RECENT EXPERIMENTAL STUDIES OF THE SHOOT APEX AND SHOOT MORPHOGENESIS

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I. INTRODUCTION

The aim of this review is to discuss experimental work on the shoot apex and shoot morphogenesis published during the last decade. Morphogenesis, the inception and development of form, is considered to be one of the central problems of biology, and the importance of gaining an understanding of its many aspects has recently received emphasis from several quarters. The development of the primary pattern of the shoot may be traced back to the activities of the shoot apex, which accordingly assumes importance as a major controlling region of the plant. Structural aspects of the shoot apex have been the subject of sustained investigation since the time of Nägeli, and a number of useful reviews dealing with this topic exist (95, 97, 122, 183, 184, 191, 393, 415, 416, 429, 431, 481, 616). Experimental investigations of the shoot apex on any considerable scale are, however, of comparatively recent date.

Major advances in the experimental study of the shoot apex have been made in the last two decades; the many recent and contemporary investigations have probably been stimulated in large part by the publication of books on plant morphogenesis by Wardlaw (612, 613), and more recently by Sinnott (483). Accounts of the earlier work can be found in these books and in a review by Wetmore and Wardlaw (671).

As Wardlaw (635) has pointed out, the shoot apex, comprising both the apical meristem and the subapical regions, should be regarded as an integrated whole. Within this region, most of the characteristic features of the mature shoot are established in miniature; they become externally evident as a result of elongation and expansion growth. As Brown (80) has cogently remarked in discussing certain recent experiments, "The observed effects are magnified by growth from primary events occurring in the apical meristem." Reported results on shoot structure thus often represent effects on the shoot apex, which in due course become manifest in the mature shoot. Moreover, experiments actually carried out on parts of the mature shoot may have effects, often of a far-reaching nature, on the shoot apex; a striking example of this is the response of the apex to photoperiodic treatments of the mature leaf. Experiments on the shoot apex can thus be of two kinds: direct experiments, carried out on the apex itself, for example surgical experiments; and indirect experiments, carried out on more mature regions of the plant but affecting the shoot apex.

The aim of experimental studies of the shoot apex is to gain an understanding of its complex functions. To achieve such an understanding, as much information of all kinds as possible is needed. Much of this is derived from direct experiments, and the more recent work of this kind has currently been reviewed by Wardlaw (639) and Allsopp (16); it is also discussed to some extent in recent books by Clowes (122) and Sinnott (483). Useful knowledge can also be gleaned, however, from indirect experiments, including those not actually intended to disclose information about the shoot apex. An attempt to integrate the findings from both direct and indirect experiments may thus

fulfil a useful function. The volume of literature on some aspects is very considerable, and this survey does not claim to be complete. It may well be undesirable to separate experimental studies from those of a non-experimental nature, which are in general no less important to an understanding of shoot morphogenesis, but the sheer volume of the relevant literature precludes their inclusion in the same article. Reference will be made, however, to work of this kind and to work published prior to the last decade, where this is helpful to the discussion.

II. TERMINOLOGY

As far as possible, the term "apical meristem" will be used to denote that part of the shoot apex lying distal to the youngest leaf primordium, thus comprising the distal and sub-distal regions of Wardlaw's scheme (629). The "subapical region" lies below the organogenic region, where the leaf primordia become visible, and is usually characterised by active growth of leaf primordia and widening of the embryonic shoot. It merges into the region of maturation. The term "shoot apex" will include both these regions, thus comprising the apical meristem and young leaf primordia. Many authors, however, do not clearly define the terms they use, and consequently it will not always be possible to adhere to these definitions with any confidence. In one paper (591), for example, the "shoot apex" may be up to two cm. long.

Young leaf primordia will be called P_1, P_2, P_3 etc., P_1 being the youngest; primordia which were initially invisible but developed during the course of the experiment will be called I_1, I_2, etc., I_1 being the first to arise (493). Sites of prospective primordia will also be referred to as I_1, I_2 etc.

Certain abbreviations in common usage to denote substances affecting growth will be employed, and will be defined at the time of their first use in the text. These are:

IAA: β-indolylacetic acid (indole-3-acetic acid)
NAA: α-naphthylacetic acid
2,4-D: 2,4-dichlorophenoxyacetic acid
TIBA: 2,3,5-triiodobenzoic acid
PhB: phenylboric acid
GA: gibberellic acid (gibberellin A_3)
Where other gibberellins are meant, or in cases where the author has not made it clear which was employed, the term "gibberellin" will be used
Amo-1618: (4-hydroxy-5-isopropyl-2-methylphenyl) trimethylammonium chloride, 1-piperidine carboxylate
CCC: (2-chloroethyl) trimethylammonium chloride.
Phosfon: 2,4-dichlorobenzyl, tributylphosphonium chloride
5-FU: 5-fluorouracil
5-FDU: 5-fluorodeoxyuridine

Other abbreviations are:

LD: long days
SD: short days
DNA: deoxyribonucleic acid
RNA: ribonucleic acid

III. PRIMARY ORGANISATION OF THE SHOOT APEX

Although shoot apices of vascular plants differ widely in detailed structure, they clearly have many functional activities and features of organisation in common (613, 639). The structural aspects of shoot organisation are well known and are described in standard text-books (122, 172, 173). The controlling factors, however, are not well understood, and the problem of organisation remains a major field for future study, the importance of which has recently received emphasis from both morphologists and physiologists (483, 526, 527, 610, 635, 640). Some years ago Sinnott (482) expressed the view that "No analysis, however complete, of substances known to have morphogenetic effects will explain their results. Only a knowledge of the organized systems upon which they act will solve the problem." More recently, Salisbury (467) has discussed the difficulty of envisaging how a chemical—for example, the flowering hormone—can control the formation of a three-dimensional structure. Yet it seems evident that various substances, together with nutrition and aspects of the environment, are among the factors that interact with the genetic constitution of an organism or tissue to bring about its orderly development. Many of the processes involved in shoot organisation remain obscure at the present time, but some information is nevertheless available.

The organisation of a shoot apex from meristematic cells occurs during embryogeny (see 621), during the formation of a lateral bud from tissues of the apical meristem or from detached meristems (596, 597, 601), or as a result of various surgical experiments on the shoot apex. Organisation from differentiated tissues may occur in the formation of some lateral and adventitious buds, and in regeneration phenomena. The present account will deal with some of the factors involved in the primary organisation of a shoot apex in embryogenesis or as a result of regeneration, and will include also various recent studies of the development of single cells or small groups of cells in culture which have contributed to an understanding of shoot organisation (445, 446, 531, 532, 654, 655). Secondary changes in apical organisation which occur at flowering will be considered in a subsequent section of the review.

EMBRYOS

The importance of environment as a factor controlling development becomes evident in very early stages of growth. Working with the fern *Thelypteris palustris,* Jayasekera and Bell (275) removed archegonia on small fragments of the prothallus before or after fertilisation, and subsequently cultured the zygotes. These gave rise to normal sporophytes, but in embryos isolated from the apical notch of the prothallus the normal sequence of development was altered, the stem apex becoming visible before the root. In other ferns no roots at all developed in embryos from excised fertilised eggs grown in vitro (451). From certain of the experiments on *Thelypteris* it was concluded that polarity had probably been established in the unfertilised egg prior to excision (275). In two species of fern, complete or partial removal of the physical constraint imposed on the zygote by the archegonial neck led to the formation of sizeable outgrowths bearing up to three shoot apices (275, 595). An abnormal

number of shoot apices also developed in cultured proembyros of barley which had formed a large number of cells before differentiation (398).

More recently, young embryos of the fern *Todea barbara,* which are not attached to the archegonial wall, have been completely removed from their normal environment and successfully grown in vitro (156). Given adequate nutrition, embryos excised 17 or 20 days after fertilisation could develop into mature sporophytes, but those excised four or five days after fertilisation, before the first division of the zygote, developed as two-dimensional, thalloid structures. Presumably, therefore, under these conditions normal organisation of the shoot apex does not take place. The normal constraint of the archegonial tissues in ferns thus appears to have an important effect on embryogenesis, although nutritional factors cannot be disregarded and there are indications that hormonal effects may also be involved (155).

The importance of nutrition and the stimulatory effect of certain growth-regulating substances on the growth and development of young embryos of higher plants is well known (442, 621). Veen (583) studied the effects of various growth regulators on *Capsella* embryos, and recently Raghavan and Torrey (440) have successfully grown globular embryos of *Capsella* in a medium supplemented with indoleacetic acid (IAA), kinetin and adenine sulphate. Embryos more than 80 μ long would grow and develop normally on a relatively simple medium, but smaller embryos required the added growth factors; the findings thus support the view that the change from heterotrophic to autotrophic growth during embryogenesis depends on the availability of specific growth factors. The ability of many embryos to grow in vitro only on media supplemented with coconut milk also lends support to this view. Pecket and Selim (411) successfully grew embryos of a *Lathyrus* hybrid, which would have aborted in vivo, in media containing appropriate concentrations of coconut milk and sucrose. The nutritional requirements of root and shoot may differ (44). The change in form from the globular to the heart-shaped embryo is due to the increased mitotic activity which gives rise to the cotyledons (428), but before their initiation a shoot apex becomes organised (243).

Organisation from single cells

Recently the growth and development of isolated somatic cells in vitro, in the presence of other cells in the medium, has been achieved. The work which is of greatest interest from the point of view of shoot organisation is that dealing with carrot phloem tissue. In 1958, Steward, Mapes and Smith (532) succeeded in obtaining cell suspensions from pieces of carrot phloem grown in rotated vessels of liquid nutrient medium containing coconut milk (the liquid endosperm of the coconut, sometimes called coconut water). These cells underwent division in various ways to give groups of cells; sometimes filamentous structures resembling young embryos of the carrot ("embryoids") were formed. The cell aggregates often gave rise to root primordia, which were formed in a central cambium-like cylinder of cells, and it was found that if such cell masses were transplanted to nutrient agar they might also form buds and shoots (531). Thus, for the organisation of a shoot apex, a semi-solid, stationary medium was

required, and also exposure to air. It is also considered that organised development is promoted by the barrier of the cambium-like cells, which restrict the access of the coconut milk, with its ability to stimulate random proliferation of cells.

After the establishment of an axis with root and shoot apices, continued development leads to the formation of mature carrot plants with storage roots (531). Ultimately, these plants may produce normal inflorescences and fruits (530, 533); this indicates the essential normality of a shoot apex organised under these exceptional conditions.

Structures resembling stages in embryo and seedling development were also obtained by Reinert (445, 446) in callus cultures from another strain of carrot, and also by Kato and Takeuchi (278) from carrot callus grown on an agar medium without coconut milk, on which a culture had previously been grown. Similar adventive embryos have been observed in callus cultures of wild carrot (660), and structures resembling normal embryos have also been obtained from callus cultures of *Cuscuta* (356); this callus originated from embryos grown on a particular medium. Haccius (230) has recently obtained embryoids, which reorganised to form usually normal adventive embryos, from the proliferated suspensor cells of embryos of *Eranthis* treated with McIlwaine's citric acid-phosphate buffer. This treatment selectively killed the fully meristematic cells of the embryo.

Perhaps the most important finding of the carrot tissue culture work is the unequivocal demonstration of the totipotency of somatic cells. As Steward and Mohan Ram (533) have pointed out, individual carrot cells supplied with appropriate nutrients show a much greater range of growth and form than they do when part of a tissue. As might be expected, this potentiality for growth and development is even more strikingly demonstrated when the individual cells are themselves derived from an embryo. An aliquot of a cell suspension culture derived from a single carrot embryo gave rise to some 100,000 embryoids (529). Embryoids formed from these cells in culture show the typical globular, heart-shaped and torpedo stages of the normal embryogenesis. Individual freed cells from carrot embryos are said to behave like zygotes (530).

The embryo-like growth of differentiated cells from several different plants (358) when grown in media containing the liquid endosperm of another species testifies to the importance in development of nutritional and hormonal factors. It is interesting that isolated cells which are themselves derived from embryos can recapitulate earlier stages in embryogenesis than those derived from more differentiated tissues.

NEOMORPHS

Structures resembling embryos and seedlings have also been obtained in two species of *Oenanthe* cultured in the presence of amino acids (654, 655). When seedlings of *O. aquatica* were grown aseptically in a liquid medium containing glycine, small green plantlets were observed in two flasks containing apparently moribund seedlings (654). These "neomorphs" originated from small nodules of tissue detached from the tips of lateral roots and possibly also from the shoot apex. The nodules developed into embryo-like structures with a root end

and a shoot end, and gave rise to lobed structures like cotyledons. After several months normal shoots arose from new growing points. Similar reversible changes in morphology occurred in *O. lachenalii* in the presence of glycine, but in the presence of leucine irreversible changes were induced in this species (655). Neomorphs were derived from nodules detached from the shoot apex, and exhibited variation in form ranging from tangles of branching threads to dwarf shoots with simple leaves. The morphogenetic effects of these amino acids were so profound that even family characters were obscured in the neomorphs. It is considered that synthesis of proteins and nucleic acids is affected.

Regeneration

In regeneration phenomena of many plant organs and tissues a shoot apex becomes organised from already differentiated cells. This process may begin very quickly after wounding or excision of the organ (145). In the endogenous buds which are formed in cortical tissues of detached roots and decapitated stems of *Ophioglossum vulgatum,* an organised shoot apex early becomes discernible (617). In the embryo of this fern, by contrast, the appearance of the primary root precedes the organisation of the shoot apex, suggesting that nutritional factors may affect the order of formation and outgrowth of the various organs (620).

Some of the most interesting regenerating materials from the standpoint of morphogenesis give rise endogenously to groups of meristematic cells, which may eventually become organised as either root or shoot primordia, according to conditions (162, 171, 435). The auxin level in the surrounding tissues is apparently one factor in determining the fate of these primordia (163, 171); but hitherto too little use has been made of these promising materials in the study of organisation. Polarity is another important factor involved. In roots of *Convolvulus arvensis* grown in vitro, buds arose endogenously in positions opposite the protoxylem poles, corresponding to the sites of lateral roots (65, 575). In cultured segments of roots, however, lateral roots were formed only at the distal end, whereas buds may be formed at any point along the length of the segment (65).

The importance of the quantitative action of chemical factors in controlling organ formation and organisation has been emphasised by Skoog and Miller (487) and their associates. Working with cultures of tobacco pith, they were able to demonstrate interactions between IAA and adenine or kinetin, a cell division factor initially discovered by these workers. For example, the presence of sufficient adenine in the medium could counteract the inhibitory effect of IAA on bud formation. By varying the relative quantities of IAA and kinetin, the growth of unorganised callus or of organised root or bud primordia could be induced. In tobacco stem segments grown in vitro, root formation was stimulated in the presence of naphthaleneacetic acid (NAA), while the formation of adventitious buds was induced in the presence of adenine sulphate (488). The effects of these substances on tissues of other species, however, are not always comparable, thus supporting Sinnott's view that the system acted upon is at least as important as the substance in controlling organisation (482).

Kinetin induces bud formation in excised roots of *Convolvulus* grown in

culture (575), and buds are also formed in cell colonies derived from suspensions of root callus of the same species when the medium contains high kinetin and low auxin concentrations (167). Kinetin, adenine and other purine and pyrimidine bases have also been shown to stimulate bud formation in leaf tissue of *Cardamine* (410). Discs of the lamina without pre-formed bud initials were treated with the various substances for 24 hours, and the number of buds subsequently formed was noted. These authors distinguish three phases in bud development: *(a)* removal of inhibition, allowing mitosis to take place; *(b)* orientation of mitoses, leading to bud formation, in which nucleic acid metabolism is implicated; *(c)* bud growth. This distinction between the inception, organisation and outgrowth of a primordium is a useful one which is too often ignored, so that it is often not clear whether the observations reported concern the initiation of an organ or phases in its subsequent development.

IRRADIATION

Certain destructive techniques offer another possible approach to the study of shoot organisation. When an already organised shoot apex becomes damaged, either mechanically or as a result of chemical treatments or irradiation, new meristems may become organised. After irradiation with gamma or X-rays or from a cobalt source, shoot apices may be more or less completely disorganised (130, 233, 365, 371, 434, 525). In maize, growth of the apical meristem is inhibited progressively with both dose and time, and ceases to function about six days after X-irradiation of the seeds (525). In *Nicotiana rustica* sublethal doses of X-rays led to total injury of the central cells of the apex and to regeneration from newly-formed lateral meristems. In more radiation-tolerant species the apical dome itself eventually broadened and divided into two (233). A high frequency of mutants was induced in the R_1 generation of coffee when the seeds were irradiated with thermal neutrons or gamma rays (377). The whole plant, including lateral buds induced to grow out, produced similar mutant characteristics, and the changes were permanent. Moh (377) argues from this that the cells forming the parts of the shoot other than the epidermis may all be derivatives of a single cell in the corpus of the shoot apex of the embryo, and that there must be one cell which dominates growth during early stages of development from the coffee embryo. However, work on root apical meristems (119, 123) has demonstrated that even cells which do not normally undergo active division may do so when the adjacent cells have been damaged by irradiation. Thus it seems reasonable to conclude from Moh's (377) experiment only that shoot organisation may under certain conditions occur from one cell in the embryo, not that it normally does so. This in itself, however, is of considerable interest.

CONCLUSIONS

The collective evidence from these various experiments seems to indicate that every cell in an organism possesses the inherent capacity to develop into a whole plant of that genotype, and that the actual development of that cell depends upon a number of supervening extrinsic factors. The importance of environment in controlling development has often been emphasised (665).

Many surgical experiments, to be discussed later, have demonstrated the self-determining nature of the shoot apex. Several factors may, however, be important prerequisites. These apparently include: certain physical factors, an adequate supply of nutrients, a system of polarised gradients, and an appropriate balance of chemical growth factors. These last apparently act as evocators, supplying the cell or group of cells with the stimuli necessary to trigger off one type of development characteristic of the species. The path of development of a cell or tissue is not irrevocably fixed but can be modified by various regulatory substances and other factors.

It is noteworthy that many of the substances other than auxin known to be involved in the formation and organisation of shoot apices are implicated in nucleic acid metabolism. Skoog (486) suggested that a mechanism for the regulation of growth may be envisaged if auxin promotes the synthesis of nucleic acids differentially according to the proportions of the required constituents present. That the processes of nucleic acid synthesis are also involved in the important changes which occur in apical organisation at the time of floral induction has recently been established; this evidence is discussed in section XI of this review.

IV. GROWTH AND FORM IN THE SHOOT

The shoots of vascular plants consist of an axis bearing lateral members; but, although constructed with this basic pattern, they exhibit remarkable variation in form. Vegetative shoots may be tall or short, branched or unbranched, radially symmetrical or dorsiventral, erect or plagiotropic. This variety of form is brought about by the expression of different genetic constitutions, which are in turn mediated through the action of various chemical substances and the relative growth activities of the apical and subapical regions of the shoot apex. Within a single species, however, or even during the ontogeny of a single plant, considerable variation in shoot form may be observed. A single genotype is thus capable of somewhat diverse developments according to the other conditions obtaining at the time. It is clear, for example, that chemical or nutritional factors may modify relative growth. Of recent years some information relating to the factors—including genes—involved in the expression of form in the shoot has accrued, although, as will be evident, considerable scope for further experiment remains. Evidence relating to the mode of action of genetic factors will be considered first, and then other factors affecting the development of form during ontogeny and in the mature shoot will be discussed.

(a) Genetic Aspects

GENES AND DEVELOPMENT. Genes exert an overall control of developmental processes, but it is sometimes possible to modify the normal expression of genetic characters by other means; indeed, experiments of this sort have contributed quite substantially to an understanding of the genic control of development. In a study of some of the many mutants of maize, Postlethwait and Nelson (432) have concluded that "switch points" in development are numerous. These are stages in development at which under normal conditions some of the complement of genes evoke a particular type of development. In

mutant plants development may have been diverted into a different pathway at a particular switch point, as can be demonstrated by morphogenetic studies. Situations also exist, however, in which the diversion may be brought about by other means: for example, in genetically male plants of *Cannabis sativa,* female flowers can be induced in sites where male flowers would normally develop by treating the plant with naphthaleneacetic acid (NAA) (246). Thus the level of auxin in the region of the floral meristems during inception of the flower primordia is evidently important, and it is probably this that is normally under genetical control. Experiments of this kind offer one means of tackling the problems of physiological genetics.

The importance of the time of action of genes during development has long been known (235). Different genes may come into action at different times. The time of appearance of genetic differences may thus vary quite widely, some being observable in the embryo or during development at the apex and others becoming evident only later.

STUDIES WITH MUTANTS. Single-gene mutants have proved to be useful materials for studies designed to elucidate the mode of action of particular genes in development. Brian and Hemming (76) showed that the height of a dwarf variety of peas, which differed from the tall form in a single gene, could be brought within the normal range of the tall type by repeated treatments with gibberellic acid (GA). Later Phinney (417) found that the height of several dwarf mutants of maize could be restored to that of normal tall maize plants by treatment with GA. It is considered that in dwarf mutants the level of gibberellin may be in part determined by specific genes which control steps in its biosynthesis (418).

Working with a radiation-induced, single-gene mutant of groundsel, Basford (53) found that GA-treatment restored the height of the dwarf plants to normal but did not restore all the morphological features of the normal phenotype. In a study of morphogenesis in the normal and mutant forms, she showed that the differences in shape and size in the shoot apices of young plants could be attributed to differences in cell number in the apical meristems and to the distribution of growth in the subapical region. There were fewer mitoses in both regions in apices of the dwarf mutant, and the rate of leaf inception was also slower. Treatment of greenhouse-grown plants with GA led to a considerable increase in the number of mitoses in the subapical region, accounting for the increase in internode length and total height, but GA had no effect on the number of mitoses in the apical meristem itself, and hence did not increase the number of leaves formed to the level typical of the normal tall form. Basford (54) later showed, however, that GA-treatment of dwarf plants grown in an environment unfavourable for growth, where the vegetative phase of development was maintained for a longer period, enhanced cell division in the apical meristem and brought both that and the node number to the level typical of tall plants grown in the same environment. Growth and development of both normal tall and dwarf mutant forms differed very considerably in the two environments. This work thus indicates that in *Senecio*

both the expression of the genotype and its morphogenetic response to GA are greatly modified by environmental factors.

Bergfeld (56) found that GA could counteract the dwarf growth of an *inhibita*-like "half-dwarf" mutant of *Antirrhinum,* but that it substantially failed to do this in the mutant *densa.* On the basis of grafting experiments he concluded that the arrested growth of the "half-dwarf" mutant could be attributed to inhibition of the action of a growth substance, while synthesis of a growth substance seemed to be blocked in the *densa* mutant. As a result of applying GA, IAA and tri-iodobenzoic acid (TIBA) to erect and stoloniferous plants of strawberry clover, in which the stoloniferous form is dominant, Bendixen and Peterson (55) concluded that the dominant gene probably controls the synthesis of an auxin inhibitor which prevents the stolons from responding geotropically. In the erect form, the homozygous recessive, this inhibitor would not be present. Applied GA was apparently capable of temporarily inactivating the inhibitor.

Studies of this kind, involving application of various hormonal substances to plants of known genotype, preferably combined with a closer study of development than is sometimes undertaken, provide a useful means of investigating gene action.

Mutants affecting the flower or inflorescence have also been used in somewhat similar studies. A single-gene mutant in pea, obtained by irradiation of the seeds, showed extensive disturbance of the floral meristem (209). The number of stamens varied from zero to eight; some consisted of a reduced carpel bearing an anther on the base of the style, while others were anthers which had a reduced style with stigma as an apical appendage. The ovary was normal and functional. In the whorl of sepals, usually only one to three members were normal sepals, the other members being in the form of more or less reduced carpels. Thus in this mutant, differing from the normal in only a single recessive gene, the normal sequence of morphogenesis in the floral meristem is grossly disturbed. This supports Wardlaw's view (630) that the clear delimitation of the phases of organogenesis in the floral meristem can probably be attributed to the action of particular genes.

Recent studies of mutants in maize are proving helpful in interpreting normal development. Some mutants resemble the effects obtained by hormonal treatments or by manipulation of environmental factors (252). Normal development of the ear of corn is very complicated; it may be summarised briefly as follows. Branch initials or alicole meristems are first formed in acropetal sequence, subtended by ridges. The branch meristems then divide to form two spikelet initials. In each spikelet there are ultimately two florets, one of which is fertile and the other sterile (66). A number of interesting mutations have been described in which the normal development is arrested or otherwise modified at various "switch points". In the mutant known as *polytypic ear (Pt)* (339), proliferation of the pistillate tissue takes place to a variable extent. Ears from heterozygous plants typically show a proliferation from the sterile floret only. Ears from apparently homozygous plants sometimes show proliferation of pistillate tissue from both florets, and other variations. Both the extent of pro-

liferation and the number of florets involved vary greatly, so that the mature ears formed by these plants show considerable diversity of form. This is an example where the time of action of the gene is apparently important.

More recent studies of morphogenesis in this mutant (432) have shown that the gene *(Pt)* apparently interrupts the normal developmental sequence at the switch point from pistil formation to ovule differentiation. The meristem is maintained in the phase of pistil inception for an extended period. Since the gene can affect ear development at four points, the variation in form observed previously (389) is not hard to understand.

The maize mutant known as *ramosa* may affect both the ear and the tassel, causing profuse branching. It interrupts development at only one point: the branch meristems do not give rise to spikelet meristems but each continues growth as a single axis (432). The lateral meristems formed on the branches develop normally as alicole meristems and may give rise to fertile grains.

POLYPLOIDS AND CHIMAERAS. While the shoot apices of plants differing in only a single gene are thus seen to differ in both form and function, those of different ploidy are, in general, less distinctive. In *Vinca rosea,* colchicine-induced tetraploid apices are considerably broader than those of the diploid plant; this is attributable to an increase in width of the cells, their length remaining comparable. Changes in the meristem following colchicine treatment are correlated with changes in form in the adult plant (131). Tetraploid apices of maize were also considerably larger than diploid ones, due to an increase in cell volume (441). A differential response to photoperiod of diploid and tetraploid races of *Trifolium* has been reported (679). Diploid mutants developed after colchicine treatment of seedlings of tetraploid sorghum; the diploid cells apparently arose anywhere within the shoot apex (115). Shoot apices of chimaeras of various plants, usually induced by treatment with colchicine, have yielded important information concerning the contribution of the various histogenic layers in the apical meristem to the organs and tissues of the shoot. This work has recently been fully reviewed by Clowes (118, 122).

CONCLUSIONS. The need for comprehensive studies of morphogenesis in closely related plants of known genetic constitution, and for cognate hormonal and biochemical investigations, was pointed out by Wardlaw (613) some 12 years ago and is indeed still evident. Many genetical studies are primarily concerned with mature form. However, a useful beginning has been made along the lines advocated by Wardlaw. Work on the morphogenesis of mutant forms (53, 54, 389, 432), in conjunction with experimental work of various kinds on the same genera (53, 252), may well throw light on the relationship between genes and hormones and their interaction in plant tissues. The complexity of these problems is evident.

(b) Ontogenetic Changes

APICAL SIZE. One of the most evident changes in the shoot apex during ontogeny concerns its magnitude; in general, there is a progressive increase in size of the apical meristem during development (8, 16, 70, 191, 603). This

may reflect an increase in cell size or in cell number, or in both. Some useful quantitative work has been carried out on the shoot apex of maize (1-3). Both during embryogeny and after germination the apical meristem increased in size, but the rate of increase in size was deceleratory during embryogeny and acceleratory after germination. These changes were due to an increase in cell number, not cell size. The dimensions and volume of the shoot apex increased in a uniform manner after germination. The rate of leaf formation was also deceleratory during embryogeny and acceleratory after germination. It seems possible that these changes may be correlated with the onset and termination of dormancy.

This precise, quantitative work on ontogenetic changes in the apical meristem has been more generally confirmed in other studies. The progressive increase in size of the apex, which is presumably in part at least a response to improving nutritional conditions (8), can be experimentally reversed. If apices of the fern *Dryopteris dilatata*[1], from which all the expanded and most of the unexpanded leaves have been removed, are kept in peat in the laboratory for periods of up to a year, they undergo progressive decrease in size (137). This change is accompanied by changes in the rates of various growth processes in the apex (137, 147).

JUVENILE AND ADULT PHASES. Many plants show conspicuous morphological differences between earlier, or juvenile, and later, or adult, stages of development. These differences in the shoot system are brought about by various changes, and a distinction has recently been drawn in woody plants between reversible changes, or "ageing", which include reduced annual growth increments, loss of apical dominance, etc., and non-reversible changes, or "maturation", which include changes in leaf arrangement and leaf shape, and the transition to flowering (379). These distinctions, although useful, are not absolute. In *Pinus sylvestris* and *Larix leptolepis,* the results of pruning treatments and experiments with ^{32}P suggest that ageing of the tree is due to increased competition for nutrients as the complexity of the shoot system increases. The actual distribution of the nutrients available to the tree is modified by apical dominance (379).

In many trees, shoots from the basal parts remain in the juvenile stage throughout life, as can be demonstrated by grafting experiments (471). Shoots from both dormant and adventitious buds from basal positions, and also from roots, show juvenile characters (472). Juvenile shoots are characterised, among other things, by retention of leaves and a greater ability to form roots when used as cuttings (651). In many species they also bear leaves which differ in form from those on the adult shoots of the same plant.

This change in leaf form with age, or heteroblastic development, has been the subject of a number of experiments; the whole topic has currently been reviewed by Allsopp (16), who has himself carried out experimental investigations on *Marsilea drummondii.* Allsopp (5) established a technique for

1. *Dryopteris dilatata* (Hoffm.) A. Gray: synonymous with *D. aristata* (Vill.) Druce and *D. austriaca* (Jacq.) Woynar. All these names have been used in the literature; in this review the name *D. dilatata* will be used throughout.

growing sporelings of this amphibious fern in aseptic culture, and was thus able to study the effects on leaf development of the nutrition of the whole plant. In sporelings of *Marsilea* there is a transition from the first subulate leaf to spatulate and subsequently bifid juvenile leaves before attainment of the quadrifid form of the adult leaf. Allsopp (7) found that quadrifid leaves were formed at earlier nodes, i.e., the course of heteroblastic development was curtailed, in the presence of increasing concentrations of glucose, sucrose or fructose in the culture medium. This effect of sugar was nutritional rather than osmotic. By depriving the cultures of either sugar or mineral nutrients, reversion to juvenile leaf forms could be induced (6). This reversal of the heteroblastic leaf sequence was more rapidly attained if short terminal regions of the cultured plants were excised and transferred to the depleted medium; if whole plants were transferred, reversal did take place but was delayed. Plants cultured on an agar medium were less vigorous than those on a liquid medium of the same composition, and the stages of heteroblastic development were extended (7). Changes in the nitrate concentration of the medium initially had no effect on growth or heteroblastic development, but at 0.2 times the normal concentration a reversion to juvenile stages finally took place. Stimulation of heteroblastic development was achieved by substitution of ammonium salts or urea for the nitrate in the medium (169).

Allsopp (6, 7, 8, 15, 16) considers that heteroblastic development may be attributed to nutritional factors, these being mediated through their effect on the size of the shoot apex. A certain minimal nutritional status is necessary for the development of the adult leaf form, whereas below that level the juvenile form develops. Since in *Marsilea* either carbohydrate or nitrogen nutrition may be limiting, it is suggested that the rate of protein synthesis may control heteroblastic development (15). White's observation (672) that the protein synthesis inhibitors 2-thiouracil and 5-fluorouracil delay heteroblastic development in *Marsilea* may be considered to support this view.

It is thus held that there is an important correlation between the size of the shoot apex and the extent of development of the leaf primordia to which it gives rise, leaf primordia formed on a large apex being capable of developing into adult leaves, whereas those formed on a small apex are considered to have only a limited capacity for growth and development. Apical regions of well-nourished sporelings with adult leaves are seen by inspection to be larger than those of less well developed sporelings with juvenile leaves, but no detailed study of changes in size of the apex in *Marsilea* has yet been undertaken (15); enlargement of the apical meristem is, however, a normal feature of the ontogeny of many vascular plants.

Wetmore (662, 663), working with excised apices of *Adiantum* sporelings, has also shown that the concentration of sucrose in the medium has important effects on leaf shape, and has suggested that this is brought about by effects on the amount of cell division in the leaf primordium.

It is clearly important to determine to what extent nutritional factors can affect the development of the leaf primordium itself, in isolation from the apex—in which case the findings are not really relevant to this review—and to what extent they are mediated through effects on the subtending shoot apex.

The technique of in vitro culture of isolated leaf primordia, pioneered by Steeves and Sussex (522), offers a possible means of investigating this problem, and Sussex and Clutter (553) have shown that the concentration of sucrose in the medium has a striking effect on the development of isolated primordia of comparable age. On media with very low concentrations of sucrose, leaf primordia excised from adult plants of *Osmunda cinnamomea* developed a dichotomous form resembling the primary leaves of sporelings, whereas with higher concentrations of sucrose they developed as pinnate leaves approaching the shape of adult fronds. From their experiments Sussex and Clutter (553) conclude, however, that heterophylly is not primarily controlled by the size of the primordium at inception, nor wholly by nutrition, but that reactions regulating the rate of leaf development must also be involved. Crotty (132) has ascribed important regulatory effects on leaf development to the older leaf primordia, which are considered to inhibit the maturation and promote the meristematic growth of younger primordia. The complexity of development in the whole apical region, the interactions between its components and the importance of regarding it as an integrated whole (635), thus become evident.

While all these experiments undeniably establish the importance of nutrition as a factor affecting heteroblastic development, some experiments on juvenility phenomena appear to implicate hormonal factors. To the present writer it seems possible that in the normal development both of these factors may act by affecting other variables, namely, rates of growth in the apical and subapical regions of the shoot.

A number of investigators of ontogenetic changes have used ivy *(Hedera helix)* as experimental material. This plant shows well marked differences between the juvenile form, with vine-like branches bearing aerial roots and palmate leaves in a distichous arrangement, and the adult form, with orthotropic shoots without aerial roots and entire, spirally arranged leaves. Flowers are borne on the adult shoots only. In 1954, Doorenbos (160) made reciprocal grafts between shoots of the juvenile and adult forms, and showed that in many cases the adult scion or stock lost the capacity to flower and displayed juvenile features on its new growth, as a result of the influence of its juvenile partner. There was no evidence of any influence of the adult form on the juvenile. Later Frank and Renner (185) showed that juvenile features were induced in adult shoots when rooted cuttings of the juvenile and adult form were grown side by side in nutrient solution, and suggested that a specific substance associated with juvenility might have been involved. The subsequent demonstration (452, 453) that adult arborescent plants of species of *Hedera* sometimes produced juvenile shoots after being sprayed with gibberellic acid (GA) suggested that GA might be such a substance, although it was considered that other factors must also be involved. Temperature has recently been shown to be important (202). Hess (see 453) has shown that two substances capable of affecting growth are present in the juvenile but not the adult tissues of ivy, and recent experiments have shown that tissues from juvenile shoots grow more rapidly than those from adult shoots when maintained in vitro on the same medium (201, 536).

These observations appear to support the view that hormonal effects may be involved in heteroblastic development, at least in some species. Wareing (651) has suggested that juvenile shoots may possibly have a higher rate of auxin production than their adult counterparts. This suggestion, in conjunction with the effect of GA in promoting juvenility in some species, is of considerable interest in view of the recent finding that auxin production in apical regions of the shoot may be greatly stimulated by applied GA (291, 292), and offers scope for experimental investigation.

The effects of gibberellin on leaf form in a number of other species are now known. In *Ipomoea* (397) and *Humulus* (676), treatment with GA leads to continued production of the juvenile type of leaf, as in *Hedera,* but in *Eucalyptus* (478) and *Marsilea* (13, 14) the adult leaf form is developed earlier in treated plants. The situation becomes still more complex when actual leaf shape is taken into account, for in *Hedera* the juvenile leaves are lobed and the adult entire, whereas in *Humulus, Ipomoea* and *Marsilea* the adult leaves are more lobed or divided than the juvenile. Thus, in different species, not only can GA treatment either curtail or prolong heteroblastic development, but it can also promote the development of either lobed or entire leaves.

A general interpretation of these apparently conflicting results is not likely to be found until it is established to what extent GA affects growth of the whole of the apical region or of the leaf primordium itself. It seems possible that this may differ according to the species. This problem offers another opportunity for studies using excised leaf culture. Wardlaw (637) has recently made the point that differences in form of leaves of the same species may be attributed to differential growth of the vertical and transverse components of the leaf primordium. As many experiments have shown (see Section VIII), GA has differential effects on these components of growth in the stem, so that it may be suggested that some of its effects on leaf form may be due to a comparable effect on the tissues of the leaf primordium itself. Gray (211) has shown that GA treatment may lead to changes in form of both simple leaves and of the leaflets of compound leaves, the pinnae of tomato leaves being entire instead of dentate. It seems probable that this may be attributable to an effect on the various components of growth in the pinnae.

On the other hand, as Allsopp (6, 7, 8, 15) has argued for *Marsilea,* leaf development may be affected by the growth of the shoot apex. In microphyllous species and in parasitic species which have scale-like leaves, the leaf primordia are of normal macrophyllous size at inception, as indeed are also the primordia of genera like *Victoria* with exceptionally large mature leaves (138, 144, 631). Thus the subsequent development of the leaf primordium in the subapical region must be important in determining its final size and form (614). It is well known that GA affects the subapical region of treated plants. In the examples cited above, GA treatment affected stem elongation in *Eucalyptus, Humulus* and *Ipomoea,* and it affected the rate of leaf formation in these three species as well as in *Marsilea* (14, 397, 478, 676). Thus in the majority of species in which GA affected heteroblastic development it also affected rates of growth in the apical and subapical regions of the shoot. Differential effects on growth in these two regions would result in a leaf primordium spending a

longer or a shorter time in close proximity to the apical meristem, which in turn would affect its development. Nutritional factors might also be expected to alter the balance of growth between these regions.

In support of the view that effects on growth in the subapical region may affect leaf development may be cited the finding of Allsopp (11)—who offers a nutritional interpretation—that in plants of *Marsilea* treated with indoleacetonitrile, stem elongation was markedly stimulated and heteroblastic development was retarded. The occurrence of an increased number of scale leaves instead of foliage leaves in plants treated with GA (146, 164, 165, 636) possibly also affords supporting evidence. The present reviewer thus concurs with Allsopp's opinion (13, 14) that GA is not a specific juvenility hormone and that its effects on leaf development are probably secondary consequences of induced changes in growth rate, but advocates studies of its effect on isolated leaves and some caution in interpreting its effects in terms of nutrition.

In a consideration of ontogenetic changes, the important question arises whether the apical meristems of juvenile and adult shoots have any inherent differences. The results of certain grafting experiments seem to suggest that they have, and some writers believe that age changes in the apical meristems of woody plants, at least, are common phenomena (472). Robbins (453) also considers that the apical meristems of juvenile and adult shoots are metabolically different. The occurrence of branches of *Hedera* with mixed juvenile and adult characters (453), however, hardly seems to support this contention. There is scope here for application of the technique of in vitro culture of excised apices.

SENESCENCE. Having developed the adult growth form and undergone active growth for a variable period of time, many plants undergo a period of senescence and then die. In a number of species the onset of senescence can be delayed or prevented by removal of developing flowers or fruits (327, 328, 471). The earlier this is carried out, the more effective it is, and it is considered that in these plants senescence may develop in response to a gradually intensifying signal in reproductive plants. Since removal of the small staminate flowers from male plants of spinach delays senescence to about the same extent as removal of the pistillate flowers, it is now considered that depletion of food reserves as a result of their mobilisation into developing flowers and fruits is not the principal cause of senescence (327). Moreover, spinach plants induced to bolt (and thus presumably to mobilise nutrients) by treatment with GA did not undergo senescence, while those given long-day treatment, which both bolted and flowered, did become senescent (274). It may be suggested that it is the number of actively growing meristems present that is important in controlling senescence.

Evidence that the shoot apical meristem is implicated in senescence phenomena is drawn from several sources. Senescence of cotyledons and young leaves of soybeans can be prevented by removal of the stem apex at an early stage (327). It has been suggested that transport of ribonucleic acid (RNA) out of ageing cotyledons is a feature of their senescence, and it has been shown that there was a corresponding increase in RNA in the growing regions of the embryo (405). Lockhart and Gottschall (336) have shown that senescence

in Alaska pea is determined by two factors. One of these is an influence of developing fruits, especially the seeds; when these were removed, stem growth was prolonged. But even when flowers were continuously removed, cessation of stem growth occurred, and this was attributed to a degenerative change in the apex itself. Grafting experiments showed that transmissible juvenile or senescence factors were not involved. It is claimed that senescence may occur in peas even in the absence of the flowering condition, but the possible presence of small flower primordia would not have been detected by the methods used. Lockhart and Gottschall make the interesting suggestion that degeneration of the stem apex may be involved in those species in which one or more lateral buds ultimately assume dominance, thus affecting the form of the shoot.

CONCLUSIONS. To sum up, the shoot apical meristem may be regarded as a dynamic system which undergoes changes in size and metabolism, often of a far-reaching kind, during the ontogeny of a plant. This is true even if the changes associated with reproduction are excluded from consideration. Apart from changes associated with the heteroblastic development of leaves, however, comparatively little is known about ontogenetic changes in the meristem, particularly those of a physiological or biochemical nature. Even the contributions of the apex to heteroblastic development are by no means fully elucidated. It is clear, however, that interactions between the various regions of the shoot apex are important during development and merit further investigation.

FACTORS AFFECTING SHOOT FORM

VARIATION IN FORM. During the adult phase of development, vascular plants differ very widely in form. The majority of differences in form can be attributed to *(a)* the degree of branching, *(b)* the extent of internodal elongation, *(c)* the prostrate or ascending nature of the shoot, and *(d)* the longevity of the terminal bud, resulting in monopodial or sympodial growth. It may now be considered to what extent these differences in form may be related to the structure, growth and metabolism of the shoot apex. Different types of shoot form may occur within a single species or even within a single plant, and the study of such examples proves especially rewarding.

The shape and size of the apical meristem in adult plants differ very widely in different species (122, 191, 616, 629). A study of published illustrations and tables reveals that in different species there is no relationship between the size of a plant at maturity and the size of its apical meristem, although such a relationship may exist in individual plants of any one species during ontogeny. Some shoot apices are bilaterally symmetrical (616). The apices of many dorsiventrally symmetrical shoots, however, are themselves radially symmetrical.

DEGREE OF BRANCHING. In what is probably the commonest type of shoot organisation, leaves are formed on the apical meristem and buds occur in their axils at a slightly lower level. In some species, however, vegetative buds are only occasional. In some varieties of *Corchorus* buds are absent or occasional; the non-branching character is controlled by a single recessive gene (290).

In certain varieties of tomato, axillary bud primordia are completely absent during the vegetative phase, but a few appear at the time of flower formation (117). In *Victoria* and the oil palm vegetative buds are apparently entirely absent (144, 444).

In many species axillary bud primordia first become evident in the axil of about P_3; they are often formed from detached meristems, i.e., portions of meristematic tissue that become separated from the apical meristem during growth. In some species, however, bud primordia may be formed on the apical meristem itself, in the axil of the youngest leaf primordium (85, 86, 148, 349). Structural aspects of bud formation are well known (122, 172, 173), but the factors controlling their development are less well understood. Snow and Snow (497) have shown that in several angiosperms the inception of axillary buds is dependent on some influence from the axillant leaf primordium, since if this is excised or destroyed its axillary bud fails to develop (see Section V).

In some species, however, bud primordia are not axillary but occupy other more or less specific positions. In species of *Lycopodium* that bear bulbils, the bulbil primordia occur in leaf sites on the apical meristem, not in axillary positions (149, 244, 401, 556). Vegetative buds are also formed in leaf positions in species of *Nymphaea,* and flower buds occupy comparable positions in both *Nuphar* and *Nymphaea* (140, 144). The apical meristem of these species thus gives rise to a complex sequence of leaf and bud primordia. This occurs also in *Victoria* and *Euryale,* in which flower primordia are also formed at the apical meristem, but in extra-axillary positions just adaxial and anodic to an older leaf primordium. In these genera the apical meristem, which is not large, gives rise to leaf and flower bud primordia in regular sequence and in close spatial and temporal proximity (144). Experimental investigation of patterns of organogenesis of this kind may well throw light also on factors controlling the commoner patterns.

Characteristic sequences of this kind occur also at the apices of certain aquatic plants in which bud primordia occur only in the axils of certain leaves. In several members of the Hydrocharitaceae, buds are formed in the axil of every second leaf; in other members of the family, buds occur at longer intervals and with rather less regularity (148). Since the bud primordia are formed at the apical meristem, in *Hydrocharis* and other genera the sequence of organogenesis is leaf, leaf + bud, leaf, leaf + bud, and so on. Such regular sequences raise considerable problems of developmental physiology. In a preliminary investigation, Cutter (146) has shown that the phyllotactic pattern can be altered by treatment with GA, but the normal association of buds with every alternate leaf persists. Treatment with kinetin leads to changes in the regular pattern of bud formation, fewer buds being formed and these being not always associated with the expected leaf axils. Various physiological treatments can thus interfere with the normal metabolism of the apical meristem, but much work remains to be done to elucidate the mechanisms involved.

APICAL DOMINANCE. The form of the shoot depends not only on the number and position of lateral buds that are formed but also on the extent to which

they grow out, i.e., the degree of apical dominance. Conflicting views are still held concerning the mechanism by which this is brought about. From experiments in which plants of *Marsilea* were grown in the presence of auxins or coumarin, Allsopp (12, where other literature is reviewed) has concluded that direct inhibition by auxin plays little or no part in the correlative inhibition of lateral buds. A similar conclusion was reached with *Coleus* (271). Recent experiments with α-naphthylphthalamic acid, however, suggest that when transport of IAA down the stem is blocked by application of this substance, lateral buds below the point of application are released from the effects of apical dominance (382). That the general level of nutrition may be an important factor—or, indeed, the principal one—in apical dominance has been claimed by several workers (12, 24, 25, 212). The rather elusive quality "vigour" is also considered to be important (675). Some experiments suggest that apical dominance may depend on an interaction between auxin and a kinetin-like substance, and kinetin can be shown to interfere with auxin uptake and transport (674, 675). The orientation of the shoot with respect to gravity has an important effect; apical dominance is lost when the normally erect shoots of woody plants are artificially maintained in horizontal or arched positions (652, 653). There is a tendency for lateral buds to grow out on the upper side of arched and horizontal plants, and it is suggested that some mechanism which diverts nutrients to the highest upwardly directed meristem is involved (653). On balance, it seems likely that both hormonal and nutritional factors are involved in apical dominance.

Gregory and Veale (212) have made the interesting observation that the lateral buds on the stem of flax have different growth potentials, this having an effect on the form of the mature shoot. The growth potential of a particular bud is associated with the degree of development of the axillary bud and "the ease of production of vascular connexions with the main stele". It is suggested that auxin passing from the apical meristem and young leaves acts by impeding or preventing differentiation of the vascular connections of the axillary buds, thus depriving them of all nutrients so that they are unable to grow. Since, however, a considerable body of experimental evidence supports the view that prevascular tissues are formed only in response to effects from an actively growing apical meristem (598, 602, 613), an interpretation involving direct inhibition of bud growth by auxin, and the consequent inhibition of its prevascular tissue, would be equally in accord with the anatomical evidence. Inhibited buds would fail to grow and fail to induce the formation of vascular connections. While agreeing with Gregory and Veale (212) that in studies of apical dominance too little attention has been paid to structural changes, the writer does not consider that their own anatomical study, although interesting, provides evidence which can distinguish between a direct effect of auxin on differentiation of the bud vascular trace or an indirect effect mediated through the inhibition of bud growth.

GROWTH-REGULATING SUBSTANCES. It is clear that the degree of apical dominance is an important—perhaps the most important—regulator of shoot form. Skoog (486) claims that form is a question of internal growth factor

distribution, which is determined in part by a chemical regulation of polarity. That auxin is one of the growth factors involved seems evident. Shoot form, however, can be affected by application of various other growth-regulating substances. For example, the habit of a plant can be readily modified by experimental treatment with gibberellin (75, 538). These effects of gibberellin are due in large part to modification of growth in the subapical region (see Section VIIIa). The form of the shoot apex itself can be altered by direct application of various physiologically-active substances, these having differential effects on the growth of the apical and subapical regions (632, 644). An extreme example of this is the formation of ring-fasciated shoot apices as a result of treatment with TIBA (204, 282, 283, 618). The induction of ring-fasciated apices by TIBA can be prevented by simultaneous application of NAA or GA (281, 282). Both TIBA and 2,3,6-trichlorobenzoic acid (TCBA) inhibited growth of the apical meristem, which sometimes resulted in the formation of more or less leafless stems (203, 283, 618). Although types of shoot apices or mature form characteristic of other species can be simulated by application of various substances, it does not follow that these substances are normal constituents of the species concerned, since the changes in form may be brought about by affecting general variables, e.g., rates of growth.

PROSTRATE AND ERECT SHOOTS. The causes of the plagiotropic habit are still somewhat obscure. Palmer (407) showed that in some prostrate and stoloniferous tropical species, plagiotropic growth is induced by light, which leads to the development of a positive geotropic response that counteracts the normal negative one, resulting in horizontal growth of the shoot. Booth (67) showed that in decapitated plants of *Solanum andigena* the buds at the upper nodes develop as leafy shoots, but if IAA and GA are both applied to the decapitated shoot, the buds develop as horizontal stolons. In the presence of either IAA or GA alone, the buds develop as leafy lateral shoots. Quite complex correlative growth effects must be involved in the potato, since the stolons normally present at the lower nodes can be caused to develop as vertical leafy shoots if the plants are decapitated and the buds are removed from the upper nodes. In stoloniferous plants of *Trifolium fragiferum,* application of GA alone led to vertical growth of the stolons (55). In other plants short-day (SD) treatment may induce a plagiotropic habit, and this effect can be counteracted by GA treatment (476, 594).

In *Dryopteris* and *Matteuccia* the horizontal underground runners give rise to compact, erect shoots with a very different growth form when they are detached from the parent shoot (637). Thus the parent shoot apparently has important regulatory effects. In *Matteuccia,* Wardlaw (637) has shown that depth of planting is important; at considerable depths bud rudiments grow out as thin elongated rhizomes, whereas when they are exposed at the surface of the soil they give rise to compact leafy buds. No doubt light is an important factor here.

Rhizomes of *Agropyron* and *Sporobolus* could be induced to develop as aerial shoots with foliage leaves by placing the rhizome tip vertically upwards (406). In this instance gravity apparently acted on the shoot apex. Gravity

also apparently affects the growth of the apical meristem in cherry and plum trees, in which fewer leaves are formed in horizontally grown specimens (653). In these two species, as well as in apple and black currant, extension growth was also less in horizontal specimens; in this instance the effect was presumably on the subapical region.

LONG AND SHORT SHOOTS. In some plants the rate of leaf formation exceeds that of leaf expansion, leading to the formation of a compact apical bud with what has been termed a "reservoir" of unexpanded leaf primordia in various stages of development. In *Osmunda cinnamomea,* which is characterised by this type of development, a leaf primordium has its inception at the apex three or four years before it finally unfolds (523). In *Leptopteris* a leaf primordium spends eight years of slow growth and development in the apical bud before it finally expands (552). This sort of shoot form is not restricted to ferns; cabbage and Brussels sprout are good examples of shoots in which the rate of leaf formation exceeds the rate of unfolding of the outer leaves, which eventually ceases (399). In these particular species or varieties, differential growth rates must have been subjected to careful selection of genetical types, since in the wild cabbage hearting does not take place.

In attempting an interpretation of this particular shoot form, Crotty (132) has suggested a "feed-back" mechanism whereby the older leaves inhibit the development of younger primordia, probably by means of auxin, and at the same time an increase in nutrients tends to create a situation whereby protein synthesis can proceed more rapidly and/or for a longer period of time before maturation is accomplished. As many experiments have shown, excision of older, growing leaves does increase the rate of formation and development of younger primordia (23, 137, 147, 523, 552). Thus it may well be that accumulating unexpanded leaves do regulate the development of younger ones, leading to this particular compact habit of growth. Plants with such an accumulation of young leaves in the bud usually possess a high system of spiral phyllotaxis and short internodes, resembling short shoots.

Some genera, e.g., *Gingko* possess both long and short shoots on the same axis, and many plants turn from the long shoot habit of growth to the short when they enter the flowering phase. Flowers are short shoots, i.e., condensed axes with no, or poorly developed, internodes (664). In *Ginkgo,* Gunckel, Thimann and Wetmore (219) have shown that the terminal buds on plants less than six years old are always long shoots; the chance of terminal buds developing as long shoots becomes progressively less as the trees get older, this affecting the whole form of the tree. The more vigorous the growth of the plant, the greater the percentage of lateral shoots which develop as long shoots. Long shoots may continue development as short shoots, and vice versa (220). Decapitation of terminal shoots usually led to the development of one or two lateral short shoots as long shoots, but the capacity of a particular tree for this response depended on its general nutrition and health. Application of NAA to the decapitated bud prevented outgrowth of the short shoots (219).

In *Ginkgo* the buds of both long and short shoots open in spring; subse-

quently some shoots elongate and form more leaves, becoming long shoots. Anatomically, the apices of both long and short shoots are the same (220), and so is their initial auxin production (218). The apices of those shoots which develop into long shoots develop a characteristic rib meristem (220), and their auxin production rises rapidly (218). Long shoots prevent the development of the lateral buds into long shoots, inhibiting the formation of the rib meristem. Gunckel, Thimann and Wetmore (219) concluded that bud development comprises two stages: *a)* the bud opens and the leaves expand, and *b)* the axis elongates. In most inhibited lateral buds both stages are prevented, in short shoots the second, and in long shoots neither stage is prevented. The development of long shoots as short shoots, however, cannot be explained solely in terms of auxin relations (664).

In short shoots of *Parthenocissus, Cercidiphyllum* and *Pteridium* only a single leaf expands in a season (154, 375, 573, 658). The apices of long and short shoots of *Cercidiphyllum* are histologically alike (573). Excision of the apices of long shoots of *Pinus, Larix* and *Cercidiphyllum* permits the development of short shoots as long shoots, as in *Ginkgo,* suggesting an inhibitory effect of the terminal long shoot (158, 159, 379, 573, 646).

In bracken *(Pteridium aquilinum)* both long and short shoots are present, but the short shoots do not possess a group of leaves with condensed internodes, as in most other genera. Short shoots are distinguished from long shoots on the basis of the length of internode. A shoot may change from one type to another during growth (154, 402). Some controversy exists concerning the adult shoot system of *Pteridium.* Some authors consider that the main axis, or long shoot, is leafless and bears specialised lateral axes, the short shoots, which produce the leaves (205, 658); other workers assert that both long and short shoots bear leaves (154, 402). Short shoots of *Pteridium,* as of other species, can be converted into long shoots by destroying the apex of the main axis (402, 658).

Gottlieb (206, 207) has studied the control of long and short shoot growth in vitro (see Section V). These experiments seem to show that, whatever may be the final conclusion in the dispute on the leafless or leaf-bearing nature of the long shoots of *P. aquilinum,* the apices of long and short shoots of this species appear to have some physiological distinctions.

In any consideration of growth and form in the shoot, the factors controlling the development of long or short shoots in the same species—indeed, on the same axis—are clearly important. An appraisal of the work on various species, discussed above, suggests that the short shoot form may be another manifestation of ageing. In *Ginkgo* the percentage of buds which develop as short shoots increases with the age of the tree (219). In *Pteridium* only long shoots are present in the young sporophyte (154, 205); moreover, the short-shoot habit is induced by a high concentration of sucrose (206), which is reminiscent of other phenomena associated with ageing (see Section IV). In some ferns *(Osmunda, Dryopteris, Leptopteris)* and angiosperms (cabbage, *Nymphaea)* the compact shoot form resembling the short shoot develops gradually in the course of ontogeny. Except possibly in the controversial case of *Pteridium,* long and short shoot apices do not structurally differ, apart from

the presence or absence of a rib meristem. That auxin relationships are involved in the control of short shoots is clear from the work with *Ginkgo;* but it has also been suggested that auxin production in juvenile and adult shoots may differ (651; see Section IVb). As in heteroblastic development, the growth of the subapical region is clearly important. It may be noted also that GA, which in some species can promote juvenile features, can also convert rosette plants—essentially short shoots—into caulescent forms, or long shoots. The onset of senescence is considered to be correlated with the formation of flowers, and the floral apex itself exhibits the short shoot form. In an attempted interpretation of this aspect of shoot development, as in many others, more information about metabolic changes in the shoot apex is badly needed.

LAND AND WATER FORMS. One final aspect of shoot form that may be briefly discussed is the occurrence of land and water forms in some aquatic and amphibious plants. Such forms may not only show distinctive leaf shapes, but also differ in a number of structural features. Control of the development of these forms has been experimentally investigated in *Marsilea* (7, 9, 10, 11, 14, 15, 190). In *M. drummondii* sporelings in vitro, plants grown in media with 5% glucose resemble the normal land form, while those from media with lower concentrations of glucose resemble water forms; plants of intermediate form do not occur (7). "Water form" plants have elongated internodes and leaves with the divided lamina expanded in the plane of the petiole, while "land forms" have short thick internodes and leaves with the quadrifid lamina expanded at right angles to the petiole; the two forms also differ anatomically. In nature, three types of leaves—submerged, floating, aerial—occur in *Marsilea* (200); floating leaves are not formed, however, by the "water form" of *M. drummondii* in culture.

Allsopp (9) has shown that the shoot apices of the water form plants from 1% and 2% glucose differ in form from those of land plants from 4% and 5% glucose. In higher concentrations of glucose, the apices are more obtuse and expand more quickly proximally, their curvature being less marked than is that of apices from lower concentrations, which enlarge more gradually. It is considered that subsequent divisions in the subapical region account for the observed differences between the apices of the two forms.

In a highly concentrated mineral nutrient medium, the osmotic pressure of which was comparable with 3% glucose, about half of the cultures developed some of the characteristics of land forms (10). On medium solidified with agar, also, the land form of leaf was finally produced, whatever the concentration of glucose in the medium (7). Various other experiments, e.g., with mannitol, indicate, however, that the carbohydrate balance is more important than the internal osmotic properties (15). It is considered that the differences between land and water forms are not merely a result of carbohydrate deficiency (9), and that it is the water balance of the developing tissues which leads to the appearance of the features characteristic of land or water forms (10). The internal sugar concentration in the developing tissues is considered to be the key factor affected by these other variables (15).

Gaudet (190) has recently described sporelings of *Marsilea vestita* which

developed as water form plants when grown in 5% sucrose and maintained under constant fluorescent light. Plants kept in darkness or far-red light for a varying number of days and then transferred to red light sometimes developed as land forms, the proportion increasing with the length of time in far-red light or darkness. Etiolation is thought to lead to some change enabling plants to be converted from water to land forms.

It is of considerable interest that very vigorous sporelings of *Marsilea,* including those grown in the presence of indoleacetonitrile (11), are often of the water form, and stunted sporelings of the land form (10). Both anatomical (9) and experimental evidence suggest that the rate of growth of the subapical region may be an important distinction between land and water forms. In support of this hypothesis, the work of Allsopp (14) on the effects of GA may be cited. This substance, which in other species affects subapical growth, suppressed the development of land form features in plants in 4% glucose and also led to the transformation of land to water forms when added to a 4% glucose medium supporting the growth of land forms. White (673) has shown that sporelings of *Marsilea* spp., grown from the earliest stages in low concentrations of the protein synthesis inhibitor 2-thiouracil, developed as land plants, even in 2% glucose. After transference to a medium containing thiouracil, adult water form plants produced land form leaves, with cognate structural features. A general inhibition or stimulation of growth may thus prove to be of some importance in affecting this particular type of shoot form.

The extent to which the differences in leaf form in land and water plants are due to general effects on the whole plant is not yet clear. Allsopp (15) considers that, while segmentation of the leaf is correlated with the magnitude of the shoot apex, (see Section IVb), the shape and structure of individual segments of the lamina is probably determined later, in response to the sugar concentration of the medium. White (673) has shown that adult plants of *Marsilea* grown in media containing thiouracil form spatulate, bifid and trifid leaves which are essentially the result of lack of separation of the individual segments during ontogeny. Their true quadrifid nature is indicated by the venation patterns. The question of the degree of independence of effects on leaf form and on growth of the shoot apex as a whole appears to require careful investigation.

(d) Seasonal Changes

In temperate regions the shoots of most perennial plants show more or less marked seasonal changes. These usually comprise leaf fall in deciduous species, the onset of dormancy in the apical bud and sometimes also in lateral buds, and subsequent breaking of this dormancy and renewed growth of the shoot. A seasonal cycle of changes thus takes place in the shoot apex.

In many woody plants the onset of dormancy in the terminal and lateral buds is a response to short-day (SD) conditions, growth continuing if long-day (LD) conditions are maintained (396, 579). Both stem extension and the development of new leaves are arrested. As in other photoperiodic responses, the leaf is the receptor of the stimulus (396). Onset of dormancy is not directly related to cessation of extension growth, at least in black cur-

rant (387). Lateral buds enter dormancy progressively in acropetal sequence. At a certain stage they exhibit "partial dormancy", since the buds do not expand after decapitation of the main stem, although the bud apical meristem is still actively growing (387). This type of dormancy might be implicated in the formation of compact leafy shoots, as cabbage. North (400) has shown that non-heading rogues of cabbage have as many closely-spaced leaves as headed plants but differ in having unfolded outer leaves.

Bud-break of woody species in spring is also under photoperiodic control. Even leafless seedlings of beech will break dormancy under LD conditions; there is a direct effect of light on the meristematic tissue of the leaf primordia in the bud (647). Bud dormancy can be broken by treatment with GA, another example of its induction of responses to LD conditions (77). An interesting example of the effects of GA has been reported by Nitsch and Nitsch (396). Peach embryos, which normally require cold treatment, will give rise to seedlings which remain dwarf if dissected out of the seed and germinated at room temperature. The terminal buds of these seedlings are dormant, but if they are treated with GA, the seedlings elongate and become indistinguishable from those germinated from cold-treated seeds. Kinetin and other kinins can also break the dormancy of buds of woody or aquatic plants (294, 657). In coffee, dormancy of the flower buds can be broken by a period of water stress preceding irrigation of the plants. It is thought that an inhibitor responsible for the dormancy many be removed in this way (20).

Control of bud dormancy has often been attributed to specific, but unidentified, growth-regulating substances in the plant. Recently, Eagles and Wareing (166) have applied an extract of birch leaves to a leaf of actively growing birch seedlings and demonstrated induction of dormancy in the apical bud and arrest of stem extension. The bud was not in any way damaged by this treatment, since subsequent treatment with GA induced it to resume growth. In bioassays with coleoptiles it was shown that the apices of plants treated with the inhibitor contained higher levels of the inhibitory substance than apices of actively growing plants. It is suggested that bud dormancy may be regulated by the balance between such a growth inhibitor and endogenous gibberellins (166).

Some plants, in particular many aquatic species, are enabled to over-winter by means of the formation of dormant buds which are the only parts of the plant to survive. Formation of the resting buds or turions of *Hydrocharis* is inhibited by light, which promotes growth, and promoted by high temperatures, which suppress growth (584). In this species and others, including woody plants, light and temperature are antagonistic factors in the control of dormancy (584, 585, 586). In *Stratiotes* high temperatures can induce a deepening of quiescence in an already resting bud and extension of the dormant state (586). Czopek (152), working with *Spirodela,* has shown that higher concentrations of sucrose or exhaustion of one or more minerals in the nutrient medium induces the formation of turions, and has concluded that inhibition of vegetative growth is the factor responsible for the induction of turion formation. The quantitative relationship between growth promoters and inhibitors seems to be important during the formation of resting buds (585).

Seasonal changes of this kind have no permanent effect on shoot form, except in cases where the terminal shoot flowers or the terminal vegetative shoot apex actually aborts, leading to resumption of growth by one or more lateral buds. Garrison and Wetmore (189) have shown that in *Syringa vulgaris* cessation of growth is the first sign of shoot tip abortion. Delay or failure of abortion is associated with vigorous growth, as it is also in *Ulmus* (374) and *Maclura* (489). In this connection the observation of Schaffalitzky de Muckadell (472) that young plants of *Syringa* show continued growth of the same terminal shoot apex for at least three years, is of some interest. Subsequently, abortion of the shoot tip occurs and the shrubby growth habit becomes established. In *Syringa* diffusible auxin is present in the shoot tip prior to abortion, but none can be detected after cessation of growth (189). In blueberry, however, abortion of the shoot tip was apparently speeded by a high level of auxin (49). In vigorous shoots of *Syringa* the abortive tip can be stimulated to resume growth by destruction of the uppermost pair of axillary buds. Shoot tips grown in vitro, however, ultimately aborted and were not stimulated to continue growth (189). In abortive shoot tips the apex ceases meristematic activity and eventually becomes necrotic (374). Tips of short shoots may abort sooner than those of long shoots of the same species (375). It seems possible that shoot tip abortion may be another manifestation of ageing of the apical meristem.

Romberger (455) has pointed out that the ability to revert the developmental pattern back to scale formation after a series of foliage leaves is lacking in aborting terminal buds. In this connection it is interesting that isolated terminal buds or apical meristems of *Castanea sativa* grown in vitro gave rise only to leafy stems and never to scaly buds; these shoots ceased growth the following year, but the apical meristem would grow if sub-cultured (124).

(e) Conclusions

The habit of a plant is one of its most characteristic features. The thesis expounded in this Section is that the form of the shoot system is very largely controlled by events in the shoot apex, this comprising the apical meristem and the subapical region. As yet, too little is known about ontogenetic and seasonal changes occurring in the apex, particularly those of a metabolic nature. The work reviewed above indicates that this remains an important field for future endeavour.

The habit of a plant is the expression of its genetic constitution in morphological terms. Many experiments have been directed towards elucidating the mechanisms by which the genes control the final form, and the study of morphogenesis in mutants and other plants of known genetic constitution has contributed not a little towards the solution of this problem. A combination of this type of study with experiments involving modification of the environment or application of growth-regulating substances has also proved fruitful.

The form of the shoot depends in the first instance on the arrangement of parts, i.e., the disposition of the leaf and bud primordia which will later expand into leaves and lateral branches. The extent of their development, and the final form which they assume, depend in some measure, at least, on the nu-

tritional status of the shoot apex, the relative rates and amounts of growth of the apical and subapical regions, and the degree of apical dominance which the shoot apex imposes. These may be affected by various environmental factors, including light, day-length, temperature, nutrition and the gravitational field. They may also be modified during the ontogeny of the plant. Various aspects of ageing in the apical meristem are clearly important. In the life of a plant there is first a general increase in size, then a period of active growth and the formation of flowers, followed by senescence and death. There is evidence that the shoot apex is directly implicated in all these phases of development. Perennial plants may also undergo seasonal changes, the shoot apex passing through successive phases of dormancy and active growth.

Some of the factors controlling these events in the apex have been elucidated in some species, but much work remains to be done. The study of shoot morphogenesis is not only of academic but also of economic importance. For example, it has recently been shown that in grazed plants of Russian wild ryegrass substantially higher seed yields were obtained; this was attributable to the increased survival of shoot apices in grazed plants, which were not subject to the high degree of frost damage observed in ungrazed plants. The shoot apices of the latter were raised to above-ground level by extensive mesocotyl growth and were consequently susceptible to frost damage (324). To consider some other examples, the factors controlling the upright, unbranched or shrubby habit of woody plants in large part determine the degree of their usefulness as timber trees. It is also important to understand the effects of artificial training and of pruning on the bearing potential of fruit trees. Understanding of the factors controlling early flowering, bolting and heading, bulb and tuber formation, etc., may be important in agriculture and horticulture. The seat of control of all these various manifestations of growth and form lies in the shoot apex, and an understanding of them should be sought there.

V. GROWTH POTENTIALITIES AND CORRELATIVE EFFECTS IN THE APEX.

The aims of most of the direct experiments on the shoot apex have been to reveal potentialities of primordia or tissues for growth and development under particular conditions, and especially to investigate the effects of one part of the apex on another and the relationships between the apex and older regions of the axis. Experiments of this kind were begun before the turn of the century but received renewed impetus 20 to 30 years ago. Apart from applications of growth-regulating substances, which will be considered later, direct experiments on the shoot apex are principally of two kinds: *a)* those involving microsurgical techniques, i.e., the placing of punctures or incisions in specific sites on the apex with a view to investigating changes in growth and interactions between parts; *b)* in vitro culture of excised apices or parts of apices under sterile conditions, with a view to elucidating their requirements for nutrients and other growth factors, and also the effect of various substances on their development. In the latter type of study some relatively crude or, in some experiments, more refined microsurgery is necessarily involved in the initial excisions of the explant. In surgical experiments, organs or tissues are only partially isolated from

their normal environment, and they obtain nutrients etc. through the plug or panel of tissue connecting them with the parent plant. Earlier experiments of these types are discussed in several books and reviews (122, 483, 612, 613, 639, 671). This resumé will deal principally with recent work, but reference will be made to earlier work where this is the basis for subsequent studies. Experiments relating to phyllotaxis and the development of leaf primordia are considered in Sections VI and VII.

(a) Autonomy of the Apical Meristem

Using the fern *Dryopteris dilatata,* Wardlaw showed that if the central conical apical meristem is isolated from the adjacent leaf primordia and lateral tissues by three or four deep vertical incisions, it is capable of continued normal growth and development (599, 602, 604, 605). This technique was later extended to eusporangiate and other leptosporangiate ferns (608, 611), and to several dicotyledons (37, 41, 608, 611, 615), with similar results. In these experiments the incisions severed the vascular and prevascular tissues of the shoot, leaving the apical meristem seated on a plug of parenchymatous tissue. It was concluded that the central region of the shoot apex is capable of autonomous development, and, except for the supply of nutrients, is not dependent on the subjacent, older tissues of the shoot. Ball (37, 41), following ideas expressed by earlier observers (320), described the apex as a self-determining region. In both ferns and angiosperms, isolated apices gave rise to leaf primordia, and new prevascular tissue was formed below the apex; in ferns this did not join up with the vascular tissue of the shoot (608), but in *Lupinus* (37, 41) and *Primula* (608) it differentiated below the incisions and joined the vascular strands of the axis. These observations support Wardlaw's hypothesis (598) that prevascular tissue is formed in response to a basipetal stimulus from an actively growing apical meristem.

In these experiments the isolated lateral regions or flanks of the apex gave rise to actively growing buds (41, 599, 602, 604). Wardlaw (626) later showed that when the apical meristem of *Dryopteris* is isolated by shallow incisions which do not sever the prevascular tissues, these buds do not develop. The conclusion (626, 643) that the prevascular tissue is the main pathway through which the regulative effects of the apical meristem are mediated is supported by Ball's (41) observations that as soon as the isolated apex of *Lupinus* had re-established vascular connections with the subjacent stem, growth of the lateral shoots was inhibited.

Completely excised stem tips of *Lupinus* and *Tropaeolum* (33, 36), asparagus (352, 353) and dodder (354) were capable of continued growth in liquid or agar mineral nutrient media with sucrose. Stem tips of asparagus were maintained for 22 months through 35 transfers, and appeared capable of potentially unlimited growth (353). Growth of asparagus stem tips was inhibited by 2,4-D (277). Ball (36) obtained entire plants with roots and leaves from excised shoot apices, including the three youngest leaf primordia, of *Tropaeolum* and *Lupinus.* Similar results were obtained with shoot apices only 0.25 mm. long of several pteridophytes and two other flowering plants (661, 667). Later Wetmore (663) showed that pieces of the apical meristem

of *Adiantum* excised above the region of leaf formation could be successfully grown in vitro on a relatively simple medium containing only mineral salts, minor elements and sucrose. Similarly excised apical meristems of other pteridophytes would also grow, but apices less than 0.5 mm. long of several flowering plants failed to grow. Larger apices of *Syringa* would undergo some growth, however, if grafted into callus of the same species, using the technique of Camus (100). This technique was later further exploited in studies of xylem differentiation (664, 670).

Wetmore (663) considered that the lack of success with small explants of angiosperm apices might be due to inadequate supplies of nitrogenous substances in the medium. Accordingly, a chromatographic study of nitrogenous substances normally present in the shoot apices of *Lupinus albus* was carried out (534), and mixtures of amino acids and amides were added to the synthetic nutrient medium in the proportions suggested by this study. On such a medium some growth occurred, but this was still not extensive. The degree of success attained in the culture of shoot apices of angiosperms was thought to be correlated with the size of the explant and especially with the presence or absence of leaf primordia (668).

In subsequent experiments with *Lupinus albus,* Ball (45) showed that some growth of excised apical meristems, some 300μ x 300μ x 100μ, without leaf primordia, could be obtained on a medium containing gamma-aminobutyric acid. Explants failed to grow when supplied with other amino acids known to be present in the apex, but in the presence of gamma-aminobutyric acid they gave rise to shoots about one cm. long with three to five leaf primordia. The best results, however, were obtained with a medium containing gibberellic acid and coconut milk, on which shoots five to ten cm. long with seven to nine leaves developed. Apical meristems of *Tropaeolum* and *Lycopersicum* failed to grow on any medium. Ball concluded that, if given an appropriate nutrient medium, the isolated apical meristem could give rise to leaf primordia in vitro. He also suggested that maintenance of the apical meristem in the meristematic state may be due to substances from the subjacent tissues, since cultured apices eventually ceased growth and lost the histological features of meristematic tissue.

Excised shoot apices of monocotyledons (352, 353, 381, 668) and gymnosperms (18) have also been successfully grown in vitro. Some of the excised parts were fairly large.

Both surgical and cultural techniques have thus demonstrated the capacity of portions of the terminal region of the shoot apex, of variable size according to the species, to undergo normal growth and development in the absence of the more mature subjacent tissues. Given a supply of nutrients, the vegetative apex is thus self-determining and autonomous.

(b) Developmental Potentialities of Apical Tissues

In many species the apical meristem gives rise both to leaf and to bud primordia, thus showing that its tissues are capable of development along at least two different paths. The factors controlling this development will be further discussed elsewhere, but some of the potentialities for growth and

development not normally expressed by the apical tissues, and some interactions between the various regions of the apex, will be considered here.

CORRELATIVE EFFECTS. Many surgical experiments have been carried out with a view to investigating the effect of the apical meristem upon the development of the lateral organs to which it gives rise. The simplest experiment consists of the destruction of the central region of the apical meristem by a more or less severe puncture. In the fern *Dryopteris dilatata* the normal uninjured apex gives rise to leaves but never to buds. After the apical cell was destroyed by a puncture, which usually resulted also in the necrosis of adjacent cells, leaf primordia continued to be formed on the meristem but one or more buds usually also developed (604, 605, 609). Subsequent work indicated that the sequence of organogenesis was affected by the extent of the damage resulting from the puncture; when the damage was extensive there was a tendency for buds to be formed immediately, subsequent primordia developing as leaves, but when the damage was more or less confined to the apical cell itself, bud formation tended to be delayed. The majority of buds were induced in a zone at the periphery of the apical meristem (642). In angiosperms also, leaf formation continues after destruction of the tip of the apical meristem (142, 346, 499, 500, 550). Such leaf primordia are sometimes abnormally orientated (346, 642). In some species, at least, a central puncture seems to induce regeneration of the flanks of the apex only infrequently (142, 419), although in *Impatiens* several new meristems were formed after this treatment (346). Although these experiments severally indicate that leaf formation can continue in the absence of the tip of the apical meristem, at least for a time, this region does exercise controlling effects on leaf formation and growth that affect phyllotaxis (499, 500).

The primordia of axillary buds are apparently not directly dependent for their formation on factors from the tip of the apical meristem, since they can be formed in tissue isolated from it (497). In fact, the outgrowth of buds, if not their inception, is usually inhibited by an actively growing apical meristem. In *Nuphar lutea,* a species in which axillary buds are only occasional, they can indeed be induced to form in leaf axils where they would not normally occur, by isolation from the apical meristem (142). Snow and Snow (497) showed that in several species of herbaceous angiosperms some factor from the subtending leaf primordium was necessary for the formation of the axillary bud, since it failed to arise if the leaf primordium was excised or destroyed at an early stage of its development. In *Cucurbita pepo,* however, this relationship did not obtain, since buds were formed on the axis when their subtending leaf was isolated at the stage of P_1 or I_1 (501). In *Hydrocharis,* in which buds are formed only in the axil of every second leaf, Cutter (146) has also demonstrated an apparent correlation between inception of a bud primordium and that of its subtending leaf primordium, since the close spatial association between them persisted even when the leaf arrangement was experimentally altered by treatment with gibberellic acid. In the woody genus *Gleditschia,* in the leaf axils of which a basipetal series of buds is normally present, Neville (390, 391) showed that destruction of leaf primordia less than one

mm. in height was followed by more or less complete abortion of these axillary buds. In most instances the axillary meristems are described as regressing to parenchyma (391). This effect on already formed bud meristems is of considerable interest, i.e., even the enlarging bud primordia in some species are apparently not self-sufficient.

The stimulatory action of foliar primordia on the growth of the buds in their axils does not seem to be restricted to the vegetative phase of development. In experiments on the inflorescence apex of *Primula,* Cusick (134) observed that no flower buds developed after excision of P_2 and younger bracts. Excision of the bracts of P_4 and older primordia did not affect the development of the subtended flower primordia.

From these experiments it appears that, at least in some species, the formation and development of an axillary bud are dependent on some activity of the subtending leaf primordium, and that this may be true in both vegetative and floral apices. Clearly adventitious buds are not dependent for their formation on effects from other organs. Neville's (391) suggestion that the degree of development of the tissue in a leaf axil depends on the resultant of apical inhibition and foliar stimulation is in accord with the views of Audus (29) concerning correlative effects in the apex. A relationship between the bud and its subtending leaf is apparently maintained throughout growth, although its nature may vary; thus bud outgrowth, as distinct from inception, is often inhibited by young leaves. In *Ipomoea,* growth of an axillary bud was inhibited by a young, growing axillant leaf but promoted by a fully expanded leaf (293).

In a recent study of *Ceratopteris,* Gottlieb (208) has investigated the correlative effects of the leaf on the meristematic groups of cells at the basal margin of each of its lobes. In the mature, but not the juvenile, leaf these give rise to new plantlets. Excised meristems cultured on a simple nutrient medium either died or gave rise to apogamous outgrowths. In the presence of appropriate concentrations of adenine or IAA, however, the excised meristems quickly developed into normal plants. On the basal medium without these additives, the excised meristems would grow if they were in contact with one vein of the leaf or if one-quarter or more of a leaf were placed adjacent to them. Evidently important correlative effects within the intact leaf are involved. In the presence of kinetin, the meristems gave rise to a granular callus which regenerated whole plants when subcultured on a medium without kinetin.

APICAL GRAFTING. Attempts have been made to apply the technique of grafting meristematic tissues, so successful in studies of animal embryogenesis, to problems of morphogenesis in the shoot apex, including correlative effects. Ball (35, 38, 39) tried to transplant the central region of the apical meristem of *Lupinus albus,* but the tissues always died. Occasionally, pieces of tissue isolated by incisions but left in situ grafted back to the tissues of the apex (39). In later experiments shoot apices were split centrally to a depth of five to ten mm. and some were then bound together with cotton twine (43). Some grafting of more mature tissues eventually occurred, beginning at the base of the cut and progressing acropetally, but the apical tissues did not graft.

Ball considered that the presence of intercellular spaces was important. Similar grafting of the subjacent pith region of bisected shoot apices of *Euphorbia* was also observed (512).

Gulline and Walker (216) later obtained successful grafts of apical tissue in *Pisum*. Tips of axillary buds were excised about 200μ below the summit and grafted back into position. They were sometimes rotated through 180°. Precautions against desiccation were essential.

This technique seems to afford a useful means of investigating the interactions between various regions of the apical meristem, or, for example, between vegetative and photo-induced shoots of the same species. Yet thus far it has been little used. In flax, buds have been induced on hypocotyls (215). However, it remains to be seen whether more extensive applications of this technique to apical tissues will yield useful information. For example, unless very early effects are involved, the situation is likely to become more complex by the development of wound tissue.

REGENERATION OF THE APICAL MERISTEM. In 1929, Pilkington (419) showed that when shoot apices of *Lupinus* and *Vicia* were bisected by a vertical incision, two new apices were usually regenerated from the flanks of the original apical meristem. This result has been confirmed in many subsequent studies (98, 239, 345, 412, 495, 512). Ball (35, 38, 39) obtained similar regeneration from the flanks of the apex of *Lupinus* after excision of a small central region. The cells on the flanks of the apical meristem thus have potentialities for growth and development that are not normally manifested; the central terminal region of the apex apparently normally prevents their organised growth as independent meristems. From a study of the vascular systems of the regenerated shoots it was concluded that the shoot apex controls the differentiation of subjacent tissues of the shoot (40). Later, Ball (37, 42) showed that regeneration of small apical meristems could be obtained from apices of *Lupinus* divided into four or six parts. He concluded that in *Lupinus,* one-sixth of the apical meristem was the smallest volume of cells that would regenerate (42). In *Phaseolus* the minimum number of cells that would develop was equivalent to approximately one-quarter of the area of the apical meristem (412), but in *Impatiens* one-sixth of the apex could regenerate (345).

Regeneration of one-sixth of the potato apex was also obtained, but when the rest of the meristem was excised, pieces of meristem not exceeding one-twentieth of the whole in area could regenerate (548). From these skilful experiments Sussex (548) concluded that competition for nutrients by the cells of the apical meristem is one of the regulatory mechanisms by which its form is preserved. Refuting the criticism (509) that inhibition of regeneration might be due to hormonal effects and not to competition for nutrients, Sussex (549) described further experiments in which the incisions were deeper and either the isolated panel or the main apical meristem was undercut. More frequent regeneration was obtained with the deeper cuts, and whichever panel was undercut grew less in a certain period of time than the other, so that in some experiments growth of the main apex was actually inhibited by the small regenerating panel. These results seem to provide convincing evidence

of the importance of nutritional competition as a factor in the normal organisation of the apical meristem. In this connection the recent trend towards recognition of nutrition as an important factor controlling apical dominance on a more macroscopic scale is of interest (12, 212, 297; see Section IV).

These experiments on the regenerative capacity of the apical meristem indicate that under certain circumstances metabolic processes take place in the cells of the flanks of the apical meristem, leading to their organisation and outgrowth as shoot apices. The results suggest that the terminal cells of the apical meristem normally prevent this. It is difficult to separate hormonal factors from nutritional competition; the terminal region of the apex probably acts as a sort of sink of utilisation by virtue of its hormonal properties. The incised central regions of the apical meristem are apparently ultimately capable of giving rise to leaf primordia, since they constitute the flanks of the regenerated apices.

The capacity for development of different regions of the shoot apex has also been investigated in vitro, using *Helianthus annuus* (664). In this species the central region of the apical meristem, overlying the pith meristem, comprises large, vacuolate cells which stain less densely than those on the flanks of the apex. When this central region was cultured, it failed to grow; the more densely staining flanks of the apex, when excised from the rest, did grow and give rise to a leaf if a primordium was present on the explant. Such explants also often rooted. As in other species, excised apices comprising the whole of the apical meristem and leaf primordia developed into whole plants in culture (664).

ALTERNATIVE DEVELOPMENTAL POTENTIALITIES OF THE SHOOT APEX. Marsden and Wetmore (360) made the interesting discovery that excised apices of the aerial branches of *Psilotum nudum* often changed after a period in culture to an axis resembling an underground rhizome in both morphology and anatomy. They concluded that the evidence was insufficient to indicate whether or not the change in the growth pattern of the apex was due to nutritional causes. The study of the factors controlling which of several alternative developmental pathways an organ or tissue will follow is a very important aspect of morphogenesis (see also Sections VII, XI, XII)); this particular example seems worth further investigation.

Gottlieb (206, 207) has carried out some interesting experimental work of a rather similar kind on the control of shoot development in *Pteridium*. In culture the leaf-bearing phase of the rhizomatous sporeling may be maintained indefinitely on media with less than 2% sucrose; above this level of sucrose the short-shoot habit becomes established, but reversion to the simple rhizome state can be induced at any time by reducing the carbohydrate (206). On medium with 8% sucrose and 2 mgm./1. kinetin, both long and short shoot apices proliferated, but the former gave rise to a smooth, globe type, the latter to a clustered, grape type, of proliferation. When cultured in the absence of kinetin the globe types did not recover, but the grape types, from the short shoot apices, gave rise to new frond-bearing shoot apices from each of the "grapes" of the cluster (207).

VI. PHYLLOTAXIS

The orderly arrangement of appendages, usually leaves, on the axis is one of the best examples of organised growth in plants, but the factors controlling these arrangements are still not well understood. In considering a dynamic pattern, for instance, the regular formation of leaves on a stem, it is necessary to achieve two things: *a)* an accurate description of the pattern, *b)* an explanation of how it comes to be formed. The experimental approach to phyllotaxis requires the first but is chiefly concerned with the second of these aims.

At the present time there are three principal theories of phyllotaxis which offer some explanation in terms of growth processes in the apical meristem or leaf primordia. These are: *a)* the theory of the first available space, which postulates that a leaf primordium arises in the first space which attains both some minimum width and some minimum distance below the tip of the shoot apex (493, 494, 495, 498); *b)* the field theory, which postulates that existing leaf primordia and the apical meristem give rise to a physiological field or fields, new primordia being formed at specific points with reference to these fields (448, 473, 606, 607); *c)* the theory of multiple foliar helices, which postulates that special mitotic properties are transmitted acropetally along a variable number of foliar helices which terminate in leaf-generating centres in the shoot apex, the sequence of activity of these centres being controlled by an organiser (422, 423, 424). The first and second of these two theories have hitherto been mutually opposed; the early experimental evidence is reviewed by Wardlaw (613, 639), Snow (508) and Richards (448, 449). Supporters of the third theory have sometimes invoked the theory of the first available space as a subsidiary explanation for particular observations (98, 345, 346).

Multiple foliar helices

The experimental evidence for and against Plantefol's (422, 423, 424) theory has been reviewed comparatively recently (143, 345, 346, 427, 508, 639); earlier literature is cited in these papers. This theory is linked with one of apical histogenesis which attributes little or no importance during vegetative growth to the cells at the tip of the shoot apex, the "méristème d' attente" or "zone axiale", the principal apical activity occurring in a lateral peripheral meristem, the "anneau initial". Several attempts have been made to destroy all or part of this initiating ring. When punctures were made in an angle of the apices of *Impatiens roylei,* which had whorls of three leaves, 20% of the specimens gave rise to stems with only two leaves in a whorl, and it was concluded that a leaf-generating centre had been destroyed (337). In the majority of such stems, regularisation back to whorls of three leaves ensued (338). The more the volume of the apex was reduced by the punctures, the more often a foliar helix was suppressed (340, 345). Regularisation took place more often in well nourished stems, and the size of the apex is consequently considered to be important (340, 344, 345, 346). Presumably the effect of low light intensity in retarding the ontogenetic change from two to three generating centres (342, 343), and the effect of a high intensity in increasing the number (112), may also be mediated through an effect on apical size.

After puncturing one I_1 site in apices of *Biota orientalis* with whorls of

four leaves, Camefort (98) usually obtained whorls with three leaves and concluded that a leaf-generating centre had been destroyed. His illustrations indicate, however, that the three leaves were symmetrically arranged, whereas a gap might have been expected in place of the missing helix. In decussate apices of *Cupressus sempervirens,* the punctured leaf site failed to develop but a decussate arrangement ensued (98); it thus seems unlikely that in this instance a leaf-generating centre could have been destroyed. Neither in these experiments nor in those on *Impatiens* (345) was an apex with only one foliar helix obtained, although this might have been expected. Even when the whole of the anneau initial was destroyed in *Impatiens,* leaving only the zone axiale, two foliar helices remained (341, 345). This experiment incidentally demonstrates the capacity of the zone axiale, considered to be inert during vegetative growth, to give rise to a whole leafy shoot.

In other experiments the zone axiale was punctured more or less severely, leaving the anneau initial intact. Numerous leaves, attributed to an increase in the number of foliar helices, were formed, and this was often followed by flattening and fasciation of the stem (339, 345, 346). Several new apices were formed; this "fragmentation" of the apex is interpreted as a consequence of dilation of the anneau initial by the increased number of generating centres (345, 589), but it may be questioned whether these apices were not in fact present before formation of the leaves. Despite the very considerable effects on growth and development of a terminal puncture, Loiseau (345, 346) concluded that suppression of the apical cells did not modify apical activity, the disorders described being only an indirect and tardy consequence of the treatment.

The difficulty of deciding which of the sets of contact parastichies constitutes the foliar helices has been the subject of some discussion (143, 425, 427, 505, 506). Vescovi (588) has recently stated that in *Sarothamnus* nothing would allow a choice between three possibilities if some branches did not show slight signs of disorganisation in the functioning of the apex. Nougarède and Loiseau (401) also stated that the direction of the foliar helices could not be distinguished in whorled specimens of *Lycopodium.* In 1959 Cutter (143) pointed out that, since several distinct types of phyllotaxis are considered to have the same number of foliar helices, the responsibility for distinguishing between these systems must fall upon the organiser, about which singularly little is known (633). Plantefol (427) has recently protested that the organiser is a physiological concept, which his critics have misunderstood. The organiser is clearly a key factor in the theory: more information about its functioning is essential to an understanding of phyllotaxis in terms of this theory, and there seems no reason why its physiological nature should be a barrier to obtaining this. A clear demonstration of the interdependence of the members of a foliar helix is also needed; otherwise the results of most experiments can be interpreted as well or better in terms of growth centres of individual leaves (627).

Supporters of the theory of foliar helices have recently suggested interpretations, in terms of this theory, of earlier surgical experiments carried out by other workers. For example, Plantefol (426, 427) has claimed that Snow and Snow (495) failed to realise the principal importance of their experiment in

which two apices with spiral phyllotaxis were obtained from bisected apices of a decussate species. According to Plantefol, this experiment resulted in the isolation, on two independent axes, of each of the two foliar helices which are normally associated in decussate phyllotaxis. On this interpretation, only one foliar helix, which must be equivalent to the genetic spiral, formerly described as "sans réalité" (423), is thus ascribed to a type of spiral phyllotaxis usually described as resulting from the presence of two foliar helices (98, 423). Moreover, some bisections of decussate apices (512) have led to the formation of either two spiral apices or two new apices with decussate phyllotaxis, which would surely present a difficulty in terms of this interpretation.

Spatial considerations

Following their pioneer surgical experiments on phyllotaxis (493-495), M. and R. Snow have recently carried out experiments on apices with less common types of phyllotaxis, and have also extended their observations on the minimum arc needed for the determination of a growth centre. Working with the bijugate species *Dipsacus laciniatus,* R. Snow (507) tested the hypothesis (448) that bijugy might be derived from a decussate arrangement by a fall in plastochrone ratio, the position of a pair of incipient primordia then being influenced by the two preceding pairs instead of only one pair. Snow removed the leaves and leaf primordia as far as the P_1's, or only to the P_2's or P_3's, and found that the divergence angles between successive pairs of leaf primordia continued to increase for five or six plastochrones, some angles being greater than 90°. One apex became spiral, and one formed whorls of three leaves. Snow (507) concluded that the inhibition hypothesis failed to account for the increased angles except those immediately following the operation, and suggested that both his observations and the normal bijugy could be explained in terms of the combined working of two factors: the contacts of the leaf primordia with older primordia or their united rims, and the pressure resulting from the contact of the vertical parts of the paired P_2's with the shoot apex.

Contemporaneously, M. Snow (491) carried out experiments on the spirodistichous species *Rhoeo discolor.* When the centre of P_1 was excised, I_1 was displaced towards it, and Snow concluded that the position of a new leaf primordium depended on a balance between the inhibiting influences of the centres of existing leaf primordia. This conclusion thus supported the theories of Richards (448) and Wardlaw (606, 607). Later, however, M. Snow (492) showed that the displacements following the operation could be accounted for by the shift of the growing-point which ensued. Normal spirodistichy could be explained in terms of a space-filling theory, without postulating physiological repulsions, by supposing that each new leaf primordium is caused to deviate laterally by contact with the flank of the next older primordium. In order to explain the initial distichy of axillary buds, the shape of the bud apical meristem was invoked (492). On any such theory the time relationships between the lateral development of the flanks of the leaf primordium, which are determined later than its centre (491), and the fixation of the position of the next younger primordium, are clearly critical.

Snow and Snow (499, 500) showed that leaf primordia of *Lupinus albus*

could be determined on arcs smaller than normal when the summit of the apex was punctured. By contrast, the removal of young leaves and leaf primordia resulted in the new primordia covering larger arcs of the circumference than usual (502). At least two factors are thus involved in determining the leaf arc: an effect of the summit of the shoot apex that increases the primary area of the apical meristem required for the formation of a leaf primordium, and an effect of developing leaves which decreases the secondary lateral extension of the leaf bases. Snow and Snow (500) have pointed out that these observations make it possible to interpret physiologically the view that a minimum free area is necessary for leaf formation, a theory "which may previously have seemed rather geometric and bare."

Recently Snow and Snow (503) have proposed a theory, based on the nature of the primary and secondary leaf arcs discussed above, which would account for the exact regulation of phyllotaxis systems, especially their return to regularity after a disturbance. The transitions from Fibonacci spiral systems to spiral systems of the accessory series 1, 3, 4, 7, 11 . . ., explained by Snow and Snow (503) on space-filling principles, also seem explicable in terms of a field theory, and in fact somewhat comparable transitions can also occur in ferns (150), in which neither primary nor secondary leaf arcs are in contact.

PHYLLOTAXIS AND APICAL GROWTH

There is a relationship between the size of the apex and the phyllotaxis of the shoot (113, 137, 340, 341, 364); this is true even in the establishment of the first whorl of leaves (539) and is often concerned in phyllotactic changes that occur during ontogeny. The vigour and nutritional status of the shoot also affect phyllotaxis (111, 112, 344). Changes from one type of phyllotaxis to another on a single axis may occur naturally, either as a regular ontogenetic change or fortuitously (98, 198, 348, 364, 577), or they may be experimentally induced (150, 495, 504, 507, 512). Following the early experiments of Snow and Snow (495) on decussate species, Soma (512) bisected the decussate shoot apex of *Euphorbia lathyris* in various planes. Some of the apices which grew from the bisected halves had spiral phyllotaxis or whorls of three leaves, or less regular systems, but there was a tendency to revert to decussation. These various observations all show that phyllotaxis is not directly controlled by genetic factors but can be more or less readily altered by various environmental factors.

Richards (448-450) analysed the radial spacing of leaf primordia in relation to various processes of growth occurring in the apex, and devised a measure, the phyllotaxis index, by which different systems of phyllotaxis can be directly compared. Changes in this value reflect changes in growth. In *Dryopteris dilatata,* which normally shows Fibonacci spiral systems of phyllotaxis, a proportion of natural populations is bijugate (592). In an attempt to repeat Snow's (507) experiments with *Dipsacus* on bijugate specimens of this fern, Cutter and Voeller (150) obtained some initial increase in divergence angle, which was comparable with Snow's results, but also subsequent more profound changes in phyllotaxis. However, bijugate apices in which the younger leaf

primordia were not destroyed, but which were simply maintained under observation in the laboratory (a procedure necessarily involving the excision of all the outer leaves and leaf primordia), also underwent comparable changes in phyllotaxis. Most became spiral, of the Fibonacci series 1, 2, 3, 5, 8 . . ., but a few gave rise to a trimerous pseudo-whorled system or became spiral, of the accessory series 1, 3, 4, 7, 11 . . . Naturally occurring spiral apices of *Dryopteris* maintained under comparable conditions merely reverted to the lower spiral systems characteristic of sporelings (137). Cutter and Voeller (150) concluded that Snow's (507) explanation of bijugy in *Dipsacus* could not apply to *Dryopteris,* since the leaf primordia were not in contact, and suggested that, for *Dryopteris* at least, an explanation was more likely to be found in a field theory of phyllotaxis. They attributed the observed changes in phyllotaxis to changes in apical growth.

More recently, apical size, phyllotaxis index and the rate of leaf inception in spiral and bijugate specimens of *Dryopteris* were compared and found to be similar (147). In the apices of the two groups of specimens these parameters also underwent similar changes over a period of time. In the spiral apices originally observed (137), such changes led to an increase in the differences in size and stage of development between successive primordia, and it is concluded that a similar effect on existing small differences between the two primordia of a pair in bijugate apices might account for the change to a spiral system (147). The observed changes in phyllotaxis would thus be indirectly attributable to progressive changes in the rates of the various components of growth in the apex occurring over a period of time.

Rees (444) has recently shown that the phyllotaxis index of oil palms from different habitats and ranging in age from three to 19 years did not greatly vary.

Treatment of *Dipsacus* plants with GA led to increased internodal elongation and a slight increase in the mean angle of divergence, suggesting that the vertical component of growth should not be neglected in studies of phyllotaxis (147). In buds of *Hydrocharis* which developed in nutrient solution containing GA, phyllotactic changes were also induced (146), probably as a consequence of extensive internodal elongation. In this connection the observation of Gifford and Tepper (196) that a slight increase in the length of internodes in the apex of *Chenopodium* follows photoperiodic induction is of interest. These authors considered that a rapid elongation of developing internodes probably often accompanies inflorescence development. In *Kalanchoë* and *Bryophyllum,* also, flower formation is always associated with increased length of newly formed internodes (524, 684). As is well known, changes in phyllotaxis often occur at the time of flowering. Schwabe (476) has recently reported a change from decussate to spiral phyllotaxis in plants of *Epilobium adenocaulon* that were maintained in daylength conditions which just failed to induce flowering. These observations are compatible with the view that the vertical component of apical growth may be of more importance in phyllotaxis than has hitherto appeared.

Effects of growth-regulating substances

The known effects of GA on phyllotaxis have been mentioned above. Treatment of plants with other growth-regulating compounds often leads to anoma-

lous phyllotaxis. For example, treatment with 2,4-D caused several aberrations, including various types of fasciation (30, 260, 590). Treatment of the shoot apex with IAA often led to the formation of joined leaves, with various changes in phyllotaxis, including a change from decussate to spiral (34, 496, 590). In plants of *Cannabis* treated with 2-thiouracil there was a transition from decussate to irregular alternate phyllotaxis which persisted for three or four nodes (253). The effect of phenylboric acid (PhB) is particularly interesting because of its capacity to suppress leaf primordia completely without damaging the shoot apex. Induced effects included a change from decussate phyllotaxis to distichy (231).

Treatments with these substances, with the possible exception of GA and PhB, which have specific effects on growth, seem unlikely to contribute substantially to an understanding of phyllotaxis, although they may cause considerable, though transient, disturbances.

Conclusions

Of the three current theories of phyllotaxis, in the writer's view the theory of multiple foliar helices is not supported by any conclusive experimental evidence. The main supporting evidence is the claimed suppression of foliar helices, but this happened rarely (in about 20% of specimens, in most of which regularisation ensued). Authors supporting this theory have had to invoke the theory of the first available space as a subsidiary explanation for certain observations (98, 345, 346).

The other two theories are evidently becoming less opposed than formerly. As many experiments have shown, a minimum free space is certainly an essential pre-requisite for the determination of a leaf site, but it is a sustaining condition, not an active factor; moreover, its creation probably depends on physiological events in the apex, as indeed the recent experiments of Snow and Snow (500, 502) suggest. The relationship between apical growth and phyllotaxis, so cogently discussed by Richards (448, 449), seem worthy of further experimental investigation.

Many surgical experiments on the shoot apex lead to immediate, transitory effects on phyllotaxis, which are often predictable. But it should be noted that several experiments have led, sometimes unexpectedly, to effects on phyllotaxis that were more than transient (147, 150, 500, 502, 507). Moreover, effects on phyllotaxis have been caused by operations on primordia relatively remote from the primordial sites affected (150, 491, 502, 507), or by treatment with GA, thiouracil or particular light régimes without operating on any primordia (146, 147, 253, 476). In particular, removal of the outer, growing leaves—a necessary preliminary to most surgical experiments on phyllotaxis—can itself have considerable effects (150, 500, 502). Factors affecting growth can thus affect phyllotaxis, sometimes profoundly, but their action may, of course, be mediated through effects on the space occupied by leaf arcs, pressure of leaf primordia on the apex, etc., although it is difficult to apply these arguments to the ferns; a field theory still seems to provide the most satisfying explanation of phyllotaxis in their group.

VII. INCEPTION, GROWTH AND DEVELOPMENT OF LEAF PRIMORDIA

Leaf primordia are formed by the apical meristem, and their inception, arrangement and early development are thus of the greatest relevance to an account of work on the shoot apex. The later stages of leaf development take place in the region of expansion and maturation, and consequently lie principally outside the scope of this review; however, as will be apparent, the course of this development is largely determined during early stages of growth at the apical meristem.

(a) Factors Controlling the Development of Leaf Primordia

The fact that some regions of a shoot apical meristem develop as leaf primordia while adjacent, apparently comparable, regions of tissue develop as axillary buds, or do not undergo any specialized organogenic development, has long puzzled morphologists. The factors which control the regular arrangement of the growth centres are discussed in Section VI. Experiments which contribute to an understanding of the factors controlling the development of these growth centres as leaf primordia are considered below. Morphologically, leaf primordia are typically characterised by dorsiventral symmetry and determinate growth.

LEAF DETERMINATION IN FERNS. Many of the experiments which throw light on the factors that control the morphological destiny of a growth centre, e.g., its determination as a leaf primordium, have been carried out on ferns. In 1949, Wardlaw (606) showed that, if presumptive leaf sites of *Dryopteris dilatata* were isolated from the shoot apical meristem by a deep adaxial incision, they would develop as buds. From I_1 positions isolated in this way he obtained radially symmetrical, solenostelic buds which quickly gave rise to leaf primordia and exhibited indeterminate growth. This important discovery forms the basis for extensive subsequent studies of the factors controlling leaf and bud development in both ferns and flowering plants. Following up this work on *D. dilatata,* Cutter (135, 139) showed that the three youngest visible leaf primordia, P_1-P_3, were also capable of development as buds if they were isolated before formation of the enlarged, lenticular leaf apical cell characteristic of the species. In different apices the formation of this cell could occur during the first plastochrone or as late as the end of the third plastochrone. Primordia isolated after formation of this cell developed as normal, dorsiventral leaf primordia. It was therefore concluded that in this species the determination of a primordium as a leaf, however this is brought about, approximately coincides with a visible histological feature, namely, the formation of a lenticular apical cell. From experiments in which the centre of leaf primordia isolated before the formation of an apical cell was marked with India ink, it was concluded that the apex of the bud did develop from this central region of the leaf primordium.

Wardlaw (623, 624) later showed that the width of the incision isolating the primordium from the apex is important. When small incisions were made in the region immediately adaxial to I_2-P_2, buds were not induced; but when two wider, lateral incisions were made on either side of the primordia so as

to leave a bridge of intact tissue immediately adaxial to them, a proportion developed as buds. The depth of the isolating incision was also shown to be important. In the previous experiments the cuts were deep and penetrated the prevascular tissue of the apex. However, Wardlaw and Cutter (641, 643) found that when primordia were isolated by shallow incisions, penetrating only a single layer of cells and leaving the prevascular tissue intact, bud development was never induced. The regulative effects of the apical cell group and adjacent primordia on the growth and development of an incipient primordium thus appear to be mediated through the prevascular tissue. These experiments support the view that prevascular tissue may constitute a channel for the rapid translocation of growth-regulating substances and nutrients. Primordia isolated by passing a needle through the adaxial prevascular tissue, leaving the superficial layer of cells intact, developed as leaf primordia, but the aim of destroying all the prevascular tissue, and that only, was not adequately achieved because of the curved surface of the apical meristem and the rigid nature of the instrument used (136). Primordia isolated basally and laterally by deep undercutting and radial incisions, which must have disturbed the normal path of nutrients, also developed as dorsiventral leaves (628). From all these experiments it is concluded that the control which the apex and existing primordia exert upon the growth and development of new primordia is probably a complex combination of hormonal relationships and requirements for nutrients and other substances, and that it is mediated through the organisation and physiological activity of the apex as a whole, and the distribution of growth within it (624, 643).

In the experiments with *Dryopteris* it was found that, whereas undetermined primordia isolated by four deep cuts developed as buds, those isolated by four shallow incisions when the apex was not in active growth usually became dispersed and disappeared; the inception of prospective primordia could also be prevented in this way (641, 643). Primordia already determined at the time of isolation continued to develop as leaves. It was suggested that the formation of abundant wound tissue in response to the cuts, especially the adaxial one, might account for the dispersal of the primordia by competing for substances necessary for their growth. Cutter (141) later showed that if the adaxial shallow incision was subsequently deepened, usually when the primordia were just at the point of disappearance, the growth centres were sometimes stimulated into renewed growth. Some failed to grow, showing that the capacity for meristematic growth could be permanently lost as a consequence of isolation with shallow incisions. A greater proportion of primordia failed to respond to the stimulus of deepening the cut when the original shallow incisions had been made close to the growth centre, which is compatible with the view that the resulting wound tissue had successfully competed for nutrients with the growth centre. Of those isolated primordia that did resume growth in response to deepening the cut, the majority developed as buds, but about a quarter developed as leaves.

These last results were analysed in several ways in an attempt to discover the factors effecting leaf determination, and no correlation could be found between the development of an initially undetermined primordium as a leaf

and *a)* its stage of development at the time of the initial isolation by shallow incisions, *b)* its external appearance at the time of deepening the adaxial incision, *c)* the time that elapsed between the initial isolation and deepening the cut, or *d)* the rate of growth of the apex during this time (141). Some of the primordia which developed as leaves in these experiments were of normal dorsiventral symmetry; some were radially symmetrical, and others underwent a phase of radial symmetry before becoming dorsiventral. Some dorsiventral primordia were abnormally orientated, possibly as a result of the change in relative growth rates in the tangential and radial directions brought about fortuitously by the incisions (141).

The cumulative results of these experiments thus show that in *Dryopteris dilatata: a)* there is a period of time during which a visible primordium is capable of more than one kind of development, although this is never manifested during normal growth (that is, the determination of a primordium as a particular organ does not necessarily coincide with the fixation of its position on the apical meristem); *b)* determination of a primordium as a leaf approximately coincides with the formation of an enlarged, lenticular apical cell (139); *c)* active outgrowth of meristematic tissue is a necessary prerequisite for its determination as a leaf (141); *d)* the controlling effects of the apical meristem, which determine in the primordium the type of development characteristic of a leaf and restrict its potentiality for bud development, are usually mediated through the prevascular tissue (643); and *e)* under some imposed circumstances (the nature of which, however, is still not clear) leaf determination can occasionally occur in primordia no longer connected to the apical meristem by prevascular tissue (141).

Experiments on the growth and development of young leaf primordia of *Osmunda cinnamomea* in aseptic culture have led to some interesting results which closely parallel and extend those obtained previously and contemporaneously from surgical experiments on *Dryopteris.* In 1953, Sussex and Steeves (554) demonstrated the possibility of culturing excised fern leaves (see Section VIIb). With improvements in technique, it became possible to culture the youngest leaf primordia closest to the apex, which surgical experiments had shown to be capable of diverse types of development in *Dryopteris.* In *O. cinnamomea* it was first shown that the five youngest primordia P_1-P_5, when excised and cultured, developed not as leaves but as shoots, which eventually became complete, rooted plants (515). In more extensive experiments on the ten youngest primordia, P_1-P_{10}, Steeves (516-518) showed that P_1 always developed as a shoot and P_{10} always developed as a dorsiventral leaf, but P_2-P_9 showed variable development, progressively older primordia giving an increasing proportion of leaves. Roots were formed at the base of shoots, and also often of leaves. The development of excised primordia was not influenced by various changes in the nutrient medium; sugars, vitamins and amino acids were tested. Morphological and anatomical observations showed that P_1 developed in the same way as any excised piece of the apical meristem other than a leaf primordium, quickly becoming organised as a new shoot apex which began to form leaf primordia. P_2 often developed in the same way. In other P_2's and in P_3-P_9 the primordium apex continued to grow without evident change

in organisation or direction of growth, and in some instances it continued to develop in this way and ultimately gave rise to a dorsiventral leaf. In primordia which eventually regenerated shoots, however, this first stage was followed by an apparent separation of the original leaf apex into two meristematic mounds of approximately equal size; the more abaxial of these became the apex of a leaf, and the adaxial mound became a shoot apex. The leaf was not entirely equivalent to the later leaves of the same shoot.

From these experiments Steeves (517, 518) concluded that leaf primordia of *Osmunda cinnamomea* are not irreversibly determined from their inception, but undergo a relatively long period of development during which they remain undetermined. Determination is gradually imposed on the primordium. He suggested that leaf determination may be effected by a specific leaf-forming substance.

On the basis of arguments too lengthy and intricate to be stated here, the present writer believes that these interesting results are open to a different interpretation. It seems to her that the continued development as a leaf primordium of the apex, or part of the apex, of an excised primordium of *Osmunda,* described by Steeves (517, 518) for some P_2 and all older primordia, implies that it was determined at the time of excision. On this interpretation the buds which developed must have been formed from meristematic tissue comprised in the leaf primordium but not from its apex, or, at least, not from the whole of it. Steeves (517) reported that if the leaf apex was damaged buds often arose from other parts of the primordium. Steeves' own observation (517) that occasionally a shoot was produced by a primordium older than some from the same plant which produced leaves may be considered to support the present writer's suggested interpretation of his results to some extent. The criterion for leaf determination must surely be whether the apex of the isolated primordium develops as a leaf or not. If this definition of leaf determination is accepted, in *Osmunda* all primordia older than P_2, and even some P_2's, would be considered to be determined as leaves. Since Steeves (517, 518) reported that the leaf apical cell is formed in *O. cinnamomea* at about the time that P_2 becomes P_3, his contention that in this species leaf determination does not coincide with this would need to be reconsidered. In this species, unlike *Dryopteris,* leaf and shoot apical cells are both tetrahedral (69, 520).

Kuehnert and Steeves (289) have shown that fragments of leaf primordia derived by vertical bisection of primordia of *O. cinnamomea* are capable of developing in aseptic culture as whole leaves. In most instances the two halves of a primordium both developed either as leaves or as shoots, but in a few cases one half gave rise to a shoot and the other to a leaf. Kuehnert and Steeves consider that these results support the view that a leaf-forming substance generally distributed within the primordium at the time of excision is responsible for the eventual determination of the leaf. This conclusion is based on the premise that the leaf primordia used, P_4-P_{10}, were not irreversibly determined at the time of excision: for the reasons outlined above, however, the present writer would question the validity of this premise.

In somewhat comparable experiments on *Dryopteris dilatata,* Cutter and Wardlaw (151) have recently shown that buds can be induced to develop

from parts of already determined leaf primordia when these are isolated from the leaf apex or this is damaged. When primordia sufficiently old to be determined as leaves were punctured or bisected and also isolated from the shoot apex, buds developed from some of the half-primordia. Buds were also induced in the presumptive petiolar regions of P_9-P_{22} by isolating portions of the marginal meristem from the leaf apex by small incisions. Some of these primordia were also isolated from the shoot apex. As in the experiments of Kuehnert and Steeves (289), some of the half-primordia also developed as leaves, thus confirming the previous finding (141) that in *Dryopteris* leaf primordia can occasionally develop from tissue isolated from the apical meristem, under some circumstances. The results of these experiments indicate that the leaf apex has an important function in controlling the development of the subjacent tissues of the leaf. In the writer's view, the leaf apex—not necessarily the apical cell itself—is probably the vital controlling region of the leaf, equivalent to the apical cell group in the shoot apex; in the process of leaf determination, therefore, it is probably what happens to the apex of the primordium that is important. The marginal meristems are clearly also important in the development of leaf shape.

Both the surgical experiments on *Dryopteris* and the cultural experiments on *Osmunda* have shown that in these species leaf primordia are not determined at their inception but retain a potentiality for bud development for a number of plastochrones. Leaf primordia are not self-determining in the sense that the vegetative shoot apex is (see Section V). In this respect the leaf primordium may be more closely comparable with the floral apex, which incidentally also exhibits determinate growth. Steeves (518) has postulated the existence of a leaf-forming substance, produced elsewhere in the apex, which becomes localised in specific regions of the apical meristem. Bünning (87) has used the concept of adaptive enzymes in interpreting the patternised distribution of various structures, and this concept could well be applied to fern leaf primordia (136). In view of recent advances in knowledge of the histochemical changes associated with the transition of a vegetative to a flowering apex (see Section XI), it is indeed remarkable that so little is known in physiological terms about the more general and equally fundamental phenomenon of the determination of a part of the vegetative apical meristem as a leaf primordium.

LEAF DETERMINATION AND SYMMETRY IN FLOWERING PLANTS. In an attempt to repeat and extend the original experiment of Wardlaw on *Dryopteris,* Sussex (547, 550, 551) isolated the I_1 position on the potato shoot apex in a number of ways. He failed to obtain the development of I_1 as a bud, but found that it developed as a centric or radial leaf primordium. If, by varying the position of the incision, a shoot apex was induced lateral to the isolated I_1 position, I_1 developed as a dorsiventral leaf primordium orientated towards this apex. From these experiments and a survey of the earlier literature relating to the formation of radial leaves (321, 495, 497, 600), Sussex concluded that leaf dorsiventrality was determined by an effect from the apical meristem similar to that operative in embryonic induction in animals. Similar centric organs

were obtained in *Sesamum* after a comparable experimental treatment (238).

Snow and Snow (501, 510, 511) failed to obtain radial leaves in potato apices, which were, however, maintained under conditions very different from those of Sussex, but did not obtain radial leaves from isolated I_1 areas in *Epilobium hirsutum.* In *Lupinus albus* and *Cucurbita pepo* isolated P_1's and I_1's developed only as dorsiventral leaves (493, 501). Snow and Snow (501) described some experiments which suggested that in *Epilobium* leaf dorsiventrality is indeed controlled by the shoot apex, but concluded that reduction of size of the area available to the primordium is a contributory factor and perhaps the principal one. They also pointed out that if leaf dorsiventrality in *E. hirsutum* is induced by the apex it must sometimes be lost again, since, although some isolated I_1 areas developed as dorsiventral leaves, some P_1 primordia isolated early on small pieces of tissue became radial.

Although, in general, Snow and Snow (501) failed to induce the formation of radial leaves in potato, they obtained some which were of radial symmetry in their basal region but were dorsiventral higher up. Somewhat similar structures, as well as more truly radial leaves, have been observed also in *Dryopteris* (139, 141) and *Osmunda* (518). This suggests that a radial leaf is an organ of restricted development.

Other observations which are compatible with this view are reported in several species. In the potato itself, two out of 123 small isolated panels of the apical meristem failed to regenerate shoots but developed as organs resembling the radial leaves (548). In *Epilobium* (495), *Phaseolus* (412) and *Euphorbia* (512) small isolated regions of the apical meristem also failed to regenerate and developed in this way. It may be questioned, indeed, whether these organs should be regarded as leaves at all; many of them are, in fact, just outgrowths with very limited meristematic activity.

It seems likely that the small size of the meristematic tissue available for growth is a contributory factor in controlling the very limited development of the "radial leaves" or "centric leaves" obtained in these various experiments (510), although Sussex (550, 551) has argued that the development of quite large centric organs from isolated I_1 sites in the potato as a result of delayed excision of the rest of the apical meristem is not compatible with this view. Since, however, I_1 was actually just emergent when the apical tissue was excised in these experiments, the important effect may already have taken place in the apex of I_1, subsequent enlargement ensuing when apical competition was removed.

In future considerations of dorsiventrality and limited growth, attention might profitably be turned to the development of the "seed scale complex" in conifers. For example, in *Pinus ponderosa* the ovuliferous or seed scales are initiated in the axils of bracts (195), and appear radial at first, but eventually become dorsiventral structures. This must take place when they are at some distance from the subtending shoot apex. It would be worth investigating the factors concerned.

Pellegrini (413) claimed that if the isolation of I_1 sites in *Phaseolus* was accompanied by excision of the remainder of the apical region, some of the I_1's developed as shoots. Shoots were not formed from P_1's treated in this

way, nor from P_1 primordia or I_1 sites isolated without removal of the apical region. This is an important result and, if confirmed on other species, would considerably affect current views of leaf determination in angiosperms. However, it will be important to obtain confirmation, since only four out of 19 primordia treated in this way developed as shoots (413, Table 1). After similar treatment of I_1 in potato shoot apices, Sussex (550, 551) obtained shoots from the isolated panels of tissue but considered that I_1 itself developed as a dorsiventral leaf primordium orientated towards this shoot.

Flower buds in *Nuphar* and *Nymphaea* and vegetative buds in *Nymphaea* occur in leaf sites in the normal development (140). These species were therefore thought to be favourable materials for an experimental investigation of the developmental potentialities of lateral growth centres. Principally using apices of *Nuphar lutea,* Cutter (142) found that I_1 sites and P_1 primordia isolated by a deep cut from the shoot apex did not develop as buds but as dorsiventral leaf primordia, sometimes with associated axillary or lateral buds which were formed from isolated apical tissues adjacent to them. Initially both the leaf primordium and the bud participated in a common outgrowth. It was concluded, however, that, although they constituted part of this general growth, the isolated leaf primordia or leaf sites did eventually develop as dorsiventral leaf primordia. Moreover, some of the isolated areas gave rise only to leaf primordia. Radial leaves were not obtained. It was concluded that in *N. lutea* lateral growth centres are determined as a leaf (or, presumably, as a flower) at about the stage of I_2.

The results of experiments involving isolation of young leaf primordia or presumptive leaf sites in angiosperms, with the exception of a few on *Phaseolus* (413), thus indicate that leaf determination takes place at an earlier stage in the ontogeny of the growth centre than it does in the two ferns investigated. This conclusion is compatible with the fact that the whole process of leaf development in these ferns is slow. In inflorescence apices, also, there is evidence that the associated bract and flower primordia are determined from the stage of I_3 (134).

(b) Other Aspects of Leaf Growth and Development

RATES OF LEAF FORMATION AND DEVELOPMENT. Factors affecting rates of inception and development of leaves have recently been reviewed by Humphries and Wheeler (263). In both LD and SD plants, transfer to inductive daylengths led to an increased rate of leaf formation (322, 475, 476, 564, 565), and it is thought that there is probably a direct relationship between onset of flowering and promotion of leaf growth. In *Sorghum,* however, photoperiod had little or no effect on the rate of leaf formation (267). In peas the rate of leaf formation is slightly accelerated in light as compared with darkness (571; see also Section IX).

Removal of actively growing young leaves may lead to increases in the rate of leaf inception and usually also of development (23, 137, 147, 270). Defoliation may result in premature expansion of leaves that would normally expand only in the following year (19, 645). In sporelings of *Marsilea vestita* the first leaf and first root both inhibited development of subsequent organs.

This dominance of the primary organs was apparently a result of lack of available nutrients, not an auxin effect (297). Various factors may have differential effects on the rates of inception and development, and this may lead to effects on form in both the leaf and the shoot (see Section IV).

LEAF FORM. Most leaf primordia are very similar at their inception, their characteristic features becoming established in the subapical region (634, 635). The primordia of scale and foliage leaves and of microphylls and macrophylls are similar at their inception (138, 181, 182, 631). The development of primordia as cataphylls or as foliage leaves may be controlled by daylength (396) or by an effect of gravity (406), among other factors. The primordia of prospective cataphylls can be caused to grow as foliage leaves by defoliation and other treatments (19, 391, 523, 542). Treatment with GA may lead to the development as scale leaves of primordia which would otherwise become foliage leaves (146, 164, 165, 636), or to the development of coiled, tendril-like structures (576). Humphries and Wheeler (263) consider that the concept of phyllocaline is partially fulfilled by the gibberellins; in Section IV of this review, where other effects of GA on leaf form have already been discussed, it has been suggested that at least some of these effects may be attributed to differential effects on growth in the apical and subapical regions of the shoot. Ontogenetic changes in leaf form have also been discussed in Section IV.

Leaf shape may also be affected by various environmental factors, among them temperature and daylength (475, 476, 477, 594), and even by insect infestation (479) and virus diseases (560). These various factors probably affect rates of growth. For example, Schwabe (475) has shown that temperature affects the rate of unfolding of leaves of *Chrysanthemum,* this being slower at lower temperatures, but has little effect on the rate of leaf inception. The less dissected character of chrysanthemum leaves which are formed at higher temperatures might thus be attributable to their more rapid expansion, resulting in their spending less time at the apex, since more complex leaves are thought to need a longer period of meristematic growth for development (8).

CONTROL OF LEAF MORPHOGENESIS. The physiology of leaf growth has recently been reviewed by Humphries and Wheeler (263), and the factors controlling early stages of leaf growth and development have already received some discussion above and in Section IV. Normally, once the site of a growth centre has been fixed, a primordium begins to grow for a continuous period. Treatment of embryos or shoot apices with phenylboric acid, however, suppresses the growth of one or both cotyledons or of one or more leaf primordia (229, 231, 232). This substance is considered to inhibit selectivly the first processes of differentiation in the leaf primordium. Inhibition of cell division in the young leaf primordia is also brought about by treatment with the pyrimidine analogue 2-thiouracil, and it is thought that after prolonged treatment leaf formation is probably progressively arrested (253). These effects are likely to be a consequence of the disturbance of nucleic acid metabolism in the leaf cells, which is also a possible explanation of the interesting effects of tobacco mosaic virus on leaf development reported by Tepfer and Chessin

(560). At certain stages of infection of the tobacco plant, narrow-bladed and "shoestring" leaves are formed. These latter are of radial symmetry and devoid of a lamina, as a result of the absence or reduced activity of the marginal meristems.

In such leaves the direction of cell divisions in the primordium appears to have important effects on leaf shape. However, there is some evidence that, at least in early growth, the form of the leaf may be determined independently of the sizes and shapes of cells and the orientations of cell divisions (225, 226). The shapes of leaves of wheat growing normally or without DNA synthesis and cell division (as a consequence of gamma-irradiation) were essentially identical, while the sizes, shapes and number of cells in them were very different. In *Callitriche,* however, polarity of cell expansion has a considerable effect on leaf shape (276). In a recent study of tobacco leaves, Haber and Foard (227) concluded that the polarisation of leaf growth cannot be attributed to the planes of cell division. They further stated "On the basis of what we now know, and lacking further information, we are inclined to suggest that in the stem apex, cell divisions do not play an essential role in the changes of polarization underlying the initiation of primordia. Of course, in the stem apex, cell divisions would still play an important role in sharply delimiting the *extent* of growth of the incipient primordium." Cell division in leaves is not restricted to the early stages of development, but continues until the leaves have reached at least half their maximal area. Cell expansion continues after the cessation of division (543).

One of the most complete contemporary accounts of leaf growth and development is that of the leaf of *Osmunda cinnamomea* (78, 79, 519, 520, 523). This descriptive work is complementary to the experimental work on the leaf primordia of this species discussed in Section VIIa and below.

The technique of culturing excised fern leaves (554), which made it possible to study the growth in isolation of a normally determinate organ over a period of time, has led to a number of important conclusions concerning leaf morphogenesis. Most of the work has been done on ferns, although it was also shown that leaf primordia of flowering plants could be successfully cultured (521). The first major conclusion from this work was that in both ferns and angiosperms the complex pattern of leaf development is self-controlled within the leaf, once it has been determined at the shoot apex (517, 519, 521, 522). In the fern *Osmunda cinnamomea* excised leaf primordia of various ages developed into miniature replicas of the mature frond. The smaller size was primarily a result of reduced cell number instead of smaller cell size (522). Increasing concentrations of sucrose supported the development of taller and heavier leaves with up to 50% more pinnae (518), and Steeves (519) considers that the small size of cultured leaves may be attributable to inadequate nutrition.

In a detailed study of the growth in culture of leaf primordia of *O. cinnamomea* of the second outermost annual set (Set III), Caponetti and Steeves (101) have shown that growth and development in culture parallel natural growth in most respects, but there is no dormant period. The time taken to reach maturity is reduced from more than a year under natural conditions

(of which six months would be passed in dormancy) to about ten weeks in culture. The differences between cultured and natural fronds are quantitative rather than qualitative. Cultured set III leaves had an average height of 4.6 cm., whereas natural fronds are usually 60 cm., sometimes 100 cm., in height (518). Successively younger primordia are capable of more prolonged meristematic growth but attain a smaller final size.

These studies on the development of older fern leaf primordia in culture have not been restricted to *Osmunda* but have included also *Adiantum pedatum* (522), *Todea barbara* (555), *Leptopteris hymenophylloides* (552) and *Ceratopteris* (208). The importance of carbohydrate nutrition as a factor affecting leaf development has been demonstrated in several investigations. In both *Leptopteris* (552) and *Osmunda* (553), excised leaves developed the simpler, juvenile form in media with low concentrations of sucrose, and the more complex, pinnate, adult form in the presence of higher concentrations. Croziers failed to uncoil on high concentrations of sucrose, but uncoiled when yeast extract was added to the medium (552). The height, fresh and dry weight, number of pinnae and pinnules, and the number of epidermal cells of the excised primordia increased with increase in sucrose concentration, but the dimensions of the epidermal cells of the pinnae decreased (553).

In the main, these experiments and those of Gottlieb (208) support the original conclusion that the pattern of leaf development, once the leaf primordium is determined at the shoot apex, is self maintained within the leaf, but certain results considered below suggest that under normal conditions this is affected at least to some extent by correlative factors from other parts of the plant. The small size of cultured leaves, which is a result of premature cessation of cell division, may be due to restriction of the supply by the rest of the plant of some substance or substances essential for the continuation of active cell division (518). Many experiments suggest that rates of growth and development of leaf primordia are controlled to some extent by the outer leaves which are normally present. Although leaf shape can be controlled at least partially by nutrition, importance is also attributed to unidentified reactions in the whole plant which regulate the rate of leaf development (553). Sporangia could be induced in leaves of *Osmunda* excised in autumn but not, or only occasionally, in those excised at other times, suggesting that some predisposition to fertility was acquired by the leaf (555). Meiosis did not occur in the sporangia induced on excised leaves but did take place on leaves of intact sporelings cultured under identical conditions (518). Steeves (518) considers that the influence of the rest of the plant on the development of individual leaves is largely of a nutritive nature, and further experiment may well substantiate this belief; however, it seems likely to be true only of leaves which have attained a certain stage of development.

VIII. EFFECTS OF APPLIED SUBSTANCES

One way of investigating processes of growth and development in the shoot is to apply particular substances, e.g., auxins and auxin inhibitors, either directly to the shoot apex or, more commonly, to more mature parts of the plant. Both naturally occurring and synthetic growth-regulating substances have been

used in this way. The results obtained are usually described in terms of effects produced in more mature parts of the shoot, but most of these are determined in the apical meristem or immediately subapical region. Wardlaw (625) emphasised the advantages of direct observation of apices treated with growth substances, but relatively few investigations of this kind have been reported. An extensive literature recording the eventual effects of growth substances in mature parts exists, and this review makes no claim to completeness. In recent years the effects of gibberellin have been so extensively reported that this substance will be treated separately.

(a) GIBBERELLIN

The gibberellins first discovered were products of the metabolism of a fungus, but they are now known to be of quite wide occurrence in higher plants. Nine different gibberellins have now been specified, the most frequently used in studies of growth being gibberellin A_3, or gibberellic acid (GA). Several reviews deal with the properties and effects of the gibberellins (73, 75, 312, 418, 537, 538, 540, 677). Gibberellins are hormonal substances, being highly mobile; their distribution is usually systemic in herbaceous but not necessarily so in woody plants (677).

EFFECTS ON PLANT HABIT. Treatment with GA may affect the habit of a plant in various ways (55, 67, 73, 75, 146, 535, 538). This is sometimes brought about by stimulation of the outgrowth of lateral buds (75, 146, 287). In cereals and in *Trifolium,* however, tillering was inhibited (72, 535). It is not always clear whether these effects of GA are on the formation or the outgrowth of lateral buds, though they are apparently usually on outgrowth; indeed, Stuart and Cathey (541) have concluded that the effect of applied gibberellin is usually not to establish new growth patterns but to accelerate those already in progress. Its effect on flowering in *Petasites* (638) is also compatible with this interpretation. At least in excised tissues, however, GA sometimes inhibits bud formation (383, 474).

EFFECTS ON THE APICAL MERISTEM AND SUBAPICAL REGION. One of the most widespread and conspicuous effects of gibberellin is stem elongation. Dwarf mutants of several species can be caused to grow to heights comparable with those of the normal tall phenotype if treated with GA (53, 54, 56, 76, 417). In bringing about stem extension, the principal effect of GA is on elongation of internodes (73, 75, 282, 309, 332, 361, 384, 404, 514, 538, 574, 594, 676, 677). This takes place not only in herbaceous but also in woody plants (18, 361, 367, 396, 478). Apart from dwarf mutants, the most striking results have been obtained with rosette plants (103, 292, 309, 310, 312, 319, 388, 460, 463, 464, 514). In the fern *Dryopteris dilatata,* on the other hand, internodal elongation was not stimulated by direct application of GA to the apex (644). The effects of GA on stem elongation are transient, and in rosette plants secondary or tertiary aerial rosettes may be formed on cessation of treatment (103, 292, 677).

These effects of GA on shoot growth are mediated through its action on

the tissues of the apical and subapical regions. In rosette plants of *Hyoscyamus* and *Samolus* treated with GA, a marked increase in the number of mitotic figures in the tissues immediately underlying the apical meristem occurred from 12 to 24 hours after treatment (319, 460, 463, 464). GA treatment may also stimulate division in the cells below terminal inflorescences (485). Thus gibberellin can function as a regulator of cell division (463, 582). In *Samolus,* GA treatment led to a partial synchronisation of cell division, peaks in mitotic activity in the cells of the pith region occurring 24 and 48 hours after treatment (461). Most of the mitotic figures induced in the subapical region of rosette plants were transversely orientated, thus contributing to extension of the length of the axis (388, 460, 463). When plants of *Hyoscyamus* treated with GA were completely defoliated, the number of induced cell divisions was reduced and the orientation of the spindle was changed. Some influence of the leaves, which could be replaced by applied IAA, was apparently necessary for the normal orientation of mitosis in the subapical region (312, 388).

In the apical meristem of *Hyoscyamus* the increase in the number of mitotic figures in plants treated with GA was negligible; in *Samolus* the number of mitoses in this region was never more than twice that in the controls, whereas in the subapical region the increase might be ten-fold (460). From these various results with GA, and from observations on rosette plants induced to bolt by suitable environmental conditions without GA, it was concluded that the subapical region of the shoot apex is largely responsible for stem histogenesis in both caulescent and rosette plants, the apical meristem contributing very little to the normal elongation of the axis (460, 461, 465). It is suggested that the subapical region may be considered as an intercalary meristem (464).

A statement of Lang (310) suggests that the presence of a living apical meristem is not necessary for the characteristic response to GA treatment, namely, stimulation of cell division in the subapical region. The tips of a number of *Hyoscyamus* plants treated with relatively high concentrations of gibberellin were killed; from Lang's description it appears that these plants elongated but failed to flower. In experiments in which shoots did not respond to GA if the shoot apex was excised, presumably the subapical region also was removed.

In addition to these effects on internodal extension, treatment with GA may lead to a decrease (538) or an increase in the actual number of internodes (4, 54, 75, 367, 404, 514, 538, 656, 676). Basford (54) has shown that in dwarf groundsel this stimulatory effect of GA on leaf formation is influenced by environmental factors (see Section IV); Wittwer and Bukovac (677) have also stated that the effects of GA on crop plants are greater in unfavourable environmental conditions. Although the principal effect of GA is clearly on mitosis in the subapical region, in species in which the number of leaves formed is increased there must be a stimulatory effect of GA on mitosis in the apical meristem itself.

FLOWERING. The capacity of gibberellin to lead to the flowering of some LD and cold-requiring plants in non-inductive conditions, first demonstrated

by Lang (310, 311), is the first major example of the chemical stimulation of flower formation (318). It is thought that native gibberellins, or comparable substances, may function as natural biochemical regulators of flower formation (314).

The effects of gibberellin on flowering must clearly be effects on the development of the apical meristem. However, the majority of papers merely report the ultimate results of GA treatment and do not deal at all with its action on the apical meristem itself. Nevertheless, since the important effects must be on this region, it may be relevant to review briefly some of the literature.

Plants which normally require cold-treatment to induce flowering have been stimulated to flower by treatment with GA (103, 310, 311, 313, 315). GA failed to induce flowering in some cold-requiring plants, however, leading only to stem elongation (60, 311, 313). Large amounts of gibberellin are usually needed to simulate vernalisation (74). GA induced flowering in unvernalised winter rye in LD, its effect being apparently cumulative (82). In other experiments only a temporary stimulation was obtained, and this was considered not to be due to a reduction in the vernalisation requirement (264). Hurd and Purvis (264) attributed the effect of GA on flowering in rye to its ability to induce renewed mitotic activity in dormant meristems.

Flowering of obligatory LD plants has also been induced in SD by treatment with gibberellin (73, 90, 311-313, 315). It did not, however, cause flowering in caulescent LD plants (315). In facultative LD plants, flowering was accelerated by gibberellin in both LD and SD (48, 323, 677, 678). Plants which normally require a period in LD followed by a period in SD will flower when kept only in SD if treated with gibberellin (89, 684). Flowering of SD plants was not stimulated by gibberellin (213, 242, 311, 313) and may be inhibited by it (72, 91, 242). However, flowering of SD *Cosmos* was accelerated (679). GA treatment also sometimes modified inflorescences towards vegetative development (83, 453).

The action of the gibberellins on flowering is difficult to interpret. Gibberellin is sometimes considered to be identical with vernalin (103), but other authors advocate caution in equating it with this (318). The observation that, in endive, vernalisation is inductive, causing a persistent after-effect, whereas gibberellin treatment is not (443), tends to support the latter view. Certainly the evidence seems to indicate that gibberellin is not the sole factor in inducing the flowering phase (242). Its primary effect seems to be on stem elongation, and it is sometimes suggested that some processes which occur in elongating stems may trigger off flower formation (110, 311-313, 317). On this interpretation the induction of flowering in GA-treated *Rudbeckia* in SD constitutes a difficulty; in this instance stem elongation did not precede flowering (678). The view that there is some relationship between flower formation and the gibberellin metabolism of the plant is supported by the finding that, while extracts from vegetative plants of *Hyoscyamus* had little or no effect on dwarf mutants of maize, extracts from plants which had just formed flower primordia showed increased activity (318). Since gibberellin does not usually induce flowering in SD plants, it is probably not identical with "florigen" nor the sole regulator of flower formation (311, 318). It may, however, be a

factor which limits formation of the flowering hormones (318, 684). General stimulation of meristematic activity may be important (264). Chailakhyan (107) has suggested that there may be two groups of substances necessary for flowering: gibberellins, necessary for stem formation and growth, and anthesins, necessary for flower formation.

REACTIVITY TO THE VARIOUS GIBBERELLINS AND SIMILAR SUBSTANCES. Plants respond differently to the different gibberellins, and their response may also be affected by environmental conditions (236). Considerable differences in the activity of gibberellins A_1 to A_9 on flower induction were observed, and different species reacted with differing sensitivities. Some of the negative results on flowering previously reported may be attributable to the use of a gibberellin relatively ineffective in that particular species (368, 369).

Of substances similar to gibberellin, allogibberic acid, a degradation product from GA, cannot stimulate internodal elongation in dwarf peas but still delays flowering, as does GA itself. Gibberic acid, a degradation product derived from allogibberic acid, shows neither of these effects (384). Steviol, a plant diterpenoid, has recently been shown to affect growth of a particular dwarf mutant of maize which had only previously responded to gibberellins (458).

METABOLISM OF GIBBERELLIN. It is of some importance to know both the site of synthesis of endogenous gibberellin and the mechanisms by which it exerts its effects on growth. It has been shown that the inhibition of stem growth by light can be quantitatively reversed by gibberellin treatment (332, 334, 335, 591). Lockhart (334, 335) concluded that light affects the level of endogenous gibberellin in the stem, and that this becomes the factor limiting the rate of elongation, but Mohr (378) holds the view that GA and the light-controlled endogenous growth system act independently. Light is thought to diminish the action of applied GA (558). From experiments with decapitated pea seedlings, Lockhart (333) concluded that the natural gibberellin factor is produced in the shoot tip. Vlitos and Meudt (591) considered that induced stem elongation is probably dependent not only on GA but also on the activity of growth factors produced in the shoot apex, since elongation diminished with excision of the shoot tip. Brian (72, 73) has proposed a scheme of the action of gibberellin which attempts to explain its relationships with the effects of light, temperature and daylength.

Transference of spinach plants from SD to continuous light led to a substantial temporary increase in gibberellin content (437). In *Hyoscyamus,* extracts from photoinduced plants gave higher levels of gibberellin-like activity; this was obtained only from young leaves or from these and shoot tips together (316).

Relationships between gibberellin and auxin metabolism have been demonstrated several times. In several species, plants treated with GA have yielded greatly increased quantities of diffusible auxin only a few days after treatment (291, 292, 396). In apical parts of *Centaurea* there was a 43-fold increase in diffusible auxin, and it was concluded that GA treatment caused an increase

in auxin production in the apical portions of the plant which led to stem elongation but had no direct effect on flowering (292). In shoot apices of *Lupinus,* application of GA led to a decrease in IAA oxidase activity (656). The observation that GA induces elongation in intact plants but not in segments, while IAA is effective on segments (75), is of interest in view of the finding that application of GA led to increased production of IAA by the shoot apex. The effects of GA and auxins on growth are evidently in some way complementary (75), but it does not seem likely that all responses to gibberellin can be related to auxin levels, as has been suggested (291).

(b) Growth Retardants

Certain substances, notably Amo-1618, CCC and Phosfon D, have effects on growth opposite to those of GA, e.g., suppression of internodal growth (105, 106, 359, 362, 459, 464, 574). Several of these substances are chemically related, and quite small differences alter their effectiveness on processes of growth (237, 288). When applied simultaneously to shoots, Amo-1618 or CCC or allyltrimethylammonium bromide and GA are mutually antagonistic (105, 106, 359, 459, 464, 574). In stem callus cultures, however, the inhibitory effects of Amo-1618 or CCC are not reversible by GA treatment (459).

In several caulescent plants, treatment with Amo-1618 led to a reduction in the number of mitoses in the subapical, but not the apical, region, and shoot elongation ceased, leading to the formation of rosettes. Treatment with GA prevented or reversed these effects (459, 464, 465). Later it was shown that not only Amo, but also CCC and Phosfon, inhibited cell division and cell elongation in the subapical region of *Chrysanthemum.* Cell expansion and division in the transverse direction were actually stimulated by these substances. Application of GA to retardant-treated plants resulted in maintenance of subapical cell elongation and division (462).

In azalea, flower formation was induced by application of Phosfon or CCC (540); in *Bryophyllum daigremontianum,* CCC suppressed flower formation, and this effect could be overcome by treatment with GA (685).

It thus seems likely that these substances may affect metabolism of endogenous gibberellins in the plant.

(c) Other Substances

KINETIN. Kinetin is a synthetic substance which stimulates cell division. There is as yet little evidence of its occurrence in plant tissues, but a substance with rather similar chemical properties has been isolated from young maize kernels (372). A factor which may be identical with this and which was also isolated from maize increased cell division in carrot explants about six-fold (326).

The principal effects of kinetin are on bud formation and growth. Bud formation has been stimulated in various tissues in the presence of kinetin (410, 474, 487). Miller (373) has pointed out, however, that kinetin should not be regarded as a specific bud-forming substance, since it is known to cause bud formation only in materials with a natural tendency for such developments. In the presence of kinetin, disorganisation of the shoot apex may take place.

In *Ceratopteris* the bud meristems gave rise to callus (208); in *Marsilea* a callus with a large number of peripheral growing points developed (295). In *Hydrocharis* fewer buds were formed in the presence of kinetin, but a greater number of these grew out as stolons (146). Another effect of kinetin and allied substances on bud growth is the breaking of dormancy (294, 657).

Kinetin also has considerable effects on the growth and development of young leaves. Wardlaw and Mitra (644) found that the youngest leaf primordia on apices of *Dryopteris* treated with kinetin disappeared completely, older ones also being modified; no new leaf primordia were formed. This investigation differed from the majority of studies in that kinetin was applied directly to the apical meristem, which was kept under direct observation throughout the experiment. In *Marsilea* the growth of young leaves was also affected (295), but the most striking effects were on leaf morphology. The leaf segments showed repeated branching, and roots were formed (17, 295, 513). Similar effects were obtained on plants grown in the dark (296). One interpretation of some of these effects is that buds or root-like organs replaced the leaflets (513).

Correlative growth may also be modified by kinetin. In *Lemna* high concentrations allowed the development of axillary fronds on both sides of the parent instead of principally on one side, thus removing normal inhibition effects (214). Interactions between kinetin and auxins have been demonstrated, and indeed Wickson and Thimann (674) have suggested that similar interactions may be the basis of apical dominance. Kinetin interferes with auxin uptake and transport (675), but direct application to a bud failed to reverse its inhibition by IAA (293). More recently, Sachs and Thimann (466) have shown that direct applications of kinetin to lateral buds of Alaska pea plants may completely release them from apical dominance for the first few days. In longer periods kinetin-treated buds on intact plants grew less than untreated buds on decapitated plants; this difference in growth could be decreased by treatment of the former with GA. Sachs and Thimann considered that kinetin was probably primarily involved in the release phenomenon and GA only subsequently. They suggested that the development of lateral buds in intact plants may depend on the balance between auxin from the shoot tip and a kinetin-like factor acting locally. The interaction between auxin and kinetin is probably not a simple one, but kinetin, or similar substances, may be intermediaries in various metabolic reactions (114).

2,3,5-TRIIODOBENZOIC ACID. One effect of low concentrations of TIBA is thought to be elimination of the normal polar mechanism for auxin transport (486); certainly its effects often seem to be indirect. Various effects on normal growth are reported, e.g., increased internodal elongation and delayed heteroblastic development (397). More commonly, however, TIBA treatment induces various teratological developments, e.g., ring fasciation (204, 281, 282). These effects could be counteracted by GA (282) or NAA (281). In tomatoes, TIBA inhibited leaf growth and completely suppressed leaf formation (283), an effect comparable with that of PhB (231). Some similar effects on leaf

formation and development were obtained by direct applications of TIBA to shoot apices of *Dryopteris* (632).

2,4-DICHLOROPHENOXYACETIC ACID. Many effects of 2,4-D are also teratological. According to the time of treatment, in *Avena* 2,4-D induced fasciation of the shoot apex, resulting in the formation of multiple inflorescences, and sometimes effects on leaf formation and suppression of organogeny in the florets (260). When young embryos of *Eranthis* were treated with 2,4-D, a proportion of twin embryos were formed, and it was suggested that the substance had destroyed apical dominance (228). In several species, 2,4-D had effects on leaf growth and development, often causing the concrescence of cotyledons or adjacent leaves (52, 228, 234).

INDOLEACETIC ACID. The effect of applied IAA in suppressing outgrowth of lateral buds is well known. Experiments with two-shoot plants afford some evidence that auxin must move both up and down the shoots (447), a conclusion also supported by work on xylem regeneration (268, 269). Direct application of IAA to shoot apices of *Tropaeolum* led to the formation of multiple leaf primordia and the development of tangentially elongated axillary buds, resembling fasciations (34). Tangential enlargement of leaf primordia was also observed in *Dryopteris* (632). In this fern, direct treatment of shoot apices with IAA led to a considerable increase in the rate of growth of the subapical region. This effect was even more marked after treatment with indoleacetonitrile (IAN) which also affected the young leaf primordia in various ways (632).

NAPHTHALENEACETIC ACID. Wardlaw and Mitra (644) showed that NAA has effects on the shoot apex of *Dryopteris* somewhat similar to those previously obtained with IAA and IAN. In apices collected in winter, NAA had a differential effect on the growth rates of the apical and subapical regions, resulting in a cup-like upgrowth of the latter, a morphological feature found in the normal development of some other ferns. With high concentrations of NAA, the apical meristem became parenchymatous and gave rise to scales. Such differential effects on growth in different regions may afford a partial explanation of the antagonistic effects of NAA and TIBA (281).

Treatment with NAA induced the development of female flowers in genetically "male" plants of *Cannabis* (246), a finding which will receive more extended consideration in Section XII.

MISCELLANEOUS SUBSTANCES. Treatment with phenylboric acid led to inhibition of the formation of cotyledons, foliage leaves and some of the lateral organs of the flower (229, 231, 232, 325). Leaf growth was also considerably affected by 2-thiouracil (253).

Bud formation on the roots of *Populus tremula* was inhibited by 2,4,5-trichlorophenoxyacetic acid and other phenoxy substances (170).

A considerable number of substances directly applied to shoot apices of *Dryopteris* had effects on growth and development. Of these, dinitrophenol,

yeast extract and maleic hydrazide led to parenchymatisation of the apical meristem and the formation of scales, and also to the formation of buds in leaf sites or other positions (625, 632). Maleic hydrazide can also prevent inflorescence formation in strawberries and cause a reversal of various processes following induction, leading to the formation of some foliar parts in the prospective flowers (569).

(d) Conclusions

Application of various chemical substances, some synthetic and others known to occur naturally in plant tissues, can lead to many modifications of growth and development. With some exceptions, such observations are often difficult to interpret in metabolic terms. The normal growth and development of a plant are now generally considered to be regulated by a delicately balanced system of chemical controls (528). For example, one view of the effect of auxin on growth is that it predisposes cells to change, other chemical regulators such as gibberellin and kinin determining the precise nature of the change (279). The effects of applied substances must thus depend in large part on the endogenous growth regulators already present in the plant treated and are difficult to interpret without a comprehensive knowledge of its native metabolism.

The usefulness in developmental studies of experiments of this kind is often diminished by a failure to observe effects on early stages of growth, especially by failure to distinguish clearly between effects on the inception or on the outgrowth or subsequent development of an organ.

IX. EFFECTS OF ENVIRONMENT

In both earlier and contemporary work, various environmental factors have been shown to have more or less considerable effects on growth and development, and indeed a recent symposium deals exclusively with this topic (179). On the whole, however, with the exception of photoperiod, these factors have been relatively little studied in relation to the shoot apex itself. Some of the principal effects of temperature and light, including duration of light, will be considered here. Any consideration of environmental factors is, of course, rendered more complicated by the frequency of interactions between them, especially temperature and daylength.

(a) Temperature

Apical growth. Apical growth may be affected by temperature in both the vegetative and reproductive phases of development. In young tomato plants an increase in temperature delayed the enlargement of the vegetative apex but accelerated the rate of leaf formation and growth (265). At higher temperatures the first two leaves of young plants apparently compete actively with the shoot apex for the assimilates available. Removal of the first two leaves at 25°C led to rapid enlargement of the apex and earlier flowering, whereas at 15°C only slight effects were observed (266). The effects of temperature on bud dormancy have recently been reviewed by Vegis (587).

The rate of development of the inflorescence may also be affected by temperature (175, 176) and by photoperiod. Y. Heslop-Harrison and Woods

(255) showed that, in male plants of *Cannabis,* low night temperatures during or just after the period of induction led to the formation of intersexual flowers, involving the development of potential stamens as carpellate or intermediate structures and a reduction in the number of floral parts. They suggested that, if there is a sudden variation in an important environmental factor, such as temperature, at the time when the growth centres of the floral organs are becoming established in the floral apex, important modifications in the numbers of parts, and in their development, may ensue. The mechanism by which this is brought about is not clear.

FLOWERING. Promotion of flowering by low temperatures, which is usually termed "vernalisation", has recently been reviewed by several workers (116, 386, 436, 468, 683). The flowering hormone believed to be formed as a result of cold treatment is known as "vernalin". Hillman (259), however, has queried the existence of vernalin on the grounds that there is no clear evidence of the transmission of a substance resulting from vernalisation alone instead of vernalisation followed by LD. Vernalisation occurs only if mitosis takes place during the cold treatment, and it is concluded that the process must occur only in cells undergoing mitosis (659). Other work on seeds suggests however, that dividing cells are not essential (468). Cytological studies of plants at low temperatures are required to elucidate this point (683). Vernalin is thought to be produced in the shoot apex or youngest leaves (317). Perception of the stimulus usually takes place in the embryo or the shoot apex (62, 308). There is thus no separation of the sites of perception and response (308), comparable with that which occurs in floral induction by daylength factors. There may, however, be a considerable separation in time between perception and response (468), since vernalisation can occur in the seed. In the contemporary idiom, this ability to retain the "message" or "instruction" to flower over a considerable period of time, i.e., the capacity to receive a stimulus and to react to it only after a lapse of time, is one of the most interesting aspects of the physiology of the apical meristem.

(b) LIGHT

The effects of light on growth have recently been reviewed by Mohr (378) and by Hendricks and Borthwick (245), and only observations relating directly to the apex will be considered here.

The size of the apical meristem of broad bean is the same whether the seedlings are grown in darkness or in light, as is the distribution of mitotic figures (94). In peas, also, the organisation of the shoot apex of plants grown in darkness, red light or white light was the same above P_2 (572). However, there were slower production and development of leaf primordia and young internodes in darkness (94, 571). In *Elodea* the apical meristems of lateral shoots produced in darkness were smaller than those formed in light, comprising fewer cells (153). Below the level of P_4 in pea there was an increase in internode length in light-grown plants (572). Raising the light intensity led to an increase in cell number in the leaves of *Vicia* (92), and to increased rates of apical enlargement and leaf formation in barley and tomato (26,265).

The effect of light comprises two phases: a sensitive effect on cell multiplication and a less sensitive one on cell enlargement (92). In light-grown plants the stimulus which suppresses etiolation is thought to be perceived in the growing region, and not by the leaves (476).

In *Marsilea* rhizomes are orthotropic when grown in the dark and plagiotropic in light or in red light (296). Plagiotropic growth is also induced by light in other species (407), and by SD in still others (476). Development of *Marsilea* as land or water forms can be affected by far-red and red light (190; see Section IV).

In cultured rhizome apices (apparently quite long pieces) of blueberry, the diageotropic habit was maintained in the dark. In the light, however, internodes were shorter and foliage leaves were formed (50). Following exposure to light, the apex is described as undergoing disruption and becoming reduced to an inoperative unit, but a greater number of leaves was formed in the light and the apices illustrated appear quite healthy, although smaller.

(c) Daylength

EFFECTS ON VEGETATIVE GROWTH. Although the principal effects of daylength are on induction of the flowering phase of development, various aspects of vegetative growth are also affected; the observations imply effects on the shoot apex, although these are not always described in detail. In a number of woody plants shoot growth ceases in SD, both stem elongation and leaf formation being arrested (396, 579, 649). Bud break may be stimulated by LD treatment (647, 648). In some woody plants the photoperiodic stimulus is perceived by the leaf as in floral induction (395, 396, 648), but in other species it may be perceived by still meristematic leaf primordia (647) or by the shoot apex (648).

Exposure of plants to inductive photoperiods for less than the period necessary for floral induction sometimes results in an increase in the rate of leaf formation and other effects on apical growth (176, 564, 565). The rates of formation of leaf primordia and axillary buds may be differentially affected (565), which must alter the degree of proximity to the apical meristem of bud inception. The formation of adventitious buds on leaves of *Bryophyllum* is also affected by daylength, provided the treatment is given while the leaf is still attached to the plant (484). Bulb formation and tuberisation are also controlled by daylength; this control is apparently centred in the shoot apex and young leaves (403, 483).

EFFECTS ON FLOWERING. The most spectacular effect of environment on the shoot apex is the photoperiodic induction of flowering, first demonstrated in 1920 by Garner and Allard (188). A vast amount of research has since been carried out on this topic, and there are several recent reviews of photoperiodic effects on flowering (61, 68, 161, 259, 308, 331, 467, 468, 683). Most of the published work on the effects of daylength on flowering does not deal with the changes in growth and development induced in the shoot apex, except by implication, and will therefore not be reviewed here. The morphological and histological changes which take place in the induced apical meri-

stem are considered in Section XI, and some recent experiments which suggest that the flowering stimulus can be perceived by the shoot apex or young leaf primordia are discussed below.

The technique of aseptic culture of shoot apices has recently been applied to problems associated with flowering, notably the effects of daylength, with several interesting results. In 1946, Loo (354) observed the formation of flowers on one apex of dodder grown in vitro. More recently, Baldev (31, 32) grew stem tips of *Cuscuta reflexa* in sterile culture, and subjected them to various periods of darkness. They flowered in continuous darkness or with a daily dark period of 14 hours or more, behaving as typical SD plants. It is claimed that the bud itself is sensitive to photoinduction (31, 32). The excised stem tips were one to two cm. long and, as the illustrations show, bore scale leaves; but in some experiments, not described in detail, segments of the stem with one bud in the axil of a scale leaf were cultured, and in some the scale leaf was excised. Flowering of the bud still occurred. However, the possibility that the more mature stem tissues might be sensitive to induction has not been excluded; in this genus they already perform some of the usual functions of the leaf, such as photosynthesis.

In a series of ingenious experiments with cultured apical buds, factors controlling flowering in *Perilla* have also been investigated (438, 439). In SD, cultured apical buds with only two pairs of leaf primordia eventually flowered, and those with only one pair showed some signs of induction, suggesting that the leaf primordia could participate in perception of the floral stimulus. In LD, apical buds formed sterile structures superficially resembling a *Selaginella* cone; the individual florets in these structures developed only the outer, non-sporogenous primordia. When older, unfolded leaves were excised and planted separately in a position adjacent to an apical bud on the same medium, and both were photoinduced, flowering was inhibited. It was concluded that flowering in *Perilla* seems to consist of two morphologically distinct stages, the formation of sterile cones (Stage I) and the formation of normal flowers (Stage II). Stage I is independent of daylength conditions. In LD, the unfolded leaves are thought to produce an inhibitor which keeps the plant vegetative. In SD, they promote flowering when attached to the apical bud but inhibit it when detached but grown on the same medium (439). In SD, flower formation (Stage II) was inhibited by the presence of IAA in the culture medium; in LD, IAA inhibited the formation of the non-sporogenous tissues (438). The somewhat complex role of the unfolded leaves could probably have been demonstrated only by this cultural technique.

In support of his view that in SD plants the product of an inductive long dark period is transported from the leaf to the shoot apical meristem and there transformed into a stable product, Carr (102) has shown that excised apices from plants given three long dark periods formed flower primordia after one month in aseptic culture. The apices of control plants remained vegetative.

REVERSION TO THE VEGETATIVE STATE. The change of the apical meristem from the vegetative to the floral phase of development is almost always permanent and irreversible. Pineapple, however, is anomalous in this respect; the

inflorescence is terminal, the vegetative apex changing to an inflorescence but subsequently reverting to vegetative growth during production of the "crown" (210). Such reversion of floral or inflorescence apices to the vegetative condition occurs in a number of plants which have been maintained in inductive daylengths for too short a period (175, 564, 680) or occasionally in flowering plants subsequently placed in non-inductive conditions (129). Treatments of this kind, however, do not always induce reversion (303). Surgical treatments of the young inflorescence apical meristem may also result in reversion (636). Changes of this kind can often be induced only at a certain critical stage of development (636, 680). Reversion of the plant as a whole to the vegetative state can be promoted by decapitation and disbudding (298, 299), but this does not involve a change in development of individual shoot apices.

PHYSIOLOGICAL EFFECTS OF DAYLENGTH. The mechanisms involved in the formation and transport of the floral stimulus have been fully discussed elsewhere (259, 308, 331, 468), but some other effects of daylength may be briefly considered. The content of gibberellin or gibberellin-like substances in the plant may be considerably affected by photoperiodic treatment (240, 241, 316, 395, 396, 437). In at least some instances, these substances occur in the shoot apex (395). Various metabolic reactions may be affected by photoperiod; for example, in inductive conditions CO_2 fixation is more active (128) and respiration is activated in young buds (127).

Lang (308) emphasised that inductive daylength conditions bring about changes in the plant leading to active promotion of flowering, and that there is not merely an inhibition of floral induction by non-inductive conditions. There is some evidence, however, that this may also take place. Guttridge (221, 222) has suggested that a vegetative growth-promoting and flower-inhibiting hormone is produced in the leaves of strawberry in non-inductive daylength conditions, acting on the growing regions. Defoliation treatments of various kinds supported the view that, in strawberry, flower formation could take place under any daylength conditions but is prevented in LD by the hormone formed in the leaves (223, 570). In the SD plant *Salvia* one day of continuous light apparently led to the production of a floral inhibitor in the leaves which was transported to the apex (59).

In *Lolium temulentum,* Evans (177) obtained evidence of the formation in the leaves of a substance stimulatory to flowering in LD, and an inhibitory one in SD. Both of these substances were thought to accumulate at the shoot apex, and the substance formed in LD was found to accelerate or inhibit flower formation according to the stage of development (178).

X. HISTOGENESIS AND METABOLISM IN THE SHOOT APEX

Little is known about the metabolic activities of that dynamic region, the apical meristem. The reason for this is less lack of interest than lack of adequate techniques for the study of so small a tissue mass. With the development of new techniques a beginning has, however, been made on relevant physiological problems. As regards histogenesis in the apex, a large body of descriptive information exists, but as yet the controlling factors are little understood.

(a) Histogenesis

DISTRIBUTION OF MITOTIC FIGURES. In elaborating his theory of phyllotaxis, the theory of multiple foliar helices (see Section VI), Plantefol (424) claimed that the foliar helices end in a flank meristem at a certain distance from the tip. This meristem, the anneau initial, was thought to perform the functions usually attributed to the classical initial cells, and he claimed that there are no initial cells at the summit of the axis. Subsequently, associated workers carried out anatomical and cytological studies on the shoot apices of a considerable number of species of vascular plants, and affirmed the presence of an anneau initial; they also asserted that the central terminal cells of the axis have no histogenic function during vegetative growth, and constitute a méristème d'attente, or waiting floral meristem, which becomes active only in the formation of a terminal flower or inflorescence (95-98, 104, 300-302). In species without terminal flowers the inactive zone was called the "zone apicale" or "zone axiale". Subsequently, however, it was concluded that the apices of all species do not conform exactly to a single model (303). Several books and reviews appraise this earlier work (122, 143, 174, 191, 199, 303, 627, 633, 639). Some discussion of the more recent experimental and analytical work may be useful, despite Salisbury's recent complaint concerning the time wasted in philosophical discussions of the controversy centred on the méristème d'attente (467).

In the course of purely anatomical studies, several workers, in addition to those mentioned in previous reviews, have pointed out the occurrence of mitotic figures at the summit of the apical meristem (70, 157, 168, 525). Using various techniques, other workers have also been unable to find evidence of an inactive méristème d'attente region (120, 168, 192, 193, 197, 307, 408, 430). By direct observations of living shoot apices, Newman (392) produced evidence of cell division at the tip of the apex. In an extension of this kind of study, Ball (46, 47) photographed the surface layers of shoot apices of *Lupinus, Vicia* and *Asparagus* at intervals of three or five minutes for periods of five to 20 days. The apices were maintained under constant conditions in aseptic culture, in diffuse light. Ball found that the cells on the surface of the apices divided, and he observed no difference in rate of division between those at the summit and those in lateral positions. He concluded that there was no quiescent méristème d'attente and that the concept of the anneau initial was not supported by his studies. These apices were maintained under more normal conditions than in many experiments, except for the intermittent brighter illumination of the apex necessary for each frame of the film, which Ball considered caused no deviation from normal growth.

Many recent studies by French workers support the concept of the anneau initial and méristème d'attente (303-306, 433, 456). Studies of enzyme distribution in apices of *Picea* also support the view that mitotic activity is greatest on the flanks of the apex and relatively low in a terminal central region (580). Lance-Nougarède and Loiseau (307), however, reported that the anneau initial and méristème d'attente were not present in the apices of four aquatic plants, *Elodea, Hippuris, Ceratophyllum* and *Callitriche;* Savelkoul (470) had previously reached a similar conclusion for *Elodea.* In these species the apex

constantly acquires new cells by the activity of the summit meristem (307). Loiseau (347) has recently carried out some interesting experiments with apices of *Impatiens*. Marks placed in the centre of the apex remained in that position for a time, then were displaced towards the circumference, this occurring more quickly as they neared the periphery. Loiseau states that the unequal speed of movement of the marks testifies to the unequal distribution of mitoses in different regions of the apex, a facet of apical growth which is indeed accepted by many workers. Both Lance (303) and Jacobs and Morrow (273) have reported a considerably lower percentage of cells containing mitotic figures in the distal as compared with the proximal region of the apex. Newman (393), indeed, stated that the anneau initial is merely a "mathematical concomitant of apical growth in a more or less massive structure." Loiseau (347) concluded also, however, that the zone axiale was not inert, apical cells being finally incorporated in the peripheral zone; but he nevertheless considered that the concept of the anneau initial best explained the observed facts. Loiseau also asserted that in small apices, in which the marks moved quickly from a central position, the centripetal mitotic inductions of lateral origin attained the centre of the meristem. This argument, which is fairly general among the French workers, perhaps emphasises the principal difference of opinion between them and other workers. This is not whether or not the central terminal cells of the apex divide but whether they have a role as initials in the development of the shoot; the French workers claim that even when these cells divide they do not function as initials, giving rise to the tissues of the vegetative shoot; this function is fulfilled by the anneau initial. Cytological evidence testifies to considerable differences between cells from these regions (95, 97, 98, 104, 300, 303). Other recent workers, however, claim an initiatory role for the terminal cells of the apical meristem (118, 122, 197, 408).

The suggestion (143) that the number of mitoses observed, and the volume of the apex over which they extend, might be correlated with the degree of change in size and form of the apex during a plastochrone, has been supported by a study of the apex of *Ephedra altissima* (408), but apices of *Lonicera nitida* showed the same mitotic index regardless of the stage of the plastochrone (168). Jacobs and Morrow (273) have demonstrated a diurnal rhythm in the growth of the apical meristem and the formation of leaf primordia in *Coleus*, but found no evidence for a marked diurnal rhythm in the percentage of cells showing mitotic figures.

Wardlaw (639) has recently pointed out that mitotic indices alone may be misleading and that information on the duration and rate of mitosis is also required. Jacobs and Morrow (273) have also emphasized that the smaller percentage of distal cells with mitotic figures may reflect either a slower rate or a shorter duration of mitosis. It has been suggested that information on rates of mitosis in the various regions could be obtained with certain methods of treatment with ^{3}H-thymidine (194). Clowes (120, 121) pointed out that the time spent in mitosis is only a small part of the duration of the mitotic cycle, and Edgar (168) has shown that the duration of mitosis is about one-ninth that of the mitotic cycle in the upper layer of the shoot apex of *Lonicera*.

Considerations of this kind are important also in interpreting the results of experiments with radioactive isotopes. Partanen and Gifford (409) demonstrated synthesis of DNA, as shown by incorporation of ^{32}P, in the terminal zone of the shoot apex of *Pinus lambertiana* as well as in other regions; they interpreted these findings as indicative of subsequent mitotic activity. Using ^{14}C-labelled adenine, Clowes (120) showed that DNA was synthesised in cells at the summit as well as lower down in the apex of *Coleus* and three aquatic species. Clowes (122) pointed out that for all the nuclei in an apex to be labelled the isotope must be supplied for a certain length of time, according to the species; in shoots supplied for shorter periods, however, the unlabelled nuclei were not restricted to particular regions of the apex. Gifford (192, 193) has shown that ^{3}H-thymidine was incorporated into the nuclei of cells in the central region of apices of *Chenopodium* and of the shoot apical cell of *Ceratopteris*. Lance-Nougarède (306), however, found that ^{3}H-adenine was incorporated in the lateral cells of the meristem but only rarely in the nuclei of cells in the zone axiale. Gifford, Kupila and Yamaguchi (194) also found that the central zone was relatively free of labelled nuclei, although the nucleus of a cell situated medianly at the tip was often labelled. In a recent study of the distribution of ^{3}H-thymidine in the apex of *Arabidopsis,* an apical middle region of low labelling corresponding to the méristème d'attente was observed (81, 370). In these experiments the isotope solution was applied for only a six-hour period, a much shorter time than those used by Gifford and Clowes. Brown, Miksche and Smith (81) point out that differential labelling of the various histological zones is more likely to be demonstrated by shorter treatment times; presumably what is thereby demonstrated, however, may be a difference in rates of mitosis in the various regions, and not necessarily an absence of mitosis at the summit.

That the cells of the méristème d'attente or zone axiale are capable of histogenesis under some circumstances has been demonstrated by the French workers themselves. Loiseau (341, 345) destroyed the whole of the anneau initial in apices of *Impatiens,* leaving only the zone axiale, and growth of a shoot with two foliar helices continued. As has already been pointed out (143), dwarf shoots of *Pinus,* in which only the inactive zone apicale remains (98, 99), can be induced to develop as long shoots bearing scale leaves (159, 646). Dwarf shoots of *Larix* are also capable of developing as long shoots (379). These experiments, like those of Ball (43) in which the cells of bisected apices of *Lupinus* underwent rapid division, demonstrate the capacity and readiness of the cells of the zone apicale to divide under particular conditions; it must be recognised, however, that such experiments do not provide evidence that these cells in fact divide actively in the normal development.

FACTORS CONTROLLING MITOSIS IN THE APEX. The effects of applied GA on mitosis in the subapical region, and under some circumstances in the apical meristem itself, have been discussed in Section VIII.

The effect of treatment by gamma rays and other forms of radiation is to inhibit mitosis in the apical meristem either partially or completely (180, 226, 233, 371, 377, 525). In some instances cells in different regions of the apical

meristem show differential sensitivity to irradiation (130, 371). Similar differential sensitivity may also occur at different stages of development (217).

Perhaps the principal factor affecting mitosis in the shoot apex is light, especially daylength. A considerable increase in mitosis in the apical meristem follows a few inductive photoperiods (196, 197, 303, 568, 666). The position in which such mitoses occur seems to be somewhat variable. The French workers consider that during transition to the flowering phase, the méristème d'attente becomes activated and rapid mitosis occurs there (303). In both LD and SD plants the first sign of mitotic activity has been reported to occur in the cells just below the central zone (196, 666). Recently Thomas (568) has demonstrated a doubling of the metaphase mitotic index occurring in apices of *Xanthium* only 24 hours after the end of a single long (inductive) dark period. A light break prevented this effect. Butler (93) has recently shown that in apices of shoots of *Vicia* maintained in either constant darkness or constant light, there is no rhythmic cell division but a higher mitotic index in the light; in apices of plants given cycles of 12 hours light and 12 hours darkness, a mitotic rhythm was observed, with maximal peaks two hours after the beginning of the light period and also in the middle of the dark period. If dark-grown plants were merely stimulated with 12 hours light and then returned to darkness, the mitotic rhythm induced by the light was endogenous. Mitotic rhythms in plants have previously been shown to occur (88, 300), but these were not endogenous.

(b) Metabolism

Much of the limited information relating to the metabolism of the shoot apex is inferred from chromatographs of extracts of shoot apices, some of which have previously been given various experimental treatments. Notwithstanding the difficulties of working with such small volumes of tissue, however, some progress has been made with a study of the physiology of apical tissues on a cellular basis.

Most of the histochemical work on the shoot apex concerns the nucleic acids, and will be considered in Section XI. In an interesting study of the distribution of peroxidase, Van Fleet (581) demonstrated localised reactions for this substance in prospective leaf sites on the apical meristem and also in the sites of future axillary buds. This approach gives some insight into the metabolic changes in the apex which must necessarily precede the visible histological changes.

EXTRACTS OF SHOOT APICES. It is known that auxin is present in growing shoot apices, especially in young leaf primordia (669), and that none is detectable after growth ceases (189). Applied GA can cause a considerable increase in diffusible auxin, especially in the apical regions (291, 292).

The effect of daylength on growth substances as shown in extracts of shoot tips has been studied in several species. Shoot tips of *Rhus typhina* in LD contained a growth-promoting substance which moved to the same R_f as IAA; the level of this substance fell after two weeks in SD. Treatment with GA led to an increase in the substance (396). LD treatment of *Rudbeckia speciosa*

led to the appearance in the shoot tip of a natural gibberellin (240). Similar qualitative and quantitative changes in endogenous gibberellin-like substances were shown to occur in inductive photoperiods in other species (241, 316).

NITROGEN METABOLISM AND RESPIRATION. In a study of the nitrogenous components of shoot apices by means of quantitative paper chromatography, it was shown that apices of the fern *Adiantum pedatum* had a lower total protein than apices of *Lupinus albus* and *Syringa vulgaris,* which both had approximately the same protein content per unit weight—a value higher than in more mature tissues. (534). There was a high content of arginine and lysine. Also using *Lupinus albus,* Sunderland, Heyes and Brown (545, 546) attempted to investigate cellular metabolism in the shoot apex. They compared growth and metabolism in three regions: *a)* the apical dome itself, above the leaf primordia; *b)* the individual leaf primordia; and *c)* units of the embryonic axis corresponding to each leaf primordium, termed internodes (544). They found that the concentration of protein in the apical dome was higher than that in the internode but lower than that in P_1 (545). Protein content per cell, however, was probably consistently higher in the internodes than in the primordia, and at least in the upper part of the apex respiration per unit protein was also higher in the internodes than in the primordia (546). It is suggested that metabolites may be synthesised in the youngest internodes, which are characterised by a higher metabolic activity per unit protein, and then transferred to the leaf primordia; some component of this complex of metabolites may inhibit differentiation in the primordium (545). Some of the observed metabolic differences between the older internodes and primordia are considered to be ultimately attributable to the differentiation of the apical dome into tunica and corpus. It is suggested that the observation that the properties of the dome are often intermediate between those of the first primordium and the first internode may be due to derivation of average values for the dome from a central tissue (the corpus), which determines internodal characters, and a surface tissue (the tunica), which determines primordial characters (546).

This interpretation is probably considerably over-simplified, especially as there is good evidence from work with chimaeras that leaf primordia are derived from the cells of the corpus as well as from those of the tunica (for a discussion of the relevant literature, see Clowes (122)). However, the outer less metabolically active and the inner more active regions of the apex perhaps need not be strictly equated with the tunica and corpus. At all events, this work is a valuable contribution to the difficult problem of metabolism in the shoot apex, especially at the cellular level.

XI. THE REPRODUCTIVE APEX: EARLY STAGES

The transition from the vegetative to the reproductive phase of development is probably the most profound ontogenetic change in plants. It has been intensively studied by physiologists, and also to a considerable extent by anatomists and histologists, but relatively little is known about the effects of the various controlling factors at the cellular level, although a valuable begin-

ning along these lines has recently been made. Among others, Steward (527) has rightly pointed out that it is necessary to know exactly how the factors shown to be important in the induction of flowering, such as a long night or a light break, affect the apex itself. For it is here that the changes in shoot growth and development which are subsequently manifested have their inception. These factors, their gross effects on the apex, and their effects at the cellular level will be discussed in this Section.

(a) Factors Affecting Flowering

Before a plant can be induced to flower, it must usually have attained a certain size or stage of development, a condition known as "ripeness to flower". In some plants this can be quantitatively measured; for example, there may be a characteristic minimum number of leaf primordia produced before flowering can occur (259). Some plants, notably many monocotyledons, reach a certain age characteristic of the species, then flower and die. For a certain variety of bamboo, the required age is about 32 years; flowering occurs at this time even if the bamboos are transplanted to another part of the world, and is thus apparently independent of environmental factors (21, 22, 259). In other plants, also, even those sensitive to photoperiod, there is evidence of a gradual progression towards the flowering condition (58, 651). In raspberry, shoot apices remain vegetative one season but flower under the same conditions one season later (262). Very little is known about the mechanisms in the shoot apex which control the time of flowering in this sense; in bamboos a long-term endogenous rhythm of some kind may be involved (259). Hillman (259) has pointed out that the death of monocarpic plants may well be due to the conversion of every shoot apex on the plant to the reproductive state.

While in some instances this type of age requirement seems to be absolute, in others earlier flowering can be induced by various means. In banana, immature lateral buds forced to grow by a technique for multiplying the plants flowered while still attached to the parent plant, when the bud apex had formed only seven or fewer leaves, or in a few instances none. These buds always developed on plants which were themselves flowering (51). In Japanese larch, training the branches in a downward position advanced the onset of flowering by one or two years (350).

ENVIRONMENT. The effects of temperature and photoperiod on flowering have been discussed in Section IX, including a few examples in which the effects of photoperiod are apparently perceived by the shoot apex or shoot tip, not by older leaves. As already pointed out, the effect of vernalisation is usually thought to be perceived by the shoot apex. These effects are apparently perpetuated in the products of the apical meristem, since all tillers of vernalised winter rye will flower even though they are formed some time after cessation of the cold treatment (308, 683).

Since environmental factors can be precisely controlled, the aseptic culture of whole plants has recently proved to be a useful means of investigating the

factors controlling the change of the apex to the flowering condition (80, 257, 258, 284, 285, 323, 357, 557, 593).

AUXIN. Except in a few plants, pineapple among them, the evidence for the implication of auxins as factors affecting flowering is controversial; it has received considerable discussion (259, 331, 467). Bergfeld (57) made the interesting observation that, whereas a decrease in the content of growth substances (IAA, IAN, and another substance) occurred at the beginning of inflorescence formation in the normal form of *Antirrhinum majus,* no such change occurred in the mutant *sterilis,* which failed to flower. On the other hand, auxin antagonists, e.g., TIBA, sometimes delay the onset of flowering in some plants (283). In day-neutral *Coleus blumei* the axillary shoots inhibit flowering, most of this effect being attributable to the auxin which they produce (271).

In most instances little is known about the mode of action of auxin on the apex itself. Interactions between IAA and daylength in controlling flowering in excised apical buds of *Perilla* (438) have been discussed in Section IX.

OTHER HORMONAL SUBSTANCES. The postulated hormone vernalin is thought to be produced in the shoot meristems and youngest leaves, whereas florigen, the hormone supposedly formed in response to photoperiod, is formed in relatively older, expanded leaves (317). Both of these exert their effects on the shoot apical meristem.

In *Iris,* experiments with cultured apices have shown that flower induction is promoted by primordial leaves and by a factor present in the scales (454). In strawberry there is some evidence that a flower inhibitor is produced in the leaves and affects the development of the shoot apex (222, 570; see Section IX). Gibberellic acid is probably also implicated in the control of flowering (see Section VIII).

In precise chemical terms very little is known about the flowering hormone, and so far it has not been successfully extracted and isolated. Recently, however, Lincoln, Mayfield and Cunningham (329) have succeeded in inducing flowering in 50% of plants treated with an extract of the flowering branch tips of *Xanthium.* They claim that this is the first reproducible demonstration of floral initiation in a SD plant as a direct result of an extract from flowering plant tissues. Later, extracts prepared from the leaves of flowering plants of the day-neutral species *Helianthus annuus* were shown to induce flowering in some 20% of treated plants of *Xanthium* (330). The authors conclude that the active principle in these extracts is florigen. Salisbury (467) has pointed out that in these experiments no extracts were made of vegetative control plants.

NUTRITION AND GRAVITY. An analysis of the distribution of male and female cones on *Pinus sylvestris* showed that female cones were produced first, on leading shoots, and several years later male cones were formed in the basal region of the lower, apparently less vigorous, branches. Male cones apparently required a lower nutritional status within the shoot than female ones. In early

stages the primordia of male and female cones, dwarf shoots and vegetative buds appeared to be identical (650). Disbudding of the terminal shoots led to earlier production of male cones in young trees (646). In *Larix leptolepis* male flower buds occurred mainly on horizontally or downwardly directed twigs, usually on the lower side, and flower formation could be induced by training branches downwards (350, 351). By inverting and fixing the branches it was possible to convert a developing vegetative bud into a flower bud, and vice versa (350). Bending the tops also led to a larger number of male flowers (366). These effects of gravity on flowering are not restricted to conifers; apple trees, and to a lesser extent cherries, flowered much more heavily when maintained in a horizontal position (652). The precise effect of the force of gravity on the apical meristem is not very clear; it has been suggested that gravity may affect the differential distribution of auxin or other hormones in the branch (259, 351).

REDUCED VEGETATIVE GROWTH. Various techniques for reducing vegetative growth have been said to stimulate flowering, and this has long influenced certain horticultural practices. Among these is ringing of trees or branches (259, 366). Kojima and Maeda (286) found that retardation of the growth of the shoot apex by pressure caused flower initiation to some extent in seedlings of *Raphanus* which had not been exposed to cold. In these experiments the seedlings were embedded in gypsum in a split bamboo cylinder, so that the roots and cotyledons were free. Inhibiting the vegetative growth of the apex by hypertonic sugar solutions or by application of maleic hydrazide also led to increased flower initiation (286). In black currant cessation of extension growth in the shoot led to transformation of the buds to the flowering condition if the bud meristems were not yet dormant (387).

Thomas (566, 567) has recently shown that formation of axillary bud primordia was promoted in inductive conditions, buds occurring in younger leaf axils. He suggested that inductive conditions might result in decreased inhibition of cell division in the apical meristem, and that this might in turn allow rapid initiation and growth of axillary bud primordia. "Such uninhibited growth of primordia might be a directly causal factor leading to inflorescence development" (567). It seems unlikely to the present writer, however, that active growth and division of the apical meristem would lead to a lesser degree of apical dominance; other factors must also be involved. In discussing the results of some experiments with thymidine analogues, Brown (80) has suggested that precocious induction of floral development may follow a temporary impairment of the active nuclei of the vegetative apical meristem. Nuclei which were normally relatively inactive would then be released to form the reproductive meristem.

(b) Changes in the Apex at Flowering

When the reproductive phase of development begins, the apex of a terminal flower or inflorescence enters upon a greatly modified sequence of morphogenetic events. It is therefore not surprising that at the change to the flowering phase

there are a number of profound physiological, histological and morphological changes in the shoot apex. As Salisbury (467) has pointed out, the flowering hormone is apparently a chemical substance capable of controlling the development of a three-dimensional structure. The problems relating to this are difficult enough to understand, but it seems to the writer still harder to envisage any explanation of the situation where the terminal shoot apex remains vegetative indefinitely but lateral meristems give rise to flowers. In various members of the Nymphaeaceae, for example, the rhizome apex continues vegetative growth for many years, but each season gives rise to a number of lateral flower primordia which are formed at the apical meristem in a more or less regular pattern characteristic of the species (144). These primordia develop the characteristic features of flowers as soon as they are formed, on the flanks of the main apex which nevertheless itself remains vegetative. Spikate inflorescences show this phenomenon also but to a lesser degree. Some sort of balance of activities in the apical meristem must be envisaged.

CHANGES IN FORM. One of the changes most characteristic of the transition to the flowering phase is a stimulation of the formation or outgrowth of axillary buds, an effect which has long been known but is shown very clearly in recent controlled experiments (129, 196, 566, 567). In the SD plant *Chenopodium album* the appearance of precocious axillary bud primordia after 4 SD was the first macroscopic change observed (196).

In this species there is also a marked two- or three-fold increase in height of the apical meristem (196). Similar changes occur in other species (176). In chrysanthemum the diameter of the apex increases from about 150 μ, characteristic of the vegetative apex, to about 3000 μ just before the formation of florets. There is approximately a 399-fold increase in area, and this takes place within a few hours (475).

RATE AND DEGREE OF PERSISTENCE OF CHANGES. Even in species in which the control of flowering has been worked out very precisely, little information about the rates of changes occurring in the apical meristem has been available until recently. In *Xanthium* anatomical changes are said to become detectable two to two-and-a-half days after the beginning of the inductive dark period, at about the time that translocation of the flowering stimulus from the leaf is completed (61, 62, 468). A significant increase in mitotic index in the apex was observed slightly earlier than this (568). Apices of plants of *Xanthium* given one SD showed no recognisable histological change until the fourth day; changes were more rapid if more than one inductive photoperiod was given (666).

In some plants such changes as are induced by a few appropriate photoperiods do not persist, but in *Xanthium* the flowering impulse can be transmitted through five to seven graft transfers (61). The self-perpetuating agent, whatever its nature, is thought to be produced by the actively dividing tissue of the apical meristem (62). In sugar-cane the floral stimulus apparently persisted in cultured isolated apices for some months (125). The persistence of

changes induced by vernalisation has already been mentioned (308, 476, 683).

HISTOLOGICAL CHANGES. The major anatomical changes associated with the transition to the reproductive phase are described in standard text-books (172, 173). Only those changes induced by precisely controlled factors will therefore be discussed here.

The initial effect observed in the apices of several species of both LD and SD plants subjected to inductive conditions was mitotic activity distal to the rib meristem, below the central zone (666). One of the species used in this investigation, *Chenopodium album,* has since been studied in more detail, with some interesting results. After four inductive SD, a mantle of three or four layers was present, a biseriate tunica being characteristic of the vegetative phase (196). After induction starch grains tended to disappear from the apex, the nucleoli of cells on the flanks of the apical meristem and in the upper corpus enlarged and became comparable with those in the cells of the terminal region of the tunica, and the concentration of RNA increased and became more uniformly distributed (197). Comparable observations on RNA have been reported previously by various French workers (see 303, 433). By autoradiographic techniques it was shown that the rate of synthesis of nucleic acid, especially RNA, increased in the apical meristem of *Chenopodium* during photoperiodic induction (197). The protein concentration in the apical meristem was much greater after four SD than in vegetative plants. In the early stages of inflorescence development the distribution of histone matched that of DNA, but after five SD or more there was a marked decrease in histone staining which was not accompanied by a change in the staining for DNA (197). Histone distribution does not change in any of the later stages of development of the vegetative apex (198).

(c) Implication of Nucleic Acids in the Control of Flowering

Considerable recent evidence implicates nucleic acid metabolism in the ultimate control of flowering. Nucleic acids are now known to be associated with many organelles, and are thus involved in many aspects of cell metabolism. It is considered likely that phytochrome controls reactions in protein and nucleic acid metabolism at the cellular level (378).

There is experimental evidence, based mainly on the supply of various nucleic acid derivatives to cultured apical buds, that flowering in SD plants is promoted by increased protein and nucleic acid metabolism (91, 107-109). Derivatives of the purine and pyrimidine type stimulated flowering in excised apices of *Perilla* as well as in whole plants (108). In *Pharbitis nil* cultured buds flowered in SD but remained vegetative in LD unless supplied with a mixture of RNA nucleosides and casein hydrolysate (91). The action of maleic hydrazide in preventing flowering in strawberries has been interpreted as a block to incorporation of uracil into the nucleic acid molecule (569). In experiments on other species, uracil, xanthine and caffeine increased flowering, while adenine and guanine had no effect; the flower-promoting effect of these purines and pyrimidines was attributed to an enhancement of the synthesis of RNA and protein (280). In *Pharbitis* uracil and guanine both stimulated floral initiation (363). Labouriau (cited in 62) found that inhibitors of RNA synthesis

inhibited transformation of the vegetative apex but did not inhibit synthesis of the floral hormone in the leaf.

EXPERIMENTS WITH ANTI-METABOLITES. A number of recent investigations using various inhibitors of nucleic acid metabolism have given very interesting results. Some of the compounds effective and ineffective in *Xanthium* are listed by Salisbury (467), who pointed out that inhibitors of both RNA and DNA metabolism were found in both groups. In 1960, Heslop-Harrison (251) demonstrated an inhibitory effect of 2-thiouracil on flowering in *Cannabis,* and Salisbury and Bonner (469) showed that 5-fluorouracil (and also thiouracil) inhibited photoperiodic induction in *Xanthium.* A given amount of the substance was more effective when applied to the apical bud rather than to a leaf. 2-thiouracil also inhibited, or at least delayed, flowering in peas (380), *Streptocarpus* (256) and *Pharbitis* (363).

Extending the earlier work with the pyrimidine 5-fluorouracil (5-FU), Bonner and Zeevaart (64) showed that in *Xanthium* this substance exerted its inhibitory effect on flowering by acting directly on the apical bud, not on the leaf, during an inductive dark treatment of the leaf. Thus processes essential to the subsequent successful receipt of, and response to, the flowering hormone must take place in the bud during this time. By means of experiments with labelled 5-FU and labelled orotic acid, it was found that 5-FU inhibited the synthesis of both DNA and RNA. The results of experiments with 5-fluorodeoxyuridine (5-FDU), which inhibits synthesis of DNA, showed that inhibition of DNA replication during the 16-hour dark period was not inhibitory to floral induction in *Xanthium,* provided DNA replication could subsequently resume. It was concluded that 5-FU inhibited synthesis of RNA in the bud, and that this process was essential to the successful induction of flowering. By contrast, the site of action of 6-azauracil, which also inhibits floral induction in *Xanthium,* is apparently in the leaf (457).

Zeevaart (682) showed that both 5-FU and 5-FDU inhibited flowering in *Pharbitis nil,* the latter substance being much the more effective. Both substances were effective even if applied many hours after the flowering hormone had reached the shoot apex, and Zeevaart concluded that the process inhibited by them was the induction of flower primordia. The inhibitory effects of 5-FU could be overcome by application of several precursors of DNA, and it was concluded that in this instance synthesis of DNA, not of RNA, was the process essential for floral induction. A study of the frequency of mitotic figures in the apical regions showed that 5-FDU almost completely suppressed cell division within 24 hours; mitosis was apparently arrested in prophase. Experiments with other substances indicated that DNA multiplication in the apex, instead of cell division, was the process essential for floral intiation. Thus, unless there is multiplying DNA in the apical region, the flowering hormone will fail to induce initiation of flower primordia (681, 682).

Brown (80) has recently shown that the thymidine analogues 5-iododeoxyuridine and 5-bromodeoxyuridine promoted flowering in cultured plants of *Arabidopsis thaliana,* whereas 5-FDU suppressed growth. In other species the inhibitory effects of 6-azauracil and 5-bromo-3-isopropyl-6-methyl uracil could

be reversed by uridine (126). Ethionine also inhibited floral initiation (126, 363).

The experiments discussed above all implicate nucleic acid metabolism in the shoot apex in the control of flowering. It is evident that an interpretation of flowering in terms of cellular metabolism is almost within reach; yet the cellular organisation of the apex remains an important factor.

GENIC CONTROL OF FLOWERING. The arrival of the flowering hormone in the shoot apex is sometimes considered to be the trigger that allows the floral genes to act (467, 681). The resulting changes in the apical reaction system may lead to further specific genic action (630). As a result of the recent work with anti-metabolites discussed above, it is suggested that the floral genes are activated during the multiplication of DNA, a view which is compatible with the well known fact that dormant buds do not respond to the floral stimulus (681, 682).

Since histone is known to be a regulator of genetic activity in that it suppresses the ability of DNA to produce RNA (63, 261), the observation of Gifford and Tepper (197) that there was a marked decrease in histone staining in developing inflorescence apices of *Chenopodium* has evoked considerable interest. Bonner and Huang (63) have stated that this fall in histone content preceded any visible signs of differentiation in the apex and was followed by an increase in RNA content. Salisbury (468) states: "Thus it appears that turning on a gene requires removal of histone, which in turn results in production of RNA and then the enzyme controlled by the gene. The observations of Gifford and Tepper seem to indicate that floral transformation consists of turning on nearly *all* the genes by removing nearly *all* the histone, which leads to the synthesis of much RNA and many enzymes." It seems to the present writer that these interpretations of the changes occurring in the induced apex should be regarded with some caution, since they take too little account of the timing of these events, which is so important in a morphogenetic sequence and was, indeed, scrupulously reported by Gifford and Tepper (197). These authors stated that *a)* "in later stages of inflorescence development (5 SD and on) there is a marked decrease in histone staining"; *b)* "after 2 SD there is a definite increase in RNA concentration of cells in the apex . . . After 4 SD, pyroninophilia again increases . . ." (197); *c)* "On the fifth and sixth days after the initiation of a short-day regime, a primordial compound inflorescence is visible . . ." (196). Changes in RNA content and in the morphology and histology of the apical meristem thus seem to precede or to occur contemporaneously with the disappearance of histone, and it may therefore be questioned whether it is not premature to attribute to the disappearance of this substance a causal role in the evocation of the genes responsible for the early stages, at least, of inflorescence development.

XII. THE REPRODUCTIVE APEX: DEVELOPMENT OF FLOWERS AND FLORAL PARTS

Whatever the mechanism of action of the flowering stimulus, it brings about profound changes in growth and development, notably a change to

determinate growth. The initial stages of flowering have received closer investigation than any other, especially in physiological studies, but the flowering stimulus triggers off a whole series of morphogenetic processes (247). The floral apex often produces a whole sequence of organs of differing morphology in a short space of time, and in close spatial relationship. In at least some species the primordia of all these organs—leaves, bracts, sepals, petals, stamens, carpels—have been shown to have a similar origin (559). Changes in the form of these organs have been attributed to physiological changes in the apical reaction system which gives rise to them (630), but as yet too little is known about the physiology of the apex.

(a) Early Floral Development

PROLIFERATION OF THE FLORAL APEX. Various types of proliferation have been observed in members of the Cruciferae, including maintenance of vegetative activity in the floral apex (224). In *Anagallis,* also, proliferous flowers were sometimes formed in plants given a single LD of 22 hours. In some flowers only sepals were formed before reversion to the vegetative state; in other sepals, petals and stamens were formed, and a vegetative apex replaced the ovary (84). In plants given treatment with GA some days after the single LD, a type of proliferation was obtained in which the apical part of the placenta dedifferentiated and gave rise to a normal vegetative apex. It is considered that the GA forces the expression of a latent potentiality for vegetative growth (83).

PHYSIOLOGICAL CHANGES IN THE FLORAL APEX DURING ONTOGENY. Wardlaw (630) suggested that the formation of a succession of distinctive floral organs is probably related to the changing metabolism of the floral apex, somewhat after the fashion of heteroblastic development of leaves. Some evidence supporting this view has accumulated from surgical and cultural experiments on floral apices.

In an interesting experimental study of *Primula bulleyana,* Cusick (133) applied various surgical treatments to the rather complex floral apex. When young developing flower primordia of this species were bisected with a median cut, two new apices were usually regenerated. The nature of the floral organs formed by these meristems in the region bordering the incisions depended, however, on the stage of development of the flower at the time of bisection. If the flower primordium was in the pre-sepal stage when bisected, the tissue alongside the wound gave rise to the full sequence of floral parts—sepals, stamens and petals, and an ovary—and two complete flower primordia were formed. Bisections of older primordia showed that the ability to form sepals adjacent to the incision was lost during the mid-presepal stage, and ability to form stamens and petals at about the late presepal stage. Flowers regenerated after such bisections showed incomplete whorls of the outer organs, e.g., sepals, and complete whorls of later-formed parts, e.g., stamens and petals, and an ovary. Cusick considered that these results supported the hypothesis that a developing floral apex passes through a succession of physiological states that regulate the formation of each kind of organ in turn. A contemporary view

of apical organisation postulates a patternisation of metabolites that precedes the visible pattern of organogenesis (578, 619, 622), and from these experiments it was concluded that, if the incision preceded the patternisation of those metabolites associated with the formation of a particular type of organ, organs of that category could be formed in the tissue bordering the wound.

The nutritional and hormonal requirements of the whole floral apex and of its various organs have recently been investigated in an interesting study of developing floral buds of *Aquilegia* in aseptic culture (561-563). From this work it seems likely that the apex will eventually be found to require different substances during the various different stages of organogenesis. Considerable growth and development of excised floral buds of *Aquilegia* were attained on a medium containing minerals, vitamins, coconut milk, sucrose, IAA, GA and kinetin. Of these substances, GA stimulated the development of all the floral organs except stamens; petals and carpels did not undergo complete development without it. The growth of stamens and carpels was considerably influenced by the concentration of IAA. Kinetin apparently had no very specific effects but was necessary for normal development of the floral bud (563). Good growth of the floral buds was obtained on media containing appropriate concentrations of all three hormones. The sepals were found to inhibit the growth of the upper organs as soon as they completely enclosed the apex of the flower, but the reason for this is not understood (561). In other species, flowers excised at still later stages of development will give rise to normal fruits in vitro (355, 385, 394).

NUMBER OF FLORAL PARTS. The characteristic number of floral parts can be altered by various experimental treatments. In *Cannabis* low night temperatures led to a reduction in the number of parts (255). In *Anagallis* flowers with four parts were formed after treatment with 2,4-D (30). Both the number and arrangement of parts were altered in flowers of *Digitalis* treated with 2,4-D (27). A greater proportion of floral buds of *Aquilegia* grown in vitro showed a reduction in the number of carpels than buds on intact plants (561). Some cultured buds did not achieve full size of the floral apex (561), and it seems likely to the writer that the various treatments mentioned above all achieved their effect on the number of parts by reducing the size of the floral apex. Reduced numbers of floral parts, in addition to other anomalies, also characterised a radiation-induced mutant of *Pisum* (209).

DEVELOPMENT OF FLORAL PARTS. Treatment of young plants of *Cucurbita* with phenylboric acid had various effects on the development of the petals and sepals (325). In a radiation-induced mutant of *Pisum* some members of the ring of sepals were in the form of more or less reduced carpels (209).

Growth of the corolla is apparently in some way correlated with that of the androecium, as is that of the calyx with the gynoecium. For example, auxin may stimulate the growth of the two latter and suppress that of the two former (249). Plack (420) demonstrated an interesting relationship between parts in *Glechoma;* she showed that the corolla of artificially emasculated her-

maphrodite flowers was much reduced in size, and concluded that corolla growth was probably affected by a hormone produced in the anthers. Treatment of emasculated flowers with GA restored corolla size to normal, and application of GA to natural female flowers led to striking enlargement of the corolla (421).

In cultured floral buds of *Aquilegia,* also, petals do not develop beyond early stages in a medium without GA (563). In view of Plack's results, it might be suggested that the frequent failure of the petals of cultured buds of *Aquilegia* to form the spur (561) might be due to the comparatively poor development and ultimate abortion of the stamens in such buds.

(b) Sex Expression

Modifications in the degree of development, or in the whole path of development, of the essential organs in a flower may lead to changes in sex expression. Because of the biological importance of such changes, the factors affecting the development of stamens and carpels have received much more attention than have those affecting sepals and petals. Modification of sex expression in flowering plants has been reviewed by Heslop-Harrison (247), who concluded that growth of stamen and pistil primordia is probably governed by auxin concentration at the differentiating apex. In some plants temperature and photoperiod may affect the auxin balance and thus modify sex expression. Some of the literature which has accumulated since the publication of this review will be considered below.

EFFECT OF PHOTOPERIOD. In several SD plants continuous SD treatment caused a change towards female sex expression, for example, by an increase in the proportion of female (pistillate) to male (staminate) flowers in monoecious species (247). In *Rottboellia,* in which one of the flowers of the sessile spikelet at each node of the inflorescence is morphologically male and the other hermaphrodite, a longer period in LD before induction in SD led to the development of fertile anthers in both types of flower, whereas only those in the hermaphrodite flowers were fertile with a shorter period (250). This depression of maleness in environmental conditions promoting earlier flowering was thus comparable with the previous results from other SD plants.

In maize, SD treatment led to formation of the first ear at a lower node. In plants simultaneously given SD and low night temperatures inflorescences resembling the cob in organisation were formed at the stem apex, indicating a transition to femaleness (252). In the andromonoecious muskmelon LD treatment increased the number of staminate and perfect flowers (71).

EFFECTS OF GIBBERELLIC ACID. In cucumber spraying with GA led to the formation of staminate flowers at several nodes of gynoecious plants, each application affecting only two to four nodes per plant (414). Plants grown in solution cultures to which allyl trimethylammonium bromide had been added formed female flowers at nine of the first 20 nodes, whereas only male flowers were produced at the first 17 nodes in controls. GA in the culture caused

reversion from female to male flowers in gynoecious plants up to the tenth node (376). Endogenous gibberellin may thus be a factor in controlling sex expression.

In *Ricinus* the opposite effect was obtained. The percentage of pistillate flowers was much increased by gibberellin treatment, and it was suggested that GA simulated the action of LD (480). It seems possible, therefore, that, like photoperiod, treatment with gibberellin may affect the auxin economy of the plant.

EFFECTS OF AUXIN. When flowering branches of *Silene pendula,* which has hermaphrodite or monoclinous flowers, were treated by introducing NAA through the leaves, most of the anthers failed to produce normal pollen and the corollas were suppressed; calyces and ovaries both enlarged (249). In hermaphrodite flowers, therefore, the auxin level in the developing flower is apparently important in affecting the relative development of the floral parts; it does not deflect them from their characteristic developmental paths.

In cucumber the floral bud is bisexual up to a certain stage of development. Both genetic and environmental factors affecting sex expression apparently alter the relationship between the development of the floral bud and the age of the subtending leaf, which is probably controlled by the hormone balance (28). There are also genetic races of hermaphrodite, monoecious and gynoecious plants, which facilitates experimentation. In excised, potentially male, flower buds of cucumber grown in aseptic culture ovaries developed only on media containing IAA, and stamen development was arrested at an early stage. Left on the plant, these buds would have developed as male flowers. IAA can thus have a direct effect on the excised bud (186). This effect of IAA could be counteracted by GA. Even without IAA, however, very young potentially male flower buds often formed ovaries in culture. Cultured potentially female and potentially hermaphrodite buds continued their normal development and were not much affected by either GA or IAA (187). Excised buds thus react differently at different stages of development, and there are apparently quite complex inter-relationships between the various floral parts, at least in some genotypes. In this work, for example, induced development of the ovary apparently inhibited the growth of stamens. Both this work and that of Tepfer et al. (561) seem to indicate that stamen primordia have quite complex requirements for growth, which are not as yet understood.

In monoecious members of the Cucurbitaceae, e.g., *Cucurbita pepo,* in which the plant normally shows a transition from the exclusive formation of male flowers to the formation of female flowers interspersed with these, treatment with NAA led to much earlier production of female flowers. Similar effects were obtained by exposing the plants to atmospheres containing small percentages of carbon monoxide (CO) (248). In *Echinocystis* a single female flower and a raceme of male flowers are formed in each leaf axil subtending flowers; treatment with NAA preferentially stimulated development of the female flower (249). The effect of auxin here is thus on the relative development of the two types of flower.

In the dioecious species *Cannabis sativa,* however, auxin does not merely

act as a regulator of development but participates in some determining process in the developing flower (249). Female flowers were induced to develop in genetically "male" plants by treating with NAA immediately preceding the differentiation of flowers (246). Treatment with CO at an early stage of development also led to the formation of female flowers in genetically male plants (254).

In plants of *Cannabis* treated with CO, intersexual flowers were sometimes formed. These bore organs structurally intermediate between stamens and carpels. Some anthers bore stigmatic tips; in others the filaments were expanded and the basal part of the connective enlarged into placentae that sometimes bore ovules. There were also some carpel-like organs bearing pollen sacs. These effects were obtained only in floral apices which had been at a particular stage of development, just forming tepal primordia, at the time of treatment (254). Similar floral organs showing a structure intermediate between stamens and carpels were observed in *Cannabis* plants given low night temperatures (255), and also in a radiation-induced mutant of *Pisum* (209). Intermediate structures such as these are comparatively rare in plants, and these observations are of particular interest since they suggest that the processes of determination in stamens and carpels may be rather gradual.

Auxin-induced sex reversal in *Cannabis* thus seems to depend on a diversion of the ontogeny of presumptive stamens from their normal path of development to that characteristic of carpels. In this instance auxin appears to influence some determining process which occurs in the floral apex at a particular stage of development (249).

In maize a high level of auxin in the vicinity of the shoot apex at the time of tassel formation or just before this can lead to the development of an inflorescence with a structure resembling that of the cob, and also to subsequent development of pistillate flowers instead of staminate ones (252). The path of development is decided at a very early stage of floral ontogeny; Heslop-Harrison (252) has suggested a scheme in which development of the inflorescence can be deflected by external factors at various points. This is not unlike the scheme of "switch points" controlled by genes postulated for the normal development of maize by Postlethwait and Nelson (432).

There is thus strong evidence from many experiments that auxin can control sex expression, either by affecting the relative development of stamen and carpel primordia or whole male and female flowers, or by actually directing the path of development of a primordium along one of these lines.

DEVELOPMENT OF THE INFLORESCENCE OF CAREX. In some work on inflorescence development in *Carex,* Smith (490) has shown that auxin and other substances may affect sex expression in this genus as well as have a number of other interesting effects on development. In *Carex* the primordia of the stamens are not present in the female flower, nor carpel primordia in the male flower, so that the induced changes must occur at a very early stage of floral development.

Development of the inflorescence of the British species of *Carex* can be interpreted in terms of the development of two different axillary primordia,

the male flower primordium (which gives rise to male flowers) and the female spikelet primordium (which can develop either as a female flower or as a lateral spike, according to which of the two meristems present within it aborts). Each species has a typical inflorescence structure. In a number of species the prospective destiny of the lateral primordia has been experimentally modified by spraying with IAA, TIBA or kinetin. By raising the auxin concentration the proportion of female flowers was increased. Kinetin led both to the development of potential male flower primordia as female spikelet primordia, thus resembling the effect of IAA, and to the development of female spikelet primordia which would normally have given rise to female flowers as lateral spikes. Thus it had the same initial effect as IAA but directed the subsequent development of the female spikelet primordia along a different path, i.e., that of lateral spikes. A somewhat comparable effect of an applied substance has been reported in rye; in plants treated with GA individual spikelets sometimes continued to grow as small spikes (264). In *Carex panicea* treatment with kinetin led to the formation of spikelets resembling those of the related genus *Kobresia* (490). Experimental modification of the balance of growth substances in the developing inflorescences of particular species of *Carex* can thus lead to simulation of the characteristic features of the inflorescences of other species, or even of other related genera.

XIII. OUTLOOK

The emphasis of this review has been on the more recent experimental studies of the shoot apex published during the last decade. This does not, however, imply any lack of appreciation of the earlier work, which indeed forms the basis of later studies and often accurately foreshadows subsequent discoveries. It will be evident from this review, however, that experimental work on the shoot apex, both of a direct and a less direct nature, has greatly increased in volume in recent years. These extensive studies have probably stemmed from a more general acceptance of a fact stated by Sachs in his "Lectures on the Physiology of Plants" as long ago as 1887: "Nevertheless, it will already be clear to the reader (and it is one of the most important facts of the whole of the physiology of plants) that all formative processes are initiated at the growing-points . . .". This relationship between the growth and form of the shoot and physiological and morphogenetic events in the shoot apex has since been emphasised and widely illustrated by Wardlaw (612, 613) and others.

The pioneering surgical experiments of M. and R. Snow and Wardlaw, which, despite their relative crudity, yielded such fundamental information about the shoot apex, have now to some extent given place to experiments of a rather more sophisticated nature, sometimes conducted at the cellular level. The use of radioactive isotopes and of irradiation techniques, for example, has made this possible. However, fundamental information about the metabolism of the shoot apex in relation to its morphogenetic activities is still lacking, and there is a real need for the development of physiological and biochemical techniques of sufficient delicacy for use on the apical meristem. These will

be the more valuable in so far as they can be closely related to the visible morphogenetic developments.

The study of shoot development has historically been concerned with the investigation of progressively smaller entities: the mature shoot, the shoot apex, the meristematic cells of the apical meristem. Some of the evidence discussed in this review indicates that studies of nucleic acid metabolism within the cells of the apical meristem are likely to be among the most profitable of the possible future lines of investigation. These substances seem to be closely implicated in several of the many facets of shoot morphogenesis. The activities of such substances, however, must be considered in their setting: as components of the cells of the apical meristem, which is itself a part of the whole growing plant. At this stage it may be well to heed the warning of Sinnott (482): "The triumphs of biochemistry in tracing the series of changes which lead to the production of specific substances under the control of genes . . . still are far from approaching the fundamental problem of biological organization and the control of development as a whole."

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