone of cell division much like that of elongating rosette plants. Because of these facts the behavior of the subapical meristem with respect to GA and other growth regulators is of first-rank importance to the problem of dormancy control (*p. 34*).

Though, as discussed above, GA greatly stimulates cell division in the subapical regions of *Hyoscyamus niger* and *Samolus parviflorus* rosettes, the situation in *Prunus* is somewhat different. Spraying branches of peach, apricot, cherry, almond, and plum with solutions of GA resulted in inhibition of cell division and retarded initiation of primordia in lateral bud meristems. At the same time growth in other regions of the shoot was greater than normal (Bradley and Crane 1960). This suggests that in woody shoots responses of terminal and lateral bud meristems to GA may be quantitatively or qualitatively different.

In spite of reports that shoot dormancy of some trees can be broken by GA treatment (Lona and Borghi 1957; Larson 1960b) there is as yet no cytological evidence that in these instances GA has its initial effect upon cell division in the subapical meristem. Al-Talib and Torrey (1959) found that GA induced some axial elongation of asceptically cultured, presumably dormant buds of *Pseudotsuga taxifolia* collected in November (Berkeley, Calif.). But GA treatment also invariably killed the buds.

Some chemicals, notably certain quarternary ammonium and phosphonium compounds, inhibit stem elongation in a variety of normally caulescent plants (Wirwille and Mitchell 1950; Marth et al. 1953; Wittwer and Tolbert 1960; Cathey and Stuart 1961) and thus appear to counteract some of the effects of GA (Cathey 1959; Sachs and Lang 1961). The stem growth retardants studied so far are of interest as research tools and as possible growth-control agents in husbandry. There are indications that some naturally occurring quarternary ammonium compounds are related to known growth retardants (Mayr and Paxton 1962; Paxton and Mayr 1962).

Preliminary work on the mechanism of action of a few growth retardants has shown that, like GA, some of them may have their effects upon cell division in the subapical meristem. For example, Amo-1618 [(5-hydroxycarvacryl)trimethylammonium chloride, 1-piperidinecarboxylate] when absorbed by the roots of growing *Chrysanthemum* plants, greatly reduces the number of cell divisions in the subapical meristem within 4 days and eliminates them almost entirely within 14 days. Plants so treated assume the rosette habit because the organogenic and more distal regions of the meristem are almost unaffected and leaf initiation continues. GA applied simultaneously with Amo-1618 and at the same concentration is able to prevent inhibition of subapical meristem activity. When applied 14 days after Amo-1618 it is able to reverse the inhibition within a few days (Cathey 1959; Sachs et al. 1960; Sachs and Lang 1961).

In contrast to compounds like Amo-1618, effects of maleic hydrazide (MH) are not specific. MH inhibits apical meristem activity as well as internodal elongation. Consequently MH treated plants, though dwarfed, do not form rosettes. GA is not generally effective in reversing MH inhibition (Sachs and Lang 1961), but it is sometimes partially effective (Bukovac and Wittwer 1956; Kato 1958). An explanation for this may be that MH exerts its effect upon a mechanism different from that controlled by GA or on the same mechanism at an earlier stage (Brian and Hemming 1957; Haber and White 1960).

The results of experiments with Amo-1618 and GA on plants such as *Samolus* and *Chrysanthemum* cannot be directly applied to woody species. Relatively few of the tested plants have responded to Amo-1618. Growth of *Acer rubrum*, *Euphorbia pulcherrina*, *Platanus orientalis*, *Quercus borealis*, and *Rhododendron* spp. is not retarded by Amo-1618, but all of these respond to another quarternary compound, (2-chloroethyl)trimethylammonium chloride (Cathey and Stuart 1961). This latter compound (also known as chlorocholine chloride and abbreviated CCC) and GA are mutually antagonistic in some systems (Wittwer and Tolbert 1960). The mechanism of such antagonism is not understood. There is some

possibility that CCC occurs naturally (Mayr and Presloy 1961; Paxton and Mayr 1962).

A very significant aspect of experimental work with gibberellins and selected growth retardants is that these regulators allow partially separate control of apical and subapical meristem activity. Thus they may aid in discovering how these meristems are separately controlled under natural conditions. Further work with gibberellins, auxins, and retardants is needed with respect to control of subapical meristem activity in buds of trees.

A different experimental approach to the function of gibberellin in growth control has been to observe changes in so-called growth inhibitor content (*pp. 150–154*) of tissue after treatment with GA. Work along this line has been limited, perhaps because there is still doubt that a cause-and-effect relationship exists between demonstrability of inhibitors in extracts and maintenance of dormancy in intact tissue (Wareing and Villiers 1961).

According to Nitsch (1957a), short photoperiod treatment of *Rhus typhina* results in a decreased content of growth promoters and an increase of growth inhibitors. Treatment with GA counteracts this effect, possibly by antagonizing growth inhibitors (*p. 96*). Fully dormant *Aralia cordata* (Imazu and Osawa 1958) and *Hydrangea macrophylla* (Stuart 1959) can be made to grow by GA treatment. These species have no distinct photoperiod requirement for growth but nevertheless have rest periods normally broken by cold treatment. In such instances GA may act to overcome inhibitors otherwise neutralized as a result of cold treatment, but there is no proof of this.

Reports have appeared concerning enhancement of amylase activity by GA treatment (Munekata and Kato 1957; Paleg 1960a, b) thus establishing a link between gibberellin action and carbohydrate metabolism. Disappearance of the inhibitor β complex (Bennet-Clark and Kefford 1953) of dormant potato tubers has also been correlated with rest breaking by GA treatment (Boo 1961) and with natural termination of rest (Hemberg 1958b). This is of interest because the inhibitor β complex includes a dialyzable inhibitor of α-amylase (Hemberg and Larsson 1961). Maintenance of dormancy by action of an amylase inhibitor has not been demonstrated in any woody plant, though occurrence of inhibitors possibly of the β complex type has been suggested in *Fraxinus excelsior* (Hemberg 1949, 1958a), *Acer pseudoplatanus* (Phillips and Wareing 1958), and *Pinus palustris* (Allen 1960).

From the above it is obvious that knowledge of the function of gibberellins in growth control is fragmentary. It must be emphasized, too, that in intact plants gibberellins do not have their effects upon systems isolated from effects of other controlling agents. Furthermore, gibberellins and auxins, should not be regarded as primary controlling agents, but as links in complex control systems. They may be remote from the reactions immediately controlling rate of cell division or elongation, and likewise remote from the primary reactions of phytochrome or other receptors of potentially morphogenic stimuli. The control of growth and morphogenesis is to be understood not in terms of reactions of a single regular but as the resultant of a complex of interacting processes.

Kinins [31]

The long-accepted belief that mature cells of the plant body were diploid has recently yielded to realization that somatic polyploidy (polysomaty) is widespread and perhaps general except in meristems. Polysomaty arises through failure of nuclear and cytoplasmic division to keep pace with deoxyribonucleic acid (DNA) synthesis and chromosome multiplication. Entrance into the polysomatic state may be a normal and important step in cellular differentiation and maturation (see Sinnott 1960; and Clowes 1961, for discussion and references).

Maintenance of diploidy in meristems, whereas polysomaty is the rule in older tissue, implies that division of the nucleus and cytoplasm (karyokinesis and cytokinesis, respectively) does not always follow chromosome multiplication and that the regulating systems controlling DNA synthesis and chromosome replication are not identical with those controlling actual cell division. On theoretical grounds, therefore, it might be supposed that meristematic cells contain not only factors regulating nucleic acid synthesis and chromosomal multiplication, but also other factors inducing karyo- and cytokinesis at appropriate times.

Experimental evidence for the existence of kinesis-inducing compounds, now referred to by the generic term "kinins," has come from tissue culture investigations (for review see Miller, 1961). A very active, specific chemical compound was isolated from commercial DNA preparations and identified as 6-furfurylaminopurine (Miller et al. 1956). This compound, known as "kinetin," has been used extensively in experimental work with higher plants (Miller 1961).

Synthetic kinetin stimulates cell division and sometimes cell enlargement of plant tissues, but only in the presence of IAA. The ratio between IAA and kinetin is very important in determining whether *Nicotiana* tissue cultures remain undifferentiated or develop buds (Skoog and Miller 1957; Wickson and Thimann 1958). On the basis of this and additional evidence Wickson and Thimann (1958, 1960) proposed that normal apical dominance (*pp. 82–83*) depends upon an antagonism between auxins and kinins within the plant (*see also p. 155*). There is also some evidence that root-initiating effects of auxins are counteracted by kinetin (deRopp 1956; Humphries 1960). In aseptically cultured *Pseudotsuga taxifolia* buds, kinetin appears to promote unorganized cell proliferation at the expense of normal leaf expansion and root initiation (Al-Talib and Torrey 1959).

In view of kinetin's ability to counteract some physiological processes normally associated with senescence (Richmond and Lang 1957; Osborne and McCalla 1961; Mothes 1961), the idea that kinetin's effects upon cell division may be quite indirect merits consideration. Growing organs have a high ability to accumulate solutes. Senescing organs lose that ability. According to Mothes

[31] The term "kinin" as used here and by plant physiologists generally (Miller et al. 1956) refers to substances apparently having certain kinds of regulatory activity over plant cell division. The same word is often used in the medical literature in a different sense, as a contraction of "bradykinin," to designate a group of polypeptide hormones occurring in blood, venoms, and other animal fluids. Plant and animal kinins are chemically unrelated.

(1961), kinetin increases the ability of cells to accumulate solutes, including such compounds as IAA. Applications of exogenous kinetin to local areas increases the ability of those areas to compete for nutrients and metabolites and may thus tend indirectly to promote cell division and growth. Inclusion of kinetin in the agar medium upon which *Pseudotsuga taxifolia* buds are cultured reportedly tends to promote basal callus development to the detriment of leaf expansion (Al-Talib and Torrey 1959). Such results would be predicted on the basis of the ideas advanced by Mothes (1961).

The idea that kinetin enhances the ability of cells to accumulate ions (Mothes 1961) is of added interest if integrated with new ideas concerning activation of biosynthetic systems when normal cells become tumorous. Six of seven essential biosynthetic systems liberated from normal control when cells of *Vinca rosea* become tumorous are, either directly or indirectly, ion-activable. Activation of the seventh, the metabolic system responsible for synthesis of kinin-type substances, appears to have different requirements (Brown and Wood 1962). Tumorous cells have very efficient ion uptake and utilizing systems and are in a favorable competitive position for nutrients with respect to normal cells (Wood and Brown 1961). The ion accumulating and translocating abilities of normal cells are presumably promoted by exogenous kinetin (Mothes 1961).

I encourage the reader to speculate upon the possibility that when quiescent cells resume active growth and division, kinins, if such regulators exist at all, are operative in activating ion translocating and accumulating systems. Increasing ion concentrations at sensitive sites may then activate biosynthetic systems as suggested by Brown and Wood (1962). How then, we might ask, is the synthesis and activity of kinins controlled? Though there are indications of interrelations with photomorphogenic mechanisms and auxin (Miller 1961), the question cannot yet be answered.

Wood and Brown (1961) also found that ion uptake and/or utilization by *Vinca rosea* cells is greatly facilitated by, and probably dependent upon, the availability of *myo-inositol*. This raises the possibility of a functional relationship or interaction between inositols and kinins. Increasing attention is being paid to inositols as growth regulators (*pp. 149–150*).

The mode of action of exogenously supplied kinetin, or any natural kinins, is unknown. Kinetin is a purine and as such might be expected to have its effects upon purine and nucleic acid metabolism (Patau et al. 1957), but other possibilities have not been eliminated (*see p. 155*).

Kinetin reportedly is effective in breaking winter rest of *Hydrocharis morsus ranae* buds (Kummerow 1958), though the effect is counteracted by added IAA. Exogenous kinetin also can overcome inhibition of development in the specialized buds on roots of *Ficaria verna* (Engelbrecht and Mothes 1962). The related compound 6-benzylaminopurine has been used to break dormancy (correlated inhibition) of axillary buds of apple (Chvojka et al. 1961). In none of these instances is there a clear indication of mechanisms involved.

There is no unequivocal proof that kinetin or any structurally related compound having similar properties actually occurs in vegatative parts of higher plants. Extracts from a number of sources promote cell division in a manner outwardly similar to kinetin.

Promotion of cell division may, however, be due to agents quite unrelated to kinetin. The assignment of a label to such unknowns does little to promote understanding.

Extracts possessing kinetin-like activity have been made from young apple fruits (Goldacre and Bottomley 1959) and the liquid endosperm of coconut or immature *Aesculus* fruits (Shantz and Steward 1955; Steward and Shantz 1959). These extracts promoted cell division and growth even in the absence of exogenous IAA from other sources. Perhaps they contained auxins as well as kinins. The occurrence in shoots and buds of woody plants of hormones regulating mitosis is possible, but evidence is not yet very strong. It is important to recognize that control over cell division may be quite indirect and that the process is not necessarily regulated by a single agent or system.

Other Possible Regulators

It must not be supposed that all important regulators of growth and morphogenesis will fit neatly into the current nomenclatural categories. There is no justification for neglecting consideration or study of compounds having apparent regulatory powers merely because they cannot be called auxins, kinins, gibberellins, or even vitamins. Leucoanthocyanins and inositols are examples of compounds known to occur in woody plants which may become recognized as important components of regulating systems after more information has been collected.

Coconut milk and the immature endosperm of *Zea* and *Aesculus* seeds contain substances promoting the growth of tissue cultures. Leucoanthocyanins have been associated with induction of cell division and growth responses by these preparations (Shantz and Steward 1955; Steward and Shantz 1956, 1959; Steward and Mohan Ram 1961). Leucoanthocyanins are not restricted to those plant parts and tissues which are, or become, highly colored. It may well be that the leuco compounds have greater physiological significance than the more obvious anthocyanins themselves. The occurrence of leucoanthocyanins in wood, leaves, and buds has long been known (Robinson and Robinson 1933).

Hillis (1955, 1956) studied the distribution of leucoanthocyanins in several species of *Eucalyptus* and found them most abundantly in areas of intense metabolism. Expanding leaves contain large amounts of leucoanthocyanin. The amount declines when expansion ceases. During active diameter growth, cambium and phloem from a trunk of *E. regnans* contained over one percent leucoanthocyanin, whereas the sapwood contained much less. After the spring growth flush had passed, leucoanthocyanin was almost undetectable in the cambium. Krugman (1956, 1959) made valuable studies of the distribution of leucoanthocyanins in the genus *Pinus*. However, no serious efforts have yet been made to determine the function of these substances in the tree.

Cell and tissue cultures derived from *Acer pseudoplatanus* cambium synthesize leucoanthocyanins rapidly when aeration is good, slowly when it is deficient. Thus the rate of gas exchange may be an important controlling factor in leucoanthocyanin formation in woody tissue (Goldstein et al. 1962). This is of interest because it

suggests a way in which an environmental variable may endow neighboring tissues with different amounts of compounds of morphogenic interest (*pp. 21, 45*).

If anthocyanin formation is a regulator of leucoanthocyanin level, then there is some evidence indicating a relation between growth effects of leucoanthocyanins and compounds involved in nucleic acid metabolism. Anthocyanin formation is inhibited by a variety of purines, including kinetin, and the inhibiton can be reversed by riboflavin (Thimann and Radner 1958). Light effects upon anthocyanin synthesis are, of course, well known and are possibly mediated through phytochrome (Hendricks and Borthwick 1959b; Kandeler 1960). Photoeffects upon leucoanthocyanin synthesis or utilization have not been specifically studied. All this is quite speculative, but nonetheless suggests an area in which we might look for a relation between leucoanthocyanin, kinetin, nucleic acid metabolism, light quality and intensity effects, and stimulation of cell division.

Another group of compounds which may be of interest as components of regulating mechanisms in trees are the cyclic alcohols, particularly the inositols and their derivatives. These compounds are widely distributed in woody plants. They occur free as D-, L-, or *myo*-inositol, as methyl ethers (pinitol, sequoiatol, lirodendritol, quercitol, scyllitol, etc.), as phosphates (phytic acid), and as the complex lipids, lipositols (for references see Ballou 1958; Angyal and Anderson 1959). Inositol derivatives accumulate in the wood of numerous species, but little is known of their origin or their distribution in buds, leaves, cambium, and roots. Burkholder and McVeigh (1945), by microbiological assay, demonstrated inositol in winter needles of 2 conifers and in dormant buds of 16 species of deciduous trees and shrubs.

Culture of tissue from tree species have sometimes revealed a requirement for, or a positive growth response to, inositols (White 1958; Steinhart et al. 1961, 1962). The fact that such requirements are not always evident may only mean that the tissues synthesize enough inositol so that it is not limiting under the culture conditions. According to Jacquiot (1951), in culture of *Ulmus campestris* cambium, the ratio of *myo*-inositol to adenine, and not the absolute level of each, is the determining factor in tissue organization and bud development. Recently the growth promoting activity of the neutral fraction of coconut milk has been found to reside very largely in its content of *myo*-inositol (Pollard et al. 1961). Furthermore, *myo*-inositol is present in large amounts in immature fruits of *Zea* and *Aesculus* along with leucoanthocyanins (Steward and Mohan Ram 1961).

The function of inositol in growing tissue is not definitely known. Amounts required are usually in excess of hormone or vitamin levels, yet too small to suggest utilization as a general carbon source. A specific structural use is indicated.[32] This concept has come to fruition in recent work with inositol requiring strains of *Neurospora crassa* (Shatkin and Tatum 1961). Electron photomicrographic and

[32] *Myo*-inositol in water solution administered through cut stems of parsley (*Petroselinum*) or strawberry (*Fragaria*) fruits is largely converted to D-galacturonosyl or pentose residues and incorporated into pectin or hemicellulose (Loewus et al. 1962). Such utilization of exogenous inositol is not necessarily related to normal use of endogenous inositol in meristematic tissue.

other data suggest inositol is a structural constituent of lipoprotein membranes including plasmalemma, nuclear envelope, mitochondrial membranes, and the endoplasmic reticulum. Inositol deficiency in inositol-requiring strains of *Neurospora* leads to membrane degeneration and gross morphological changes.

If inositol is also required for membrane formation in higher plants it should be detectable in expanding buds and other areas of rapid cell division and membrane synthesis. Indeed, Burkholder and McVeigh (1945) found inositol in dormant buds and that the amount increased tenfold or more during bud break. Folic acid and other vitamins increased to a lesser extent. The involvement of inositol in the formation of membranes or in the maintenance of their integrity could be related to the observations of Wood and Brown (1961) that ion uptake and/or utilization is facilitated by inositol and may even be dependent upon its availability (*p. 147*). The nature of any such relation is, however, still obscure.

Though leucoanthocyanins and inositols may have pronounced effects upon cell division and growth, neither they nor auxins, kinins, or gibberellins can be considered as prime movers of growth control mechanisms. Many synergisms and interactions are to be expected with apparent control shifting from one limiting factor to another as conditions change. Mere correlation of increased content of a presumed regulator with increased growth activity leaves the important cause and effect question unanswered. Furthermore, every change in level of the presumed regulator is itself cause for suspecting a more remote regulator, though it does not necessarily mean that such exists.

Endogenous Growth Inhibitors

Superficially the cause of dormancy may be considered from two points of view. Dormancy might be caused by the presence of growth inhibitors or by a deficiency of substances essential to the growth process. Though at first they seem poles apart, upon close examination these two viewpoints reveal only one underlying physiological problem—that of the nature of metabolic differences between dormant and actively growing tissue.

Deficiency of an essential substance may derange metabolism in such a way that growth inhibitors accumulate. Conversely, it is also possible that substances having no direct effects upon growth processes in short-term assays may interfere with synthesis or function of regulators and cofactors necessary to normal, long-term growth. Inhibitors may accumulate because of a deficiency of some factor essential to their degradation or to the utilization of their precursors in other reactions. The deficiency of this factor, in turn, may have been caused by a specific inhibitor of its synthesis or by some more remote deficiency or inhibitor.

Thus, when all superficiality is removed in the search for ultimate causes, it is not really possible to distinguish between presence of inhibitors and lack of essential substances or so-called growth promoters. It probably will not be possible until more is known about the biochemistry of growth control. Certainly there is no justification for prejudice for or against either growth promoters or inhibitors in favor of the other.

Continued study of endogenous growth inhibitors is justified and desirable if it is continuously related to the whole subject of metabolic differences between growth and dormancy and is not regarded as a discrete subject in itself. Increased emphasis is needed on design and interpretation of inhibitor assays and on distinguishing reversible and specific inhibitions from mere toxicity responses. Even with such emphasis, isolation and identification of an endogenous inhibitor is only one step in elucidation of a complex control mechanism.

What is the meaning and significance of the term "growth inhibitor" as it is commonly used in the literature? Too often it has been applied to some unidentified and uncharacterized substance contained in a relatively crude preparation having the power to reduce the growth rate of, or counteract the effect of IAA upon, *Avena* coleoptiles. The question as to whether such substances actually functioned as growth inhibitors in the tissues from which they were derived has only sometimes been asked and but rarely answered. The term "growth inhibitor," I believe, has been so much misused or misunderstood that its present usefulness is quite limited. As it is used here it means, in effect, "so-called growth inhibitor" or "a substance which by known or unknown means, not necessarily related to normal physiology, reduces growth in some test system."

Endogenous growth inhibitors have already been mentioned incidentally in relation to photoperiodism, auxins, and gibberellins. The discussion here complements what has been said earlier and summarizes recent developments. For discussion and review of early thinking and research on plant growth inhibitors and *Ermüdungsstoffen* (fatigue substances) see Reinitzer (1893), Weber (1918), and Linser (1940).

The decades of preoccupation with the auxin enigma diverted attention from other aspects of growth control but also led to the realization that endogenous growth inhibitors, or at least auxin antagonists, do exist. Indeed, auxin itself in high concentrations may act as a growth inhibitor (Skoog 1939; Eggert 1953). The possibility of dormancy being maintained by excess auxin is not appealing because dormant tissues are not characterized by high auxin content (*p. 128 ff.*). However, growth inhibitors which contain combined auxin and which may be hydrolyzed to yield active auxin have been reported (Stewart 1939; Libbert 1955).

Growth inhibitors have been obtained from vegetative tissues of a number of woody species including *Fraxinus excelsior* (Hemberg 1949); *Acer pseudoplatanus* (Phillips and Wareing 1958, 1959); *Pinus palustris* (Allen 1960); *Cornus florida*, *Rhus typhina* (Nitsch and Nitsch 1959); *Syringa vulgaris* (Guttenberg and Leike 1958); *Quercus pedunculata* (Allary 1959, 1960, 1961); *Betula pubescens*, and *B. lutea* (Kawase 1961a, b). Whether or not these various inhibitors are chemically related is not known.

The demonstrated presence of growth inhibitors in some buds and shoots suggests, of course, that under some conditions inhibitors might accumulate sufficiently to nullify growth promoters and thus become an important factor in dormancy induction. Ether extracts of *Fraxinus excelsior* buds contain compounds which overcome the effects of auxin in *Avena* tests. Extracts made in October are

highly inhibitory, but those made in February from buds collected outdoors (Stockholm, Sweden) have little effect. Treatment with ethylene chlorhydrin as well as exposure of the twigs to cold neutralizes or destroys the inhibitors (Hemberg 1949).

There is considerable evidence that seasonal changes in growth regulators are susceptible to photoperiodic control (Waxman 1957; Phillips and Wareing 1958, 1959; Nitsch and Nitsch 1959; Kawase 1961a, b). Growth regulators produced by trees grown under different photoperiodic conditions during summer can have effects upon time of bud break and amount of shoot growth the following season, even though all plants are subject to the same environmental conditions during winter (Waxman 1957). This phenomenon is evidence that plants are able to integrate the effects of environmental factors over long peirods and may explain some instances of unresponsiveness to short experimental treatments of varied photoperiods, etc. (*p. 95*). Carryover of growth regulators from one season to the next may be an important factor in success of transplanting trees to different latitudes or climatic zones.

No endogenous growth inhibitor from vegetative buds, shoots, or leaves has yet been isolated and rigorously identified. However, Hendershott and Walker (1959) isolated a growth inhibitor from dormant peach (*Prunus persica*) flower buds and identified it as naringenin (4′,5,7-trihydroxyflavanone). Decreased content of this substance on a fresh weight basis was correlated with emergence of the buds from rest (Hendershott and Bailey 1955), although no cause and effect relationship was established.

Dennis and Edgerton (1961) also found substances in peach flower buds which inhibited growth of *Avena* coleoptiles, but there was no correlation between inhibitory activity of extracts and the rest status of the buds. The inhibitors were confined to the bud scales. Applications of aqueous naringenin did not inhibit bud opening in spring. The function of naringenin in peach buds is therefore quite uncertain; nevertheless, it is interesting that naringenin contains the same carbon skeleton as leucoanthocyanins which are suspected of having growth regulating power (*p. 148 ff.*).

Naringenin is also structurally and sterically related to hydrangenol which has been found to enhance the growth-promoting effect of GA on isolated leaves of *Hydrangea macrophylla* (Asen et al. 1960). But naringenin, rather than enhancing GA activity, has been reported to antagonize it. Phillips (1962) was able to inhibit the dormancy-breaking effect of GA on peach buds with naringenin. It seems possible, however, that concentrations of both substances were unphysiologically high.

Jones and Enzie (1961) isolated from dormant peach flower buds a cyanogenic substance inhibitory to growth of *Pisum* stem sections. They tentatively identified the compound as mandelonitrile (*dl*-benzaldehyde cyanohydrin). The natural function of mandelonitrile, like that of naringenin, is unknown.

Hemberg (1949, 1958a, b) proposed that bud dormancy is due to specific growth-inhibiting substances, and that breaking of dormancy by chemicals or cold treatment is dependent upon decrease in endogenous inhibitors. Other investigators have contributed a small body of evidence indicating a decrease in inhibitor content

during the course of winter and a minimum content during the period of rapid shoot extension (Hendershott and Bailey 1955; Blommaert 1959; Guttenberg and Leike 1958).

The actual seasonal fluctuations of growth-inhibitor content of *Acer pseudoplatanus* buds were followed throughout the year by Phillips and Wareing (1958) who reported a minimum when buds were expanding, a gradual rise to a maximum in October, then a gradual decrease during the winter. These are again only correlations. Causal relationships have not been demonstrated. Pollock (1953) suggested that metabolic changes induced by low temperature might result in gradual disappearance of inhibitors. This suggestion is still a reasonable one, but has not yet been proven correct.

Some workers have assumed that cold treatment by some indirect means destroys growth inhibitors (but not growth promoters) remaining in the dormant tissue from the preceding growing season (Nitsch and Nitsch 1959), and this may be so in some species. Alternate explanations are (1) that cold treatment does not destroy the inhibitors but induces production or activation of promoters to overcome the inhibitors, or (2) that low temperature promotes metabolic changes such that the reaction blocked by the inhibitor is no longer important in controlling growth.

Such alternate explanations are made necessary by the finding that transition from rest to imposed dormancy is not always accompanied by disappearance of inhibitors. In *Syringa vulgaris* decline in inhibitor content does not begin until *after* the end of the rest period (Guttenberg and Leike 1958). In *Quercus pedunculata* also the end of rest is not manifested by any change in the β inhibitor complex content (Allary 1960, 1961). In both *Syringa* and *Quercus* the inhibitor disappears during the period of most active extension growth, but it reappears immediately afterward.

The fact is that in some species the full amount of so-called inhibitors is still present after the cold requirement has been satisfied. This suggests that cold treatment may permit production of growth promoters able to nullify the inhibitors without destroying them. Richter and Krasnosselskaya (1945) obtained preparations from developing buds of *Fraxinus* and *Tilia* reportedly capable of breaking dormancy in other twigs when introduced under the bark. Control experiments with water showed much lesser effects apparently associated with wound responses.

These results were confirmed by Danilov (1946) who, furthermore, broke dormancy of *Fraxinus* buds with homogenates of unfolding buds of *Quercus* and *Betula*. In such experiments nullification of remaining inhibitors may be an important factor. It is known that swelling buds contain a variety of vitamins and growth promoters (Dagys 1936; Burkholder and McVeigh 1945), and it is not surprising that homogenates of active buds can promote development of dormant buds. It is not certain that such substances can completely substitute for cold treatment.

Additional evidence that emergence from dormancy may involve accumulation of growth promoters which overcome the effects of growth inhibitors comes from a study of seeds and embryos of *Fraxinus excelsior* (Wareing and Villiers 1961). This work is of special merit because inhibitors and promoters were bioassayed, not

only with *Avena* coleoptiles, but also with *Fraxinus* embryos themselves.

Dormancy in *Fraxinus excelsior* seeds is apparently maintained by an inhibitory agent present in the endosperm and embryo. Inhibitor, reportedly absent from dry seeds, is metabolically produced after the seeds have imbibed water. Embryos from hydrated seeds which have not been cold treated are dormant, but that dormancy can be broken by leaching the excised embryos for 48 hours. A very important point is that application of embryo-derived inhibitor to leached embryos reestablishes their dormancy. Thorough embryo leaching, however, is not a necessary part of the normal germination process. Furthermore, leached but unchilled embryos produce only stunted seedlings.

A cold treatment of 5 to 6 months is essential to normal germination. No significant reduction in inhibitor content of embryos accompanies this treatment; instead, a germination promoter appears. This promoter is able to overcome the dormancy of unchilled and unleached embryos and also the stunted growth habit of leached but unchilled embryos. It thus appears that in *Fraxinus* seeds chilling is accompanied by accumulation of a germination promoter which not only overcomes an inhibitor but is in itself necessary for normal growth. The inhibitor is not actually destroyed by cold treatment.

On the basis of these results with *Fraxinus* seeds, Wareing and Villiers (1961) suggested the desirability of studying possible accumulation of growth promoters as well as disappearance of inhibitors during chilling of buds.

Little is known about the fate or function of growth inhibitors during the production of lammas shoots. In *Quercus pedunculata* they disappear just before lammas shoot growth begins, but they reappear before the new leaves have completely unfolded (Allary 1960). Whether the inhibitors appearing in new shoots in summer are similar to those present in winter is not known. Another interesting problem awaiting study is the function of growth inhibitors in the normal shoot tip abortion of numerous species (see Garrison and Wetmore 1961; *pp. 62–65*).

Answers to the problems alluded to above may be expected to come more largely from the comparative study of metabolic systems in dormant and growing tissue than from direct study of the so-called growth inhibitors which can be extracted from buds, leaves, or twigs.

Interactions

When the existence of biochemical growth regulators in plants was first established it was not unreasonable to expect that a single substance responsible for induction and breaking of dormancy might be found. Present knowledge of the importance of the photoperiod, of the existence of phytochrome, and of the multiplicity of endogenous growth regulators which probably exist in plants, make such an expectation seem naive.

It is now apparent that the control of growth is the resultant of many factors and that these factors interact or complement each

other in complex ways. The expanding literature dealing with this problem of interaction and interrelation is quite speculative and confusing. It would be presumptious and pointless to attempt a comprehensive review and discussion here. It must suffice to refer to a few important lines of thought.

Interpretation of experiments designed to show interactions, synergisms, or lack thereof, among the various growth regulators and light or temperature conditions is fraught with difficulty. This is true largely because the mechanisms of action of the known endogenous growth regulators has not been elucidated and because other regulators, as yet unknown, undoubtedly exist and contribute to the difficulty of interpreting experimental results.

The auxin concept is still an important part of contemporary thinking on growth control, but it is becoming increasingly obvious that auxin is not the preeminent regulator it was once presumed to be. Though the mode of action of auxin within the cell remains unknown, some progress has been made in understanding the nature of the problem. This progress has been consolidated and evaluated by Kefford and Goldacre (1961). These authors propose that auxin is a predisposing agent, regulators of other classes being the actual determinants of growth.

When auxin arrives in a cell after being transported from a distant site of synthesis via an auxin transport system, the reaction of the cell depends upon the presence of other regulators such as gibberellins and kinins. The type of reaction is determined by the nature of these regulators and their concentrations relative to each other and to auxin. Predominance of kinins favors cell division whereas predominance of gibberellins favors cell enlargement.

These concepts arose from results of experiments with isolated plant parts. It is very difficult to establish the validity of such interrelations in intact plants in which the interactions of the growth regulators themselves are further complicated by the presence of auxin destruction mechanisms (*p. 127*) and poorly understood auxin transport systems. To this is added the additional complication of responses to environmental factors such as photoperiod and temperature which may not be entirely mediated through the growth regulators mentioned.

There is considerable literature concerning a supposed interaction between kinetin and red light. It was, in fact, suggested that red light and kinetin may have their effects through the same biological mechanism (Miller 1956). Further developments, however, did not support this idea (Miller 1961). Powell and Griffith (1960) found that although both kinetin and red light promote growth of *Phaseolus vulgaris* leaf disks, kinetin stimulates growth by cell enlargement whereas red light induces growth by an increased rate of cell division. Hence kinetin, often considered to be a cell division regulator, can promote cell enlargement independently of division (*p. 147*). Kinetin does not merely substitute for red light. Leaf disks treated with both red light and kinetin grow significantly more than those treated with kinetin alone.

The hypocotyl hooks of *Phaseolus* have also been used as test objects. Opening of the hook is dependent upon cell elongation on the

688-803 O—63——11

concave side. This is promoted by red light. Cell division does not seem to be involved. Regardless of the different responses they elicit in other systems, kinetin, GA, and IAA all act to inhibit hook opening in both darkness and in red light. There is no evidence of any interaction between red light and the added growth regulators (Klein 1959). Such results illustrate the magnitude and complexity of problems still to be solved.

There is also a great amount of literature concerning interaction between gibberellins and light. Lockhart (1956) and Gorter (1961) have shown that GA at physiologically saturating levels can almost entirely overcome light-induced inhibition of stem elongation in *Pisum sativum*. Lockhart (1961) believes that light-induced growth inhibition, when photosynthesis is not limiting, results from a deficiency of endogenous gibberellin, and that both high-intensity (blue, far-red pigment) and low-intensity (phytochrome) light inhibition have their effects via the gibberellin system.

Lockhart (1960) also reported that visible light inhibits elongation of *Pisum sativum* stems by decreasing cell wall plasticity, that GA prevents light-induced growth inhibition, and that it prevents light-induced plasticity decrease. From these results he concluded that GA has its action on cell wall plasticity and that the level of endogenous GA is itself light controlled.

Lockhart's (1958, 1960, 1961) interpretations of the interrelations between light and GA are not accepted by all, however. Phinney and West (1960b) and Mohr and Appuhn (1961) have pointed out that the growth-promoting effect of GA in *Sinapis alba* seedlings involves increased cell division as well as cell enlargement and cannot be due to effects on wall plasticity alone.

Mohr and Appuhn (1961) also reject the idea that the photomorphogenic pigments exert their effects via the endogenous gibberellin system. They observed that the phytochrome system is still effective in controlling hypocotyl growth of *Sinapis alba* even when seedlings are continuously supplied with physiologically saturating concentrations of GA. This is readily demonstrated by irradiation with red or far-red light at the end of the photoperiod. Furthermore, the physiologically saturating concentration of GA is about the same for dark-grown or light-grown seedlings. This would not be expected if light inhibition of stem growth operated via reduction of endogenous GA level.

Mohr and Appuhn (as reported by Mohr 1962) have gone further and concluded that regulation of endogenous GA level is not part of the mechanism by which photomorphogenic pigments inhibit elongation of stem cells. They believe that exogenously supplied GA has its effects upon stem growth via some pathway different from that of photomorphogenesis, and have also suggested that GA, in some tissues at least, may not be an endogenous regulator at all.

What these paragraphs really indicate is that our knowledge of endogenous growth regulators (including photomorphogenic receptor pigments), and their interactions under various conditions, is so inadequate that intelligent discussion of the subject is not yet possible.

Nonperiodic Temperature Effects

Chilling Requirements

Long before anyone suspected the significance of photoperiodic and thermoperiodic conditions, early physiologists and horticulturists had already discovered an initially surprising fact. It seemed apparent that the low temperatures of autumn were responsible for induction of winter dormancy, yet it was found that plants protected from the winter cold often remained dormant longer than those exposed to the rigors of winter outdoors. This meant that warmth actually prolonged dormancy whereas low temperature shortened it. Such observations were contrary to expectations and difficult to explain.

The early observations were probably a byproduct of the introduction of greenhouses rather than results of planned research projects. Similar observations were reported repeatedly before their significance was realized and the concept of a chilling requirement for breaking of dormancy was formulated and widely accepted. The first such report by a competent scientist was probably that of Knight (1801 p. 343) who noted that grapevines grown in a greenhouse during summer and fall remained dormant in winter, whereas vines brought indoors in early winter vegetated readily. For other early references see the review by Vegis (1961).[33]

Many years after Knight's observations, Krašan (1873) working with *Salix nigricans* and Askenasy (1877) using *Prunus avium* brought cuttings indoors at intervals throughout the fall and winter. Both noted that buds on twigs brought indoors in early autumn were apt to remain dormant and finally dry up whereas those on twigs collected progressively later sprouted with less delay.

In work with numerous tree species, Askenasy (1877) recognized the important change occurring during winter as a physiological one (manifested in the response to subsequent warm-temperature treatment) rather than an anatomical or morphological change. But neither Askenasy nor Krašan arrived at the concept of a definite chilling requirement. It was Howard (1910) who made the first really thorough study of the effects of length of exposure to outdoor cold upon sprouting of cuttings brought indoors.

From October 28 to November 4, 1905 (in Columbia, Mo.), Howard collected and brought into a greenhouse twigs of 234 deciduous species to determine which would grow under the influence of warmth alone. Within 9 days 42 species sprouted. In the next few weeks 83 more species sprouted, but there remained 109 which made no growth. From January 8 to 10, 1906, Howard again collected twigs from the 234 species and additional ones for a total of 283. Within 9 days 142 species grew. Within a few weeks 244 showed some growth, but 39 others remained dormant.

[33] Vegis (1961) wrote an extensive review (in German) on the *Kältebedürfnis* (cold requirement) in growth, seed germination, and bud development. Though only a fraction of his review is concerned with buds of woody plants, his coverage of the entire problem of the chilling requirement is admirable.

Sixty-three of the more resistant species were again collected on February 26. This time 49 grew with little delay and additional ones with some delay. A final collection of the nine most resistant species (*Acer campestre*, *Alnus viridis*, *Carya aquatica*, *Carya porcina*, *Diervilla canadensis*, *Fagus sylvatica*, *Fraxinus americana*, *Fraxinus excelsior*, and *Fraxinus ornus*) was made on March 17. With the exception of *F. ornus*, these then grew.

Though he had all the essential facts at hand, Howard (1910) did not formulate the concept of a chilling requirement. The overriding controversy of that era was whether dormancy was autonomic (due to internal causes) or aitionomic (due to environmental conditions) (*p. 72*). Howard, who was influenced by Klebs, believed that his results supported those of Klebs (1913, 1917) showing that induction of vegetative rest periods could be but controlled by manipulation of the environment.

Whereas Klebs came very close to discovering photoperiodism, Howard overlooked the influence of light and concluded that plants did not really require the rest period and would not become dormant if not forced to do so by the cold of winter. Indeed, this is so for some species (*p. 98*), but, as we now know, many others can be forced into dormancy by short photoperiod treatments given at normal summer temperatures (*p. 95 ff.*). Howard also ascribed an important role in dormancy control to "habit." He believed that long-established habits could break and induce dormancy even when plants were protected from low temperatures. Later, in an analysis of physiological changes accompanying breaking of rest, Howard (1915) gave little indication of recognizing exposure to low temperature as an important factor in rest breaking under natural conditions.

Meanwhile Simon (1906), Molisch (1909), and others working with whole plants rather than cuttings, observed that many species brought indoors early in autumn sprouted much later in spring than those left outdoors. They ascribed this to lack of exposure to cold rather than to any specific inhibiting effects of the higher indoor temperatures. Weber (1916b) found that dormancy of *Tilia* and *Fraxinus* can be prolonged to more than 18 months simply by protecting the plants from cold. In later work he found that only the tops require chilling and that chilling of roots alone does not promote bud break in the unchilled tops (Weber 1921). Certainly these workers appreciated the need for chilling in breaking the dormancy of many species, but the most lucid and convincing exposition of the overall concept was made by Coville (1920).

After 10 years of experimentation, Coville (1920) arrived at several general conclusions which, in large part, are still valid. (1) Most trees and shrubs of cold climates become dormant in fall without requiring exposure to cold, but (2) lack of winter chilling results in delayed bud break in spring, and (3) the effects of cold exposure are limited to those parts actually chilled. Coville also believed that the effect of cold was intimately associated with the transformation of stored starch into sugar as a result of changes in membrane permeability. He believed that breaking of dormancy by various other treatments was also basically ascribable to such per-

meability changes. The concept of low-temperature mediated inactivation of growth inhibitors was a later innovation.

During the early decades of this century the economically important problem of delayed foliation of fruit trees in mild climates attracted a great deal of attention (for references see Vegis 1961, p. 243). This difficulty was finally ascribed to insufficient winter chilling (Weldon 1934; Chandler and Tufts 1934; Chandler et al. 1937), and the concept of a chilling requirement moved from the theoretical realm to become of the utmost practical significance to the orchardist.

Horticulturists are now able to state the chilling requirements of many varieties of fruit trees as the number of hours needed below 7° C. (for references see Samish 1954). It is recognized that flower and foliage buds may have different requirements. Numerical statement of the chilling requirement is, however, not on a firm theoretical base. Effects of many short periods of chilling (as during cold nights) are not strictly cumulative. The rest-breaking process is at least partly reversible, and a warm period can counteract the effect of a preceding cold period (Bennett 1950; *p. 160*).

Thus, by 1935 the essentials of the modern concepts of both photoperiodism and of the chilling requirement were available to research workers interested in dormancy and growth control in trees. But research in the field was not active. Photoperiodism was studied mostly with respect to control of flowering, and the work of Molisch, Klebs, Weber, and Howard on bud dormancy was not followed up. Furthermore, the work of these authors was almost exclusively on deciduous species. That some evergreen species might also have chilling requirements was not obvious.

Gustafson (1938), almost by accident, found that 3-year *Pinus resinosa* transplants kept in a greenhouse during winter made little growth the following summer. He ascribed the prolonged dormancy to lack of low-temperature exposure, but noted that this could be overridden by subjecting the plants to 16-hour photoperiods. Three-year *Picea canadensis* transplants, however, began growing when brought into the greenhouse in fall in spite of short photoperiods. This species may have no chilling requirement and no rest period, with its dormancy being only quiescence.

It is interesting that chilling requirements are not more obvious in some photoperiodic experiments (e.g. Kramer 1936). This may be related to the fact that long photoperiods can drastically reduce or eliminate the chilling requirement and also to species and ecotype differences. Ecotypic differences in chilling requirements do exist (Perry and Wang 1960) and may be widespread. Thus caution must be used in stating that a species has or does not have a chilling requirement.

Our knowledge of species requiring or not requiring chilling is still fragmentary. Relatively few conifers have been studied in this respect. Howard's (1910) studies made with cuttings collected at various times in fall and winter are still the most extensive source of information on hardwoods. However, broad surveys were also made by Moroz (cited by Vasil'yev 1961, p. 165) in the Lenin-

grad area. In these experiments twigs were cut early in fall and artificially chilled until the buds would open when the twigs were placed in a warm room. Moroz concluded that species and varieties from southerly regions need more chilling than those from northerly regions, though others have reported the opposite to be true. Kárpáti and Kárpáti (1961) reported that a majority of investigated native deciduous trees and shrubs of Hungary have definite chilling requirements.

What is the effect of exposure of dormant plants to warmth before chilling requirements have been satisfied? Is warmth merely neutral in that it delays fulfillment of the required number of hours of chilling, or is it active in that it partly nullifies previous chilling? Simon (1928) reported that exposure of *Hydrocharis morsus ranae* buds to 21° C. for several weeks, after the chilling requirement had been largely satisfied at 10°, inhibited sprouting. The inhibitory effect of high temperatures on this species was confirmed by Matsubara (1931). The chilling requirement of pear buds is increased when warm periods are alternated with cold periods (Bennett 1950).

Experimental work convinced Vegis (1948, 1955) that when the temperature of resting buds is raised above a certain level the physiological effect is one of increasing the intensity and duration of rest. If warm-temperature treatment is given immediately after rest has been broken by chilling, sprouting will occur in a narrow temperature range. If the upper limit of that range is exceeded for an appreciable time, rest will again be induced.

Viewed in this manner, the end of rest is not sharp but grades off into a state of "relative dormancy" (Vegis 1961) which is temperature dependent. At first, sprouting is possible only within a narrow temperature range above which rest is re-induced. With increasing quiescent or after-rest periods the range of growth-promoting temperatures becomes wider. Finally rest can no longer be re-induced by warm treatment (see also Vegis 1961). Thus warm treatment is not necessarily neutral, but may sometimes have a rest-inducing effect acting in opposition to the rest-breaking effect of chilling.

Present evidence, in my opinion, justifies the following views as a basis for further research. The breaking of rest by exposure of plants to low temperature is a gradual process. The actual end of rest can probably not be sharply fixed in time. During the depth of rest, growth is blocked throughout the whole physiological temperature range. As the end of rest is approached, growth becomes possible within a narrow temperature range. Temperatures above that range counteract previous chilling and reverse the rest-breaking process (Chandler et al. 1937). Temperatures below the range maintain quiescence while further promoting rest breaking. As the breaking of rest becomes more complete, the temperature range over which growth is possible becomes wider until finally reinduction of rest by high temperature is quite unlikely (*but see p. 164*). Low temperatures can, of course, always impose quiescence quite aside from any effects upon the depth of rest (*p. 163*).

All readers, especially those who handle resting plant material in the field or laboratory, are urged to consider the practical im-

plications of the above views. Could not failure of some spring plantings result from too early exposure to temperatures high enough to reinduce rest if the effect were not counteracted by a subsequent cold period?

If we are correct in supposing that some growth is possible within a narrow temperature range before rest is completely broken, it does not follow that the actual temperature within that range is unimportant. Pollock (1962) germinated unchilled peach seeds at carefully controlled temperatures and found that a difference of as little as 3° C. during a few critical days could determine whether plants would be normal or dwarf (*p. 163*). We have, unfortunately, almost no information upon the behavior of buds allowed to develop into shoots under controlled temperatures after various amounts of rest breaking treatment. It is common knowledge, however, that shoots entering the growing season with incompletely satisfied chilling requirements elongate less than similar shoots not so handicapped (Chandler et al. 1937).

Rohmeder (1962) has assembled considerable evidence that the growth rate of forest tree seedlings during the first few years is related to the rate of seed germination. All treatments which accelerate the germination process increase the rate of seedling height and volume growth for at least three or four years. Slow germination because of unsatisfied chilling requirements or other unsuitable conditions may have initial deleterious effects upon growth.

Unsatisfied Chilling Requirements and Dwarfing

The general problem of seed and embryo dormancy is outside the scope of this review, but some discussion of physiologically dwarfed seedlings grown from embryos with an unsatisfied chilling requirement is justified here. By removing the seedcoats and placing the embryos under conditions favorable to germination it is possible to obtain seedlings from freshly harvested seeds of *Prunus persica* and other species, even though intact seeds will not germinate until after cold treatment (Flemion 1934; Davidson 1935).

Seedlings from the unchilled embryos are usually characterized by an abnormal and dwarfed growth habit. Internodal elongation is minimal. Typically also cells of leaf midribs fail to elongate normally, whereas laminar development is little affected. Twisted, deformed leaves result from the uncoordinated growth. This tendency, however, is overcome by placing the excised embryos in continuous light at 70° to 75° F. (Lammerts 1943). Under ordinary greenhouse conditions, autumn planted dwarf seedlings may be sufficiently chilled by low night temperatures to allow them to revert to normal growth in spring. Such observations might suggest involvement of photoperiodic effects.

The extensive work of Tukey and Carlson (1945), however, definitely showed that photoperiodic factors are not predominant and also that dwarfing is most persistent in the main epicotyledonary axis. Axillary buds on dwarf plants may give rise to normal shoots even though the original leading shoot remains dwarfed. If carefully protected from chilling, dwarf *Prunus persica* seedlings may remain dwarfed for many years (Flemion 1959), but reversion to

normal can be induced at any time by several months of cold treatment.

Physiological dwarfing associated with insufficient chilling results from a type of epicotyledonary dormancy or shoot growth blockage. Root growth is not necessarily suppressed. An extreme example is afforded by *Paeonia suffruticosa*. In this species unchilled seeds produce roots but usually no visible shoots (Barton and Chandler 1957). *Prunus persica* dwarf seedlings with pronounced dormancy in the epicotyl region likewise have actively growing roots.

Grafting experiments have shown that control of dwarfing is exercised by the shoot apical region (Flemion and Waterbury 1945). Such control is not necessarily mediated via endogenous growth inhibitors susceptible to inactivation during periods of low temperature. Were such inhibitors or dwarfing factors present initially one would expect them to be diluted out or metabolically degraded with time. There is no evidence that this occurs (Pollock 1962). Furthermore, there is no significant difference between complements of methanol extractable growth regulators in normal and dwarf *Prunus persica* seedlings (Holmsen 1960).

Whereas it is correct to say that in dwarf seedlings the mechanisms of axial internodal elongation are inhibited, on the cellular level it may actually be cell division which is blocked (*p. 34*). There is no significant difference in length of pith cells in dwarf and normal plants and most of the difference in internode length must be attributed to failure of cell division in the dwarfs (Holmsen 1960).

This brings to mind the situation in *Hyoscyamus* and *Samolus* rosettes in which a kind of dormancy exists in the subapical meristem (*pp. 143–144*). When the subapical meristem is activated by treatment with GA, it is cell division which is initiated first (Sachs et al. 1960). Under some conditions successive applications of GA to dwarf *Prunus* seedlings causes internodes to elongate but the plants may revert to the dwarf condition after treatment. Reversion may be prevented by combining GA treatment with long photoperiods (Flemion 1959). GA treatment of unchilled peach seeds does not overcome dwarfing (Mes 1959; Flemion 1959).

The effect of light upon physiological dwarfs is also of interest. Dwarf *Prunus persica* seedlings do not elongate when placed in constant darkness, but when the tips alone are darkened extreme etiolation occurs. Likewise, 2 hours of light alternating with 22 hours of darkness causes otherwise dwarf plants to develop long, spindly internodes (Flemion 1959). Thus it seems that dwarf characteristics are not manifested when the apical meristems alone are subjected to darkness or whole plants subjected to very short photoperiods.

Dwarf strains of *Pisum* and *Phaseolus*, which can be made to appear normal by GA treatment, also show the dwarf characteristics only when grown in normal intensity light (Lockhart 1958; Simpson and Wain 1961). It is reasonable to assume that both endogenous regulators of the gibberellin type and photomorphogenic reactions are basic to the cause of physiological dwarfing.

After the probable involvement of photomorphogenic agents and growth regulators is admitted, however, it is still true that the

inception of physiological dwarfing is temperature controlled. Pollock (1962) found that subsequent expression of dwarfing in *Prunus persica* is controlled by the temperature prevailing during the first 2 to 9 days of germination. For example, resting seeds with part of the seedcoat and associated endosperm tissue removed before germination at 22° C. produced almost entirely normal seedlings. Germination of similarly treated seeds at 25°, only 3° higher, resulted in severe dwarfing. Pollock suggested a "self-replicating system" in the apical meristem region as the controlling agent. This system is presumably transmitted only by cell division and has maximal temperature sensitivity for only a short time during early plant development.

The reader is encouraged to consider the far-reaching morphogenic effects of small differences in temperature during germination (Pollock 1962), not in terms of effects upon preexisting growth regulators, but upon those mechanisms determining which items of genetic information shall be operative. Synthesis of certain groups of enzymes and ultimately of specific regulators and metabolites could be determined in this way. The sensitive period when such determination is readily effected may be short, but the effects can persist through many cell generations. The mechanism of such persistence is not well understood. One factor may be that the biochemical and biophysical environment of daughter cells developing within a tissue mass is largely determined by conditions already existing in surrounding cells (*p. 21*). Potentially reversible changes at the chromosome level may also be involved (Brink 1962).

Conversion of a potentially dwarf plant to normal by exposure to a suitable temperature during a sensitive period may be considered as an example of a developmental phase change. The term "phase change" also encompasses the sometimes pronounced shifts in ontogenetic pattern observable when a vegetative plant becomes reproductive or when a formerly juvenile shoot or branch assumes adult characteristics. Once established, phases can be maintained for long periods by "somatic cell heredity" (Brink 1962). The nature of such heredity is still quite obscure.

High Temperature and Rest Induction

Arguments can be made supporting the hypothesis that high temperatures are a significant factor in induction of rest in young tissues which are surrounded by structures limiting gaseous diffusion. Indeed, short photoperiods which induce dormancy under natural summer temperature conditions are sometimes ineffective at low temperatures. This was already reported by Moshkov (1935) and was again demonstrated by van der Veen (1951).

Experiments by van der Veen showed that 3 months of artificial winter at 5° C. and 9-hour photoperiods did not induce terminal bud formation and rest in *Populus*. The plants merely became quiescent and resumed growth when returned to room temperature. Such results have been interpreted as indicating that high temperatures are a necessary condition for rest induction and that short photoperiods and cold treatment per se are insufficient. Cold treatment alone also does not induce in *Picea excelsa* needles those changes

in chloroplast structure characteristic of needles on dormant conifer needles (Genkel' and Barskaya 1960).

Vegis (1956) proposed that in *Hydrocharis*, and in most woody species investigated, light and temperature act antagonistically. In his opinion, high temperature promotes induction of rest in buds whereas light (long photoperiods or continuous) promotes continued growth. This presupposes that structures surrounding the meristem are only slightly permeable to diffusing gases. Presumably rapid respiration at high temperature results in anaerobic conditions which in turn lead to cessation of growth and possible production of inhibitors (see also Pollock 1953).

By this line of reasoning a closed bud becomes a prerequisite for entry into rest. There is no problem with lateral buds because they are held dormant during the summer by correlated inhibition and their meristems are surrounded by structures limiting oxygen supply. What, however, provides the stimulus for the formation of the closed terminal bud before high temperature and anaerobic conditions can act to induce rest? Photoperiodic stimuli? Some kind of internal competition? Foliar inhibition? Water deficit? The question is still open.

The discussion of temperature effects above pertain to temperate zone plants. In some tropical species having seasonal dormancy there may be a warmth requirement rather than a chilling requirement. Humphries (1944) observed that high rather than low temperatures have a dormancy breaking effect upon *Theobroma cacao*. The physiology of this has not been thoroughly investigated.

Warm Baths as Rest Breaking Agents

According to the hypothesis of Vegis (1961) and others, discussed above, it is possible that high temperature and limited gas diffusion to the meristems is a significant factor in the induction of dormancy. Somewhat paradoxically, however, it is known that high temperatures when combined with low-oxygen tension, in the form of a warm bath treatment of the shoots, can also break rest.

For warm bath treatment, the tops of inverted plants are immersed in water at 30° to 40° C. for 8 to 16 hours. This method of dormancy breaking was developed empirically by unremembered gardeners, but it was Molisch (1908–1909) who first attempted to elucidate the physiology involved. He found that the warm bath combination is actually necessary. Warm air incubation and room temperature water bath treatment given separately are not effective. According to Molisch (1909) also, the warm bath method has extremely varied effectiveness depending upon season, species, and type of bud.

Upon *Syringa* and *Forsythia* the treatment breaks bud dormancy even before leaf fall in autumn. In numerous other genera it is ineffective until after leaf fall. *Aesculus* and *Fraxinus* do not respond to the warm bath until January, *Tilia parvifolia* and *Fagus sylvatica* not until March when their true rest periods are probably already over. In *Corylus avellana* warm bath treatment breaks dormancy of male catkin buds in October (Prague, Czechoslovakia), but has little effect upon female catkin or leaf buds until December.

This variability of response again indicates the complexity and multiplicity of dormancy control mechanisms and does not subtract from the theoretical significance of the warm bath method of rest breaking.

Molisch (1909) recognized that oxygen solubility in water at 30° to 40° C. is very low whereas demand by tissues at these temperatures is high. Nonetheless he was not convinced that anaerobiosis had any essential part in rest breaking. Using vacuum chambers, Boresch (1924) found air at 30° and 50 mm. Hg pressure to be just as effective as a warm water bath. Vacuum at room temperature or 30° air alone was without effect. Furthermore, with the aid of pressurized systems, Boresch (1926) discovered that when oxygen content of the water is increased to approach that of free air the warm bath's ability to break dormancy is lost. The implications were obvious.

It was well known that respiration at elevated temperatures with limited oxygen supply leads to accumulation of acetaldehyde and ethanol in the tissue. Boresch (1926, 1928) demonstrated the accumulation of these compounds in catkin buds of *Corylus avellana* during warm bath treatment and also the effectiveness of acetaldehyde, ethanol, acetone, formaldehyde, and related compounds as rest-breaking agents.[34]

With this information is was logical to postulate that metabolic changes induced by accumulations of acetaldehyde or ethanol are key factors in causing bud opening after warm water bath treatment (Boresch 1928). Indeed the action of some chemical agents in breaking dormancy may likewise be mediated indirectly through accumulation of acetaldehyde. For example, cyanide (Weber 1918; Gassner 1926; Denny and Stanton 1928) by inhibition of metal-containing terminal oxidases may promote accumulation of pyruvate and formation of acetaldehyde and ethanol. Even if these interpretations are correct, a great amount of unknown biochemical mechanism still lies between acetaldehyde and initiation of elongation growth. Research in this area has not been active.

Nonperiodic temperature effects upon dormancy are real but not simple. In nature environmental temperatures usually have a periodic component also which in its interactions with photoperiods and endogenous rhythms further complicates interpretation.

[34] A table of chemical dormancy-breaking agents, with literature references, has been compiled by Doorenbos (1953).

PART III. EPISODIC GROWTH AND DORMANCY OF ROOTS

GROWTH AND DORMANCY IN ROOTS

Definition of the Problem

The structure of the root tip is simpler than that of the shoot tip. The root apical meristem does not initiate primordia of lateral appendages. There are no bud scales, no nodes and internodes, and there can, therefore, be no structures comparable to buds. Episodic growth and dormancy in shoots, accompanied as it is by formation and subsequent outgrowth of buds, is easy to see and follow. But in roots it is difficult to measure growth in situ or to recognize and delimit in time any dormant state without causing considerable changes in root environment. Furthermore, the environment of undisturbed individual roots of the same tree may be widely different with respect to temperature, moisture, oxygen, and carbon dioxide levels. Such variation reduces the value of observations made on only small numbers of roots.

It is generally accepted that shoot dormancy is controlled by environmental factors rather than being a manifestation of an internally controlled cycle. There is no reason for supposing that root growth follows an endogenous cycle, nor is there any strong evidence suggesting it. The remaining possibilities are that roots respond to their own environments, to factors or stimuli transmitted from the shoots, or to both.

Roots are obviously dependent upon shoots for a primary supply of fixed carbon. The very fact that the existence of the still hypothetical root growth hormone, rhizocaline (Went 1938), was postulated is indicative of root dependence upon shoots for some growth factors also. Shoot influences upon root growth cannot be denied. The effects of root environmental conditions are also too obvious to be denied. The problem, therefore, is one of defining the manner in which shoot influences and root environment interact to control root growth and dormancy. The problem of whether roots are ever dormant in the same sense that buds are dormant in winter is a semantic one which need not interfere with anatomical and physiological investigations.

Seasonal and Episodic Root Growth

The natural philosopher Duhamel du Monceau (1758, 1760), in what are some of the earliest books on the culture and physiology of trees, discussed seasonal differences in root growth. Upon examination of trees dug up periodically during winter he noted that whereas some small roots seemed to turn brown and die others grew to take their places. He concluded that root growth is possible in

winter and may be extensive in mild years. Duhamel also noted that root growth may begin before bud break in spring and continue after autumn leaf fall. On the basis of these observations he recommended fall and winter rather than spring planting of trees.

During the following century numerous botanists published additional evidence that tree roots grow in winter (for references see Resa 1877, 1878; Ladefoged 1939). Some took the position that small roots, like leaves, are cast off and renewed periodically with new roots appearing in winter as well as during other seasons. Others preferred to believe that all parts of the plant grow in spring and become dormant in fall. The weight of evidence, however, favored some winter root growth. Dove (1846), in an attempted physiological explanation of winter root growth, proposed that root growth is favored in fall and winter because soil temperatures then are higher than air temperatures, whereas the converse situation in summer favors shoot growth.

The eminent forest botanist Thomas Hartig agreed that root growth can occur in winter and that length growth of fibrous roots commonly precedes bud break in spring. Hartig (1863a, b) also noted that the new growth on elongating roots is of larger diameter than the older part and is conspicuous because of its translucent whiteness. He correctly ascribed the diameter differences largely to the cortex. The growing root tips are swollen in the sense that they have a turgescent cortex. This may shrink and become brown after a few months.

If growth is slow, browning may extend to the apex and the root may appear dead, thus accounting for reports that roots die and are replaced each year. The phenomenon of cortical collapse and browning is now known to be of significance in the onset of root dormancy in some species, but it attracted little attention for more than 40 years after Hartig (1863a, b) mentioned it.

After making a study in which root systems of sample trees of about 10 species were partially exposed at intervals during the winter, Resa (1877, 1878) proposed that trees have two main periods of root growth, one in spring beginning before the leaves appear, and one in fall. In hardwoods, according to Resa, the fall period may really be continuous with the spring period, for growth is only slowed by the cold, whereas in conifers a period of winter inactivity intervenes.

Wieler (1893, 1894) did not accept Resa's results as being conclusive or credible. Why should new roots grow in fall and winter when water requirements are much reduced? What need have trees for new roots then? Wieler also objected to Resa's methods. Surely some roots were damaged by digging, and such wounding itself could induce new growth which would not otherwise have occurred. Also, Resa had examined only parts of the root systems of his sample trees. Wieler made observations on tubbed seedlings of seven woody species 2 to 4 years old. By examining each complete root system once and then discarding the plant he eliminated wound effects.

Although some of Wieler's data suggest that growing roots were present in winter, he relationalized these as individual variations. He believed the commonly observed browning of root tips to be correlated with cork formation within the formerly white zone of

young root tips as well as in the leaf abscission layers. Failure of some roots to turn brown in winter was not taken as an indication of new growth, but was likened to the failure of some leaves to fall after their purpose has been served. Wieler (1894) interpreted his data as indicating that root growth occurs in spring and summer, but not in late fall or winter.

The controversy about whether root length growth occurs in winter was accompanied by a similar controversy concerning root thickness growth. Mohl (1862) reported that root cambial activity continued into midwinter, but Hartig (1863b) disagreed. After an extensive study of several hardwood and coniferous species in the environs of Leningrad, Gulbe (1888) reported that cambial activity in *Quercus pedunculata* roots may continue until mid-November, but that it ends in late October in most species. Using cambial activity as a criterion he concluded that roots are completely dormant in midwinter.

According to Cockerham (1930) distal parts of roots of *Acer pseudoplatanus* trees growing in Leeds, England, maintain slow but continuous production of xylem and phloem throughout the year. In the upper and middle regions of the roots the surge of xylem-producing activity propagated downward from the stem is superimposed upon this slow activity in early summer. Of course, some of the disagreement between authors may be ascribed to differences in climate between areas where observations were made. There is still very little reliable information on the seasonal distribution of cambial activity in roots.

Meanwhile, a study similar to that of Resa was made in Denmark by Petersen (1898). He also found active root growth early in spring, and declining growth during leaf expansion and shoot growth, with a minimum in July. The most intensive growth occurred in August and September. Petersen, however, could not confirm Resa's reports of root growth in midwinter. The idea of two root growth periods per year was also supported by Hämmerle (1901) after extensive study of the behavior of *Acer pseudoplatanus* roots.

Büsgen (1901) too made studies on forest trees using Resa's periodic digging method. In addition he planted 5-year-old trees in zinc-lined boxes having glass sides, allowing determination of root growth rates. Again two main growth periods were evident. Büsgen (1901) collected in tabular form many published data in addition to his own. He concluded that in spite of some disagreement most data were compatible with Resa's original concepts. Büsgen blamed the midsummer decline in root growth upon water stress and suggested that low soil temperature was the important factor in slowing or halting growth in winter.

The very carefully executed and detailed studies of Engler (1903) in Switzerland with 16 forest species yielded results of permanent value. Engler was fully aware of the difficulties of determining whether or not a root is growing merely by its appearance when dug up or washed out. The presence of white tips is no guarantee of continuing growth because browning does not immediately follow cessation of growth.

By observing roots through glass plates, Engler determined that browning required 1 to 3 weeks in conifers and 3 to 6 weeks in

hardwoods to advance to the tip after growth had ceased. The longer times were observed in winter. Because data obtained by digging or washing out plants could give no accurate information on time limits of growth periods or growth rates, Engler made extensive observations using root boxes having glass sides and wire mesh bottoms.

The root boxes were buried in slit trenches and inclined 20° from the vertical to increase the number of roots visible on the lower side. Observations were made by lifting out the boxes every second day. In spite of precautions, the winter soil temperature in the boxes was lower than in undisturbed areas nearby. The soil froze to a greater depth and root growth probably stopped earlier in the boxes than outside. Soil settlement also was a problem. The shortcomings of the method, however, were taken into account in interpreting the results.

Engler (1903) concluded that vigorous root growth occurs in spring and fall periods separated by a 3- to 8-week summer intermission of little growth. According to his results, spring root growth usually begins before bud break. Exceptions are *Larix* and *Carpinus* in which it begins later. In conifers the fall growth period ends in October, but hardwoods may, in mild years, continue slow root growth throughout the winter. Total growth during the spring period is usually greater than in fall, particularly in conifers.

With regard to the reasons for episodic root growth, Engler (1903) pointed out that growth periods are dependent upon both soil temperature and water tension. Water is probably limiting in summer and soil temperature in winter. Differences between species, years, and localities are to be expected. He suggested that the more persistent fall and winter root growth of hardwoods is probably related to the greatly reduced transpiration after leaf fall.

In conifers water stresses may be severe in winter and water may not be available for root growth. Engler admitted a possibility that the winter dormancy of conifer roots might be genetically determined, but thought it unlikely that the summer dormancy was so controlled. He saw no evidence of genetically determined summer or winter rest periods in roots of hardwoods. The results of less extensive studies by Goff (1898) and Cranefield (1900) in Wisconsin were compatible with those of Engler.

On the basis of in situ studies of root growth of large specimens of *Acer saccharinum*, *Tilia americana*, *Carya laciniosa*, and *Quercus alba*, McDougall (1916) also arrived at conclusions similar to those of Engler (1903). Namely: (1) Root growth begins in spring whenever the soil is warm enough, and stops in fall when it becomes too cold. (2) The summer dormant period, when and if it occurs, is due to water stress and is not endogenously controlled. The work of Hesselink (1926) with *Pinus sylvestris* and *P. laricio austriaca* provided additional evidence that the summer intermission in root growth does not occur when water is not limiting.

The possibility of winter root growth in hardwoods gradually came to be accepted after repeated demonstrations of its occurrence. Doubt concerning winter root growth in conifers lingered somewhat longer. Harris (1926) found winter root growth of apple and filbert trees in the field in British Columbia and Oregon when soil temperatures rose to 40° F. or above. Crider (1928), working in

Arizona, grew trees for several years in wood and concrete boxes having glass observation plates behind light-tight doors. He confirmed winter root growth in six species including the conifer *Cupressus arizonica*, but found none in several other species.

Stevens (1931) studied length growth of roots of 4- to 6-year-old *Pinus strobus* under plantation conditions in New Hampshire. The method involved repeated exposure of the root tips by careful digging and brushing followed by measurements from a reference point. It is not surprising that Stevens obtained no evidence of winter root growth in the field because all the measured roots were in the upper 8 inches of soil, and the ground was frozen to at least that depth all winter. However, roots of similar trees in a greenhouse (natural photoperiods) grew just as fast in winter as in summer. Thus there was no evidence that winter root dormancy is essential in *P. strobus*, or that it is induced by internal factors.

Stevens (1931) also brought out the important point that some dormant and some growing roots are present at practically all times. Whereas the ratio of dormant to active roots varies with the seasons, either state is possible at any time within limitations set by temperature or water stress. Furthermore, there is some tendency for synchronization among the several tips associated with the same branch root. Stevens mentioned the coloration and diameter changes associated with inception and breaking of dormancy but did not speculate upon their anatomical or physiological significance.

Buried observation chambers with windows sloping inward enabled Turner (1936) to obtain measurements of the root growth of *Pinus echinata* and *P. taeda* seedlings planted in the soil just outside. Some measurable root growth was made during every 8-day period for 2 years (Fayetteville, Ark.). There were no periods of complete dormancy, but both the number of growing roots and the growth rate was less in midsummer and midwinter than during other seasons. The low air temperature of winter reduced growth at shallow depths but had less effect upon deeper roots. Again there was no evidence of an endogenous tendency toward dormancy of roots in winter, the significant factors being soil and air temperature, and water stress.

Roze (1937), working in the colder climate of Riga, Latvia, observed no winter root growth on *Pinus sylvestris* and a *Picea* species. He ascribed this to low winter temperatures.

Ladefoged (1939), in addition to publishing results of his own experiments, reviewed much of the literature on episodic growth and dormancy in tree roots. From his own data, obtained by periodically exposing and measuring roots in the field, Ladefoged concluded that roots of *Fagus sylvatica* continue to grow slowly throughout mild winters in Denmark. Those of three other hardwood and three conifer species showed no winter growth under similar conditions. The considerable within-species variation was probably largely due to local climate and soil factors. Such factors appeared to have much greater influences upon deciduous than upon evergreen trees.

Ladefoged found young *Fagus sylvatica* under old trees to be exceptionally variable for hardwoods. The individuals even within small areas showed no synchrony of root-growth periods. In such cases it is possible that soil environmental factors are overridden by subtle internal factors in the seedlings. Ladefoged also made

the interesting observation that roots of stumps of felled *Larix* and *Abies* trees began growing at the same time as those of intact trees. This is additional evidence that soil environmental factors are very influential in controlling root growth.

By 1940 it could be considered established that roots do not generally show a regular cycle of growth and dormancy determined by internal factors, but that a kind of dormancy exists in individual roots at various times. It was also obvious that winter root growth is possible in many species and that a state of rest or quiescence in the shoot does not necessarily preclude root growth.

In some deciduous species, however, root growth may be dependent upon the presence of nonresting buds on the shoot. For example, in *Acer saccharinum* seedlings root growth is inhibited after autumnal leaf fall and remains so until the chilling requirement of the buds has been satisfied. At least one nonresting bud, which may still be quiescent, must be present for the initiation of root growth in spring. Root development is completely suppressed if all nonresting buds are removed (Richardson 1958a).

It has not been shown that the same dependence of root growth upon presence of nonresting buds exists in deciduous trees beyond the seedling stage. It is also doubtful that root growth of conifers generally is completely inhibited while the buds are at rest. In *Libocedrus decurrens* seedlings, root dormancy is not readily correlated with shoot growth or a chilling requirement, but winter root dormancy may be related to short photoperiods (Wilcox 1962c).

Valuable detail has been added in recent decades (see Heikurainen 1957; Richardson 1957, 1958b; Stone and Schubert 1959; Wilcox 1954, 1962b, c), but there is still a dearth of basic information concerning episodic growth in roots. After reviewing available information Ladefoged (1939) did not feel justified in denying the existence of autonomic control mechanisms. Instead, he proposed that control of root growth is the resultant of autonomic and environmental factors.

Reviewing the same line of information today does not result in much further enlightenment. Mechanisms by which dormancy is induced and broken are particularly obscure. There exists, however, an additional little-known line of observational and experimental work bearing upon the problem. This is discussed below.

Anatomical and Physiological Aspects

Tips of dormant tree roots are often an opaque brown, whereas tips of growing roots are apt to be white or only lightly colored. Dormant root tips are also often anatomically different and have layers of cells with suberized (and lignified) walls distributed so as to form a continuous sheath over the whole tip. How do these differences come about? What is their physiological significance? Can the suberized tips revert to the growing condition, or do they die to be replaced by others? What induces and controls deposition of suberin in only certain layers of cells? What functional capacity do suberized roots have? Questions like these were being asked by a few people at the turn of the century. A few of these questions have since been answered, but most still await careful study.

688-803 O—63— 12

The existence of living roots with suberized apices in Bromeliaceae, Hippocastanaceae, and Sapindaceae was mentioned in the early literature (Jörgensen 1880; Klein and Szabó 1880; and Waage 1891), but was not specifically associated with a reversible type of root dormancy. Büsgen (1901) observed the rate at which browning advanced along the root and noted that it reached the very tip when growth slowed and stopped. He found the advance rapid in *Langwurzeln* (long roots, pioneer roots) and very slow in *Kurzwurzeln* (fiberous roots, short roots, feeding roots).

Meanwhile detailed studies of root anatomy were initiated in laboratories at Marburg, Göttingen, and Bromberg in Germany. Results of much of this work were published in dissertations or journals of limited circulation (see review by Alten 1910), never aroused wide interest, and have been almost forgotten. Nevertheless, the work encompassed several significant contributions to the understanding of root dormancy.

One such contribution was the discovery that root browning may be a superficial aspect of anatomical changes occurring within. Müller (1906) studied the dormant roots of a large variety of perennial monocotyledonous plants. The brown coloration could be bleached out with reducing agents, but anatomical differences remained. The apical meristems appeared to be isolated from outside by layers of cells with lignified and suberized walls. The formation of these layers was referred to as *Metakutisierung*.[35]

Müller (1906) believed that suberization of root tips probably prevented inward or outward passage of nutrients and also reduced movement of water (but see Kramer 1946). He succeeded in getting a suberized root to renew growth in nutrient solution, but did not eliminate the possibility that under natural conditions suberization might often lead to irreversible changes and senescence.

Plaut (1909) found that metacutization, somewhat similar to that discussed by Müller (1906) in monocotyledonous angiosperms, is also common in dormant roots of gymnosperms. The latter group, however, shows considerable variation in distribution of metacutized layers. Plaut (1909, 1910, 1918) described four distinct types of metacutization in gymnosperms (fig. 7). These types may be briefly described as follows:

Type I.—The outer layers of the root cap metacutize and become continuous with the suberized exodermis (hypodermis). This is the common type in various genera of cycads, in many dicotyledonous angiosperms, and possibly also in some *Pinus* species.

Type II.—The suberized exodermis is absent. Metacutized layers form in the cap, but not necessarily on the surface. By means of a bridge across the cortex these become continuous with the suberized cells of the secondary endodermis. The cortex and any cap cells outside the metacutized layers may turn brown, collapse, and die. Examples of species exhibiting this type of metacutization are *Podocarpus totara*, *Agathis robusta*, and *Pseudolarix kaempferi*.

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[35] Following the precedent set by Wilcox (1954) the anglicized form "metacutization" will be used in this discussion.

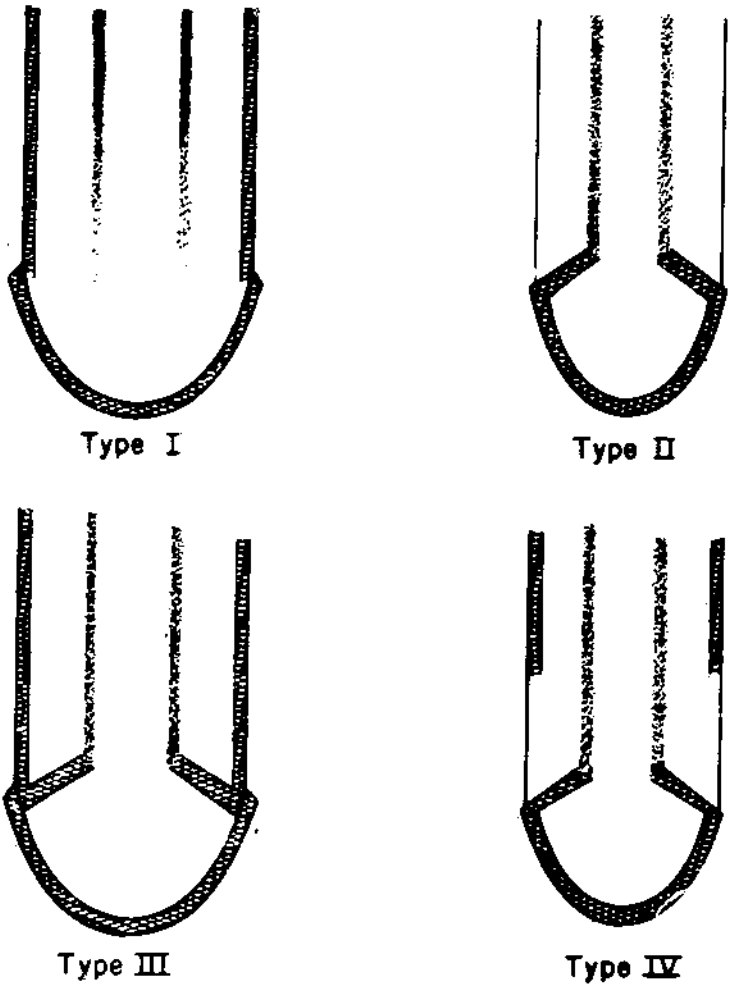

FIGURE 7.—Types of metacutized root tips. Cross hatching represents extensive suberization. Stippling represents endodermis. See text for discussion. (Schematic after Plaut 1918.)

Type III.—An exodermis is present and the metacutized layers of the cap become continuous with it as in Type I. In addition the metacutized cell layers in the cap are linked with the endodermis as in Type II. Examples are *Ginkgo bilboa*, *Taxus baccata*, *Athrotaxus selaginoides*, *Sequoia gigantea*, *Cryptomeria japonica*, and *Juniperus prostrata*.

Type IV.—An exodermis is present but does not participate in the final phase of metacutization, which proceeds as in Type II. An example is *Araucaria excelsa*.

Plaut (1910, 1918) also studied the nature and distribution of metacutized layers in roots of *Alnus glutinosa*, *Fagus sylvatica*, *Quercus sessiliflora*, *Betula alba*, and a wide variety of other dicoty-

ledonous angiosperms. He found less variation than in gymnosperms. He observed the metacutized layers of the cap to become continuous with the exodermis, as in *Type I* of gymnosperms, but he never observed a juncture with the endodermis.

Whereas it had previously been tacitly assumed that a white tip was indicative of a growing root, Plaut (1918) found this criterion to be unreliable in dicotyledonous plants. Fully metacutized tips of some species may remain white, so that microscopic examination of stained sections is necessary before their growth or dormancy status may be determined. In *Buxus sempervirens* and *Calycanthus floridus*, Plaut observed the metacutized tips to remain white throughout the winter. In other species he found surface cells of dormant tips to acquire a brown pigmentation similar to that of the older parts. In all cases browning appeared merely as an incidental phenomenon often accompanying but not causally related to metacutization.

The concept of the metacutized root tip, not as an indicator of senescence, but as a dormant structure from which renewed root growth could originate, was arrived at independently by Kroemer (1918) and Plaut (1918). Plaut described bursting of the suberized cap as growth is resumed and also observed that in some species, including *Ribes sanguinea*, *Taxus baccata*, and *Rhododendron viscosum*, an individual root may form and break several metacutized caps in succession.

Kroemer's (1918) extensive studies of *Vitis* roots convinced him also that metacutization is not always an indicator of senescence, that metacutized tips can make renewed growth, and that they may be found both in summer and winter. Reversion of some suberized root tips to active growth has been confirmed by others also (Aldrich-Blake 1930; Cossmann 1939; Barney 1951; Wilcox 1954, 1962b).[36]

Plaut (1910, 1918) speculated that the low soil temperatures of winter are probably a factor in inducing metacutization of root tips, but he did not conduct experiments to determine the correctness of this view. After experimenting with *Funkia sieboldtiana*, Mager (1913) concluded that soil water stress and high salt concentration favored metacutization of tips.

Although Plaut's papers concerning root dormancy have only rarely been cited, his concepts were largely confirmed by independent observations. Aldrich-Blake (1930) described the suberized winter root caps of *Pinus halapensis* and recognized them as dormant structures which were broken and cast off in spring. Some of the observations of apple and peach roots by Nightingale (1935) concerned extensive suberization and cessation of growth of root tips in soil at 35° C. It seems likely that high-temperature-induced dormancy was being observed. In experiments in which *Pinus taeda* were grown with their roots at controlled temperatures, Barney (1951) found that roots ceased growing after only a few days at 35° C. The tips

[36] In *Libocedrus decurrens* the ability of dormant roots to revert to active growth is related to the extent of vacuolization in cells of the apical initial group during dormancy. If all the cells become highly vacuolate the tip becomes senescent and renewed growth is unlikely, but often the cells remain densely protoplasmic and able to resume growth with consequent bursting of the metacutized layers. The basis of such differences in behavior is not known (Wilcox 1962b).

of such roots were covered with layers of suberized cells, possibly indicating that metacutization had occurred.

Wilcox (1954) also found Plaut's concepts to be substantially correct and applicable to *Abies procera* roots. In this species a brown cap forms over the root apex within a few days after growth ceases. Browning is accompanied by metacutization corresponding to Plaut's Type II in which the suberized layers of the cap become continuous with the suberized secondary endodermis. In agreement with Stevens (1931) and Ladefoged (1939), Wilcox found that there is little synchrony between different parts of the same root system. Some active and some dormant roots are apt to be present at the same time.

Individual roots of *Abies procera* enter dormancy and undergo the accompanying metacutization at various times of the year. Growth of some roots may be resumed by bursting of the suberized cap. Data from various sources indicate that, in spite of lack of synchrony under normal conditions, most roots will become dormant when conditions are particularly unfavorable and most will grow when conditions are unusually favorable.

Thus the existence of a kind of dormancy in roots has been confirmed, but the physiological mechanisms controlling its induction and breaking are only beginning to be studied.

Control of Root Growth

It is, of course, known that root growth is generally inhibited by soil temperatures that are too high or too low, by water stress, or by oxygen deficiency in the root zone. Very little is known, however, about endogenous mechanisms which control root growth in woody plants when environmental factors in the root zone are not limiting.

The supply of carbohydrate necessary for root growth must come either from stored reserves or more directly from photosynthetic products. If stored reserves are used, root growth may be largely independent of light intensity and photosynthetic rate of the shoot in short-term experiments. But if photosynthate is used more directly, root growth may respond very rapidly to changes in light intensity. This may explain why root growth of *Quercus borealis* seedlings is much less responsive to the light intensity to which the shoot is exposed than is root growth of *Acer pseudoplatanus* seedlings (Wassink and Richardson 1951).

In *Acer saccharinum* seedlings, root growth is quickly inhibited by severe curtailment of photosynthesis, but after a week the growth rate may return to over half of its original value. The renewed growth, dependent upon stored carbohydrates, is uninfluenced by shoot temperature. Nevertheless, either defoliation, decapitation, or removal of a ring of bark from the main stem is followed by complete cessation of root growth.

When new leaves develop on defoliated plants, new roots appear which again grow at a rate uninfluenced by shoot temperature and light intensity. Such results suggest that shoots, and particularly leaves, supply substances essential for root initiation and development aside from carbohydrate supply (Richardson 1953a, b). At least two such substances are indicated in *Acer saccharinum* seedlings. Excision of the apical meristem completely inhibits forma-

tion of new roots, but has no effect on elongation of existing roots. Defoliation inhibits root elongation without necessarily inhibiting formation of new lateral roots (Richardson 1957).

The requirement for an intact apical meristem for new root formation can be physiologically replaced by IAA applied to the cut surface after apical excision. The requirement of leaves for normal root elongation can be overcome by growing seedlings in humus-rich soil. Richardson (1958b) has suggested that the substance controlling root elongation, supplied by leaves, or absorbed from humus-rich soil, may be a B vitamin. The substance necessary for new root formation, normally supplied by the apical meristem (in *Acer*), is an endogenous regulator at least partly replaceable by synthetic IAA (Richardson 1958b).

The above line of evidence does not justify the conclusion that vitamins or hormones from the shoot control root growth in all species. In vitro culture of isolated roots has shown that vitamin and cofactor requirements vary a great deal between species. Growth of isolated roots of *Acacia melanoxylon* (Bonner 1942) and *Robinia pseudoacacia* (Seeliger 1956) requires that thiamin (vitamin B_1), pyridoxine (vitamin B_6), and nicotinic acid (niacin) be added to the nutrient medium. Isolated roots of numerous other woody species (including the conifer *Thuja orientalis*) will not grow even when supplied with these substances (Bonner 1942).

Isolated *Pinus serotina* roots, on the other hand, synthesize those vitamins required for growth although added pyridoxine may promote growth slightly (Barnes and Naylor 1959). Went (1938) used the term "rhizocaline" for substances synthesized in the shoot and essential for root formation, but this merely attaches a name to the unknown. Such usage should not be interpreted as indicating existence of a widely distributed specific substance controlling root development.

The theory that root growth is controlled by vitamins or hormones from the shoot suffers from the disadvantage that dormancy and growth episodes of the various roots on the same plant are not synchronized. This can be explained only by assuming a considerable degree of autonomy in the individual root tips.

Wilcox (1962c) studied the effects of various concentrations of IAA, and of extracts of growing and dormant root tips, on roots of *Libocedrus decurrens*. Root extracts were always inhibitory, but IAA in the range from 10^{-9} to 10^{-6} moles per liter was stimulatory. Slowly growing roots were stimulated more than rapidly growing ones. IAA treatment, however, did not elicit renewed growth in dormant roots.

Bioassay (*Avena* straight-growth test) of root extracts revealed growth accelerators, possibly including IAA, as well as growth inhibitors. Surprisingly, the dormant roots were richer in growth accelerators and poorer in inhibitors than growing roots. This again illustrates the inadequacy of present knowledge of growth control in roots. Wilcox (1962c) suggested that a number of hormonal factors are operating. Certainly the control system is not a simple one.

IN RETROSPECT

The reader who expects a pithy summary, replete with sweeping truths about the behavior of meristems, the control of growth and development, and the physiological basis of dormancy, will be disappointed. Even the greatest perseverance in analytical and synthetic efforts cannot compensate for the inadequacy of ideas and the gaps and discrepancies in available information. Yet the fund of seriously proffered ideas and information bearing upon these subjects is quite large, and rather detailed in some areas. What is its utility if it does not enable us to understand the basic processes involved in the control of dormancy, growth, and morphogenesis?

The utility of present information and ideas lies in two general areas—practical applications, and furtherance of basic research. In biology, incomplete understanding of a subject, even if based upon erroneous concepts, is superior to a total unawareness of it. For example, our knowledge of photoperiodism is quite incomplete, yet the effort expended in acquiring that knowledge has already been justified by practical applications of it in floriculture and in growing plants for various research purposes. Except perhaps in the production of planting stock and establishing it in the field, widespread application of the information and ideas discussed herein to practical forestry or horticulture is not an immediate prospect. Awareness of the present knowledge, and an appreciation for the limitations of the ideas upon which it is based and interpreted, however, confer upon it a great utility in enabling us in our research to ask well-defined and pointed questions in areas of the most moment.

What kind of questions is it most meaningful and profitable to ask? Anyone contemplating research on meristems, morphogenesis, or growth control needs to ponder this himself. There is perhaps some value in testing emerging generalities by variants of oft-repeated experiments with additional species and under diverse conditions. Results will add stature to the existing isolated peaks of information. In my opinion though, ultimately greater, even if initially more disturbing, contributions will arise from questions concerning the basal solidity of such peaks and the still obscure relationships between them. All of us, by the practice of wisely moderated skepticism, need to guard against the subconscious veneration of presently accepted ideas or supposedly established facts. Many such ideas and facts must be revised and invalidated as a prerequisite to the development of new and more intellectually satisfying ideas and during the gradual evolution toward truth.

Whether the reader agrees or disagrees with the ideas discussed in this bulletin is of no great importance provided that he has been motivated to examine them critically. If after reflecting upon the information and ideas discussed herein the reader is in an improved position, in his thinking and research, to ask potentially answerable

questions with a high degree of relevance, this publication will have been justified. As the direction of differentiation of a cell depends to a large extent upon its environment, which is in turn partly determined by its neighbors, so also do the questions we may ask depend upon our backgrounds, our special interests, and the immediate circumstances of our research environments. It should be understood, therefore, that the questions I have brought out in the text and repeated, in part, below are not necessarily *the* questions but merely *some* questions to be considered.

Cell division, cell enlargement, cell differentiation, and morphogenesis are largely localized in meristems and their ancillary regions. What controls the orientation of planes of cell division in apical meristems? How is the orientation of division in the outer layers of cells more strictly controlled in angiosperm shoot apices than in most gymnosperm shoot apices? What microenvironmental stimuli trigger what kinds of physiological processes in local areas of the shoot apical dome during the initiation of primordia? What factors determine whether a primordium shall rapidly develop into a scale, a leaf, or lateral bud, or whether it shall long remain a primordium? When does primordial differentiation become unalterably fixed in direction?

Initiation of primordia on the shoot apex also implies the delineation of internodes. Bud dormancy, in the classical sense, is largely localized in these internodes and the primordia they bear. How is internodal elongation controlled? By what means are both leaf and scale internodes restrained from elongating during bud formation, or subsequent periods of correlated inhibition or rest? When the restraint is finally released, why is elongation often confined to internodes between leaves whereas those between scales remain short? What are the linkages between the reactions of phytochrome and the control of internodal elongation? Do leaves produce substances of a hormonal nature which have a controlling influence upon internodal elongation?

Once rest has been induced, many buds require exposure to low temperatures for a considerable time before normal development becomes possible. Many seeds have similar requirements, and cold treatment is effective only after inhibition. What is the physiological-biochemical basis of the chilling requirement? How is the metabolism of buds or seeds changed by chilling? What is the significance of so-called growth inhibitors? Are we to suppose that if they were not present there would be no restraints upon growth?

The example of dwarf peach seedlings grown from unchilled embryos is instructive in that it cannot be explained by invoking the growth-inhibitor concept. Cells of unchilled buds or seeds are genetically no different from those in which the chilling requirement has been satisfied. But is it not possible that certain segments of the genetic information are inoperative in unchilled tissues and that, therefore, the blocking of some physiological processes is more fundamental than suggested by the term "growth inhibitor"? Can we be sure that resting buds do not lack some metabolites necessary for growth, or even the enzymes and cofactors necessary to synthesize them?

Some root apices reportedly contain a quiescent center within a bell-shaped promeristem. The cells of the quiescent center are

potentially meristematic, but not actively so. Why does the quiescent center become active when the surrounding promeristem is destroyed by X-rays or microsurgery? Is it primarily because the environment is changed, or because of a nonspecific wound effect? What physiological characteristics do cells in a quiescent center have in common with cells in the subapical region of a resting bud?

What is the complement and function of growth regulators or hormones in woody plants? Are indolic auxins preeminent? Are gibberellin-like compounds or kinins widely distributed in woody plants? Are we justified in thinking of these compounds as primary regulating agents if we know neither their locus of action nor the manner in which their synthesis or activity is controlled?

Recent developments in a number of seemingly unrelated fields, when integrated, may provide leads of value to research on morphogenesis and growth control in trees. For example, it has long been known that inositol content of buds increases in spring. Inositol has also been found beneficial to, or is actually required by, various tissue cultures, and there is evidence that it may be involved in membrane synthesis. Meanwhile some evidence has accumulated consistent with the idea that kinetin enhances the ability of cells to accumulate solutes, including auxin and presumably also inorganic ions. Interestingly, six of the seven biosynthetic systems found to be liberated from normal control when cells of *Vinca rosea* become tumorous are reportedly ion-activable. This group includes the auxin synthesizing system. The one apparent exception is the system responsible for synthesis of kinins. Its activation has unknown requirements.

If natural kinin, like exogenous kinetin, can enhance the ability of cells to take up ions, then release of the kinin synthesizing system from control could result in activation of the six other systems. Furthermore it has been observed that ion uptake and utilization by some cells is greatly facilitated by, and possibly dependent upon, the presence of inositol. We may therefore speculate upon the possible existence of a functional relationship between kinin and inositol.

Does perhaps the efficacy of kinin depend upon the relative availability of inositol? Could the role of auxin, though important, be a subordinate one? What can we learn about the metabolic aspects of dormancy breaking from work on changes in biosynthetic systems associated with the appearance of rapidly proliferating tumors in previously normal tissue? The search for possible interrelations of this kind requires cognizance of research in diverse fields, but it could lead to new levels of understanding.

Progress in research on morphogenesis and the activities of meristems in woody plants is hampered by an insufficiency of knowledge about these subjects in general. If we wish to advance our knowledge of the growth and morphogenesis of trees and eventually to enhance our ability to influence these processes to our advantage, we must not be averse to looking for answers in places far removed from the trees in which the problems are first brought to our attention. But as we become involved in academically diverse areas we must take pains to maintain communication, to cultivate a broad perspective, and to remember that we are all basically biologists.

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688-803 O—63——13

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688-863 O—63——14

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