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AUXIN CONTROL OF VASCULAR PATTERN FORMATION IN REGENERATING PEA ROOT MERISTEMS GROWN IN VITRO[1]

John G. Torrey

MUCH INTEREST has centered around the origin and determination of the precise alternate and radial arrangement of the vascular tissues in the roots of angiosperms. In an earlier paper (Torrey, 1955) an account was given of experiments with excised pea roots grown in vitro in which the usual triarch arrangement of the vascular tissues was altered following isolation of the extreme apical region of the root by excision. A proportion of half-mm. root tips grown in culture showed a reduction in vascular strand number from the triarch to a diarch or monarch arrangement. Upon prolonged elongation of such root tips, transition back to the normal triarch vascular pattern was observed. It was concluded from these studies that the vascular pattern is determined, not by inductive influences from the mature vascular tissues of the root, but rather arises as a product of the activity of the apical meristem. This conclusion has been supported by other recent experimental studies on root meristems by Reinhard (1954, 1956). It was further argued from measurements of root dimensions (Torrey, 1955) that the complexity of the vascular arrangement is related to the dimensions of the apical meristem at the time of pattern inception.

The present paper reports further experiments which bear upon the problem of vascular pattern determination—in particular, upon the experimental modification of vascular patterns by affecting the activities and dimensions of the apical meristem. It is believed that these experiments give further evidence for the view that the vascular pattern is determined in the apical meristem. The results point to a possible mechanism whereby changes in vascular tissue patterns may be controlled in intact roots by a relatively simple physiological change in the root tip.

The experiments described here follow the lead in studying the problem of vascular tissue determination given by Jost (1931-1932) who reasoned that, if the vascular pattern is determined in the apical region of the root, one might be able to modify that pattern by excising the root tip and allowing a new meristem to regenerate. In experiments with regenerating seedling roots of *Zea mays* and *Vicia faba*, Jost was able to show, in fact, that a different number of strands was formed by the regenerated tip, usually a smaller number than in the intact root, although a return toward the usual number of vascular strands often occurred beyond the regeneration zone.

Using isolated pea roots maintained in sterile nutrient medium under carefully controlled environmental conditions, similar decapitation experiments have been made and the regeneration of new root tips has been allowed to proceed in the absence or in the presence in the surrounding medium of the growth hormone, indoleacetic acid. Since it has been suggested by the work of van Overbeek (1939) and others that auxin is produced in the root apex of isolated pea roots in culture, it was thought of particular interest to determine the effect during the course of tip regeneration of externally supplied auxin. The results of these simple experiments are described.

MATERIALS AND METHODS.—The method for culturing pea roots in a sterile synthetic nutrient medium as well as the procedure followed for 0.5–mm. decapitation of the isolated roots have been described (Torrey, 1954, 1955). In the following experiments essentially the same materials and methods were used; however, in the present work, after 0.5–mm. decapitation, the root bases were maintained in culture and a study of the regeneration of new root tips under various conditions was made. The procedure may be described briefly as follows:

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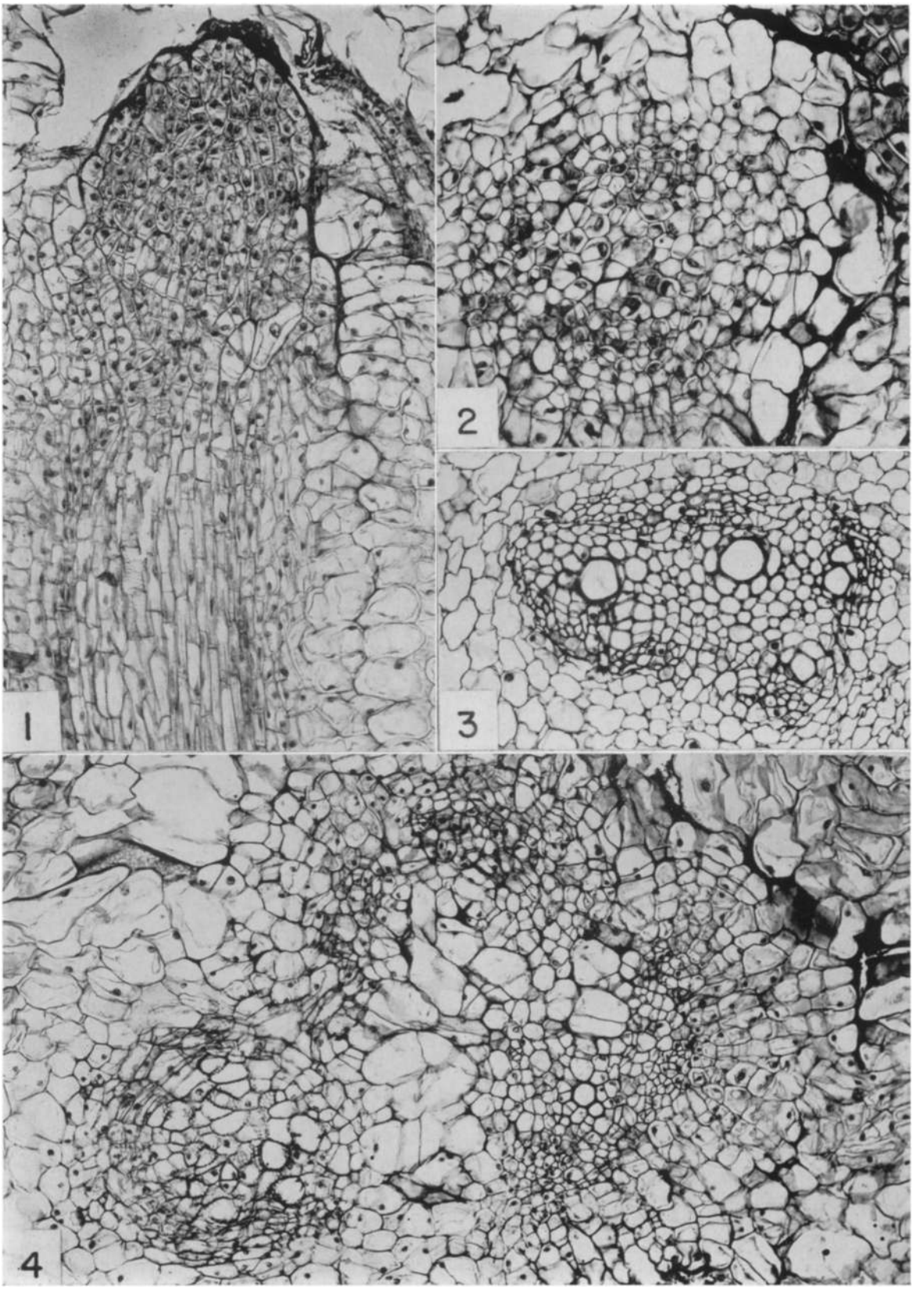
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5.0–mm. root tips were excised from sterilized seeds of the garden pea, *Pisum sativum* var. 'Alaska,' after germination for 48 hr. The excised tips were grown in the dark at 25°C. in a synthetic nutrient medium on agar for one week to an average length of 55–60 mm. The terminal 0.5–mm. tips were excised under a dissecting microscope equipped with an ocular micrometer and were discarded. The remaining decapitated root bases were used as the experimental material. The excised tip included the root cap, the apical meristem and a portion of the procambial cone proximal to the apical initials as described earlier (Torrey, 1955). At the level of decapitation, the concentric pattern of the central cylinder was observable in sectioned material, the triarch pattern of the enlarged future metaxylem elements was evident and the first immature protophloem elements had been cut off by division of the protophloem mother cells, as has been described in detail (see fig. 2, 2a; Torrey, 1955).

In preliminary experiments, regeneration of the decapitated roots was allowed to take place under various conditions. Whole root bases (averaging 55 mm. in length) or root segments 3, 5 or 10 mm. long, cut from the tip end of the cultured roots after half-mm. decapitation, were cultured on the surface of agar nutrient medium in Petri dishes or were floated in liquid nutrient medium. Under all these conditions, regeneration proceeded at much the same rate and was apparently unaffected by the morphological and environmental differences. As a routine procedure, therefore, half-mm. tips were removed from one week old roots; then further 10–mm. segments were cut from the apical end of the roots after decapitation. These segments were transferred to freshly prepared nutrient agar medium where regeneration of tips was allowed to occur. Subsequent elongation of the newly regenerated roots was allowed to proceed in the same or in different media for various periods of time. The "control medium" used for culture of the root segments during regeneration was the improved medium reported by Torrey (1954) modified to include only 4 per cent sucrose. The "auxin media" contained indoleacetic acid (IAA) at different concentrations, which had been added after cold Seitz-filtration to the autoclaved control medium.

At the end of the experimental period, whole roots were killed and fixed in formalin-acetic acid-alcohol with aspiration. The roots were dehydrated in an ethyl-butyl alcohol series, embedded in "Tissue-Mat" and sectioned on a rotary microtome at 8 μ. Serial sections were mounted on large slides to facilitate the study of the large number of sections involved and were stained with Heidenhain's hematoxylin and safranin. Length measurements were made by counting the number of sections and multiplying by the section thickness.

Previous studies of regeneration of decapitated roots.—Regeneration of the tip of decapitated roots was studied in seedling roots of a number of different genera by Simon (1904). His ingenious experimental studies were followed by careful anatomical analyses. They showed clearly that complete regeneration of the tip occurs, provided the decapitation does not remove too large a proportion of the procambial tissue proximal to the apical initials. Thus in *Zea mays* the primary root tip would regenerate completely in 3 days if 0.5–0.75 mm., including root cap, were excised, but only partial regeneration would occur if 0.75–1.0 mm. were removed. During complete regeneration, the peripheral procambial tissues (i.e., the future pericycle) were particularly active in forming the new meristem, with only the tissues of the central cylinder contributing to the new meristem; in partial regeneration cell divisions in the region of the future pericycle were less frequent and not oriented to form an organized meristem. Rather, several meristems were formed, so that regeneration was difficult to distinguish from lateral root formation. By destroying the incipient pericycle of decapitated roots of *Zea mays* or *Vicia faba* with fine glass capillaries, Simon showed that no regeneration of the tip occurred. Destruction of the tissues of the cortex or the center of the central cylinder did not prevent complete regeneration of the root tip. In his studies, Simon did not follow the subsequent pattern formation by regenerated tips.

Němec (1905) in an extensive treatise on regeneration phenomena in wounded and decapitated root tips of a number of different plant species also found that the capacity of roots to regenerate new tips depended largely upon the amount of tissue destroyed in the apical region. He reported multiple tip formation following splitting of root tips.

Jost (1931-1932) confirmed in detail the studies of Simon (1904) and reported further experiments on decapitated roots of *Zea mays* and *Vicia faba* in which particular attention was paid to the number of vascular strands formed in the regenerated root. In *Vicia* it was found that complete regeneration

Fig. 1–4. Regeneration of new tips by decapitated pea roots cultured on control nutrient medium (lacking auxin) for various periods of time.—Fig. 1. Approximately median longitudinal section through a regenerating tip after 3 days. ×175.—Fig. 2. Transverse section of a regenerating tip after 1 week, cut in the region of the apical initials. ×350.—Fig. 3. Transverse section of a regenerated root after 4 weeks, cut in a region of mature vascular tissues showing a disorganized vascular tissue arrangement. ×350.—Fig. 4. Transverse section of a regenerated root after 2 weeks, cut through mature vascular tissues, showing the divergent orientation of regenerated meristematic areas centering around the 3 original xylem strands. A diarch root tip which was continuous with the strand at the lower right persisted as the dominant root axis. ×175.

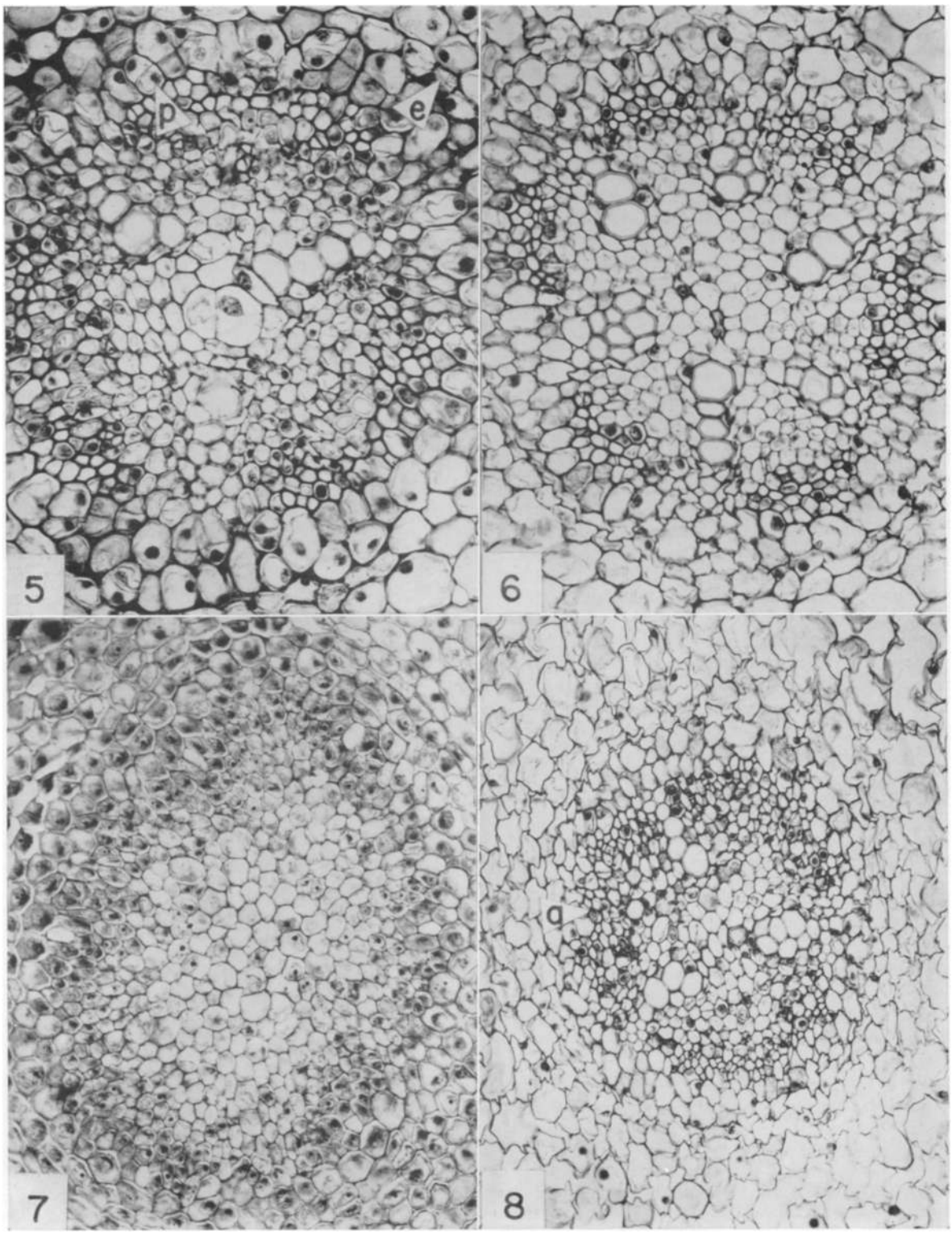


Fig. 5–8. Transverse sections showing the central cylinder region of regeneration of decapitated pea roots cultured for 8 days on auxin-containing nutrient medium. All ×350.—Fig. 5. Section cut in region of the original triarch root base near the decapitation level. The 3 original vascular strands are evident. Note the pericyclic proliferation (*p*), the rupture of endodermal cells (*e*), and the darkly staining materials occurring in the intercellular spaces surrounding the endodermis (Root 2, table 1).—Fig. 6. Same root as fig. 5 with section cut approximately 2 mm. more distal, showing the

of the root occurred if up to 0.8-mm. of the root tip were removed. In certain cases a reduction in vascular strand number was observed in the regenerated roots.

Regeneration of decapitated pea roots in control medium.—On the control nutrient medium, regeneration of new tips after decapitation of 0.5-mm. tips proceeded quite rapidly, usually requiring about 3 days for formation of a new macroscopically-visible growing point to form. Histological analyses of a number of these newly formed tips at various stages of their formation showed that there was considerable variation in the regeneration response. In general, two types of regeneration were evident. In the first type of response observed, regeneration of the tip proceeds from tissues of the central cylinder. Rapid cellular divisions in the procambial tissues of the root result in the formation of a rounded mass of meristematic cells which projects from the cut surface, aligned in the direction of the old tip. The pericycle appears to contribute most actively to the meristematic mass, the central tissues of the procambium less actively. Although cell divisions occur in the cortical tissues, these soon cease and do not contribute to the new meristem. In fig. 1 is shown in approximately median longitudinal section a regenerated root tip 3 days on control medium after decapitation. Some organization is already apparent in the new apical meristem which is aligned in the axis of the root. In fig. 2 is shown a transverse view of a newly regenerated meristem one week after decapitation. The section was cut in the region of the organizing meristem, immediately proximal to the region of apical initials. Again the origin of the new meristem from procambial tissues is evident; the meristem shows no contribution from the cortical tissues, which appear to be separated from the newly formed root by enlarged cells and crushed tissues. In subsequent development, a completely organized apical meristem is formed, a root cap is produced and the new root elongates. Such roots show only slight morphological discontinuity between the old root base and the newly regenerated root.

Regenerated roots of this first type manifest internal differences as concerns the continuity of the vascular tissues. In 5 roots studied in detail in transverse serial sections, there was found almost complete continuity of the triarch vascular pattern between the old and the new tissues. In 13 roots of the first type studied in sections, the vascular pattern of the newly formed tissues was triarch, but showed no precise relationship or continuity with the pre-existing triarch vascular pattern of the decapitated root. Thus the new vascular pattern formed was unrelated to the old pattern, although it was triarch. Usually in such roots, the region of regeneration showed a disorganized vascular tissue arrangement which, however, effectively bridged the region from the organized tissues of the old root to those of the new tip. In fig. 3 is shown in transverse section the mature central cylinder of a root in such a region where no well-organized pattern of vascular tissues can be seen. In some regenerated roots, such nondescript vascular arrangements could be traced all the way into the meristem.

The second type of regeneration response in the control medium was even more variable and difficult to assess. In this type of regeneration, no terminal meristem was produced, but rather the regeneration of new tips was by lateral root formation. These new meristems were formed from the pericycle, usually opposite the protoxylem points of the decapitated root in the same manner as lateral roots are formed normally. These meristems were oriented initially at right angles to the main axis of the root or at an angle slightly less than 90°, pointing toward the distal end of the root. Usually one such lateral root assumed the dominant position during regeneration, became oriented in the direction of the main axis of the root and finally substituted for the decapitated tip. In 4 roots studied, two or three lateral roots developed and elongated about equally. In 15 other cases of this type, a single lateral root persisted from the regeneration region to continue the main axis of the original root. In fig. 4 is shown in transverse section a regenerated root cut distal to the level of original decapitation. Three meristematic areas are evident, each centering around the xylem strands of the original triarch root. The oblique orientation of the regenerating lateral root at the bottom left is clear. A diarch root, organized from the regenerating region at the lower right persisted and became oriented along the axis of the original root, forming the new regenerated tip. In 8 other cases, regeneration involving lateral root formation was even less precise and little or no organized vascular pattern continuity could be found between old and regenerated root, although some continuity between vascular tissues was always found.

Thus, regeneration of decapitated root bases in the control medium shows a wide range of variation. The two general types of regeneration response described have been separated into types arbitrarily; they fit into the range of regeneration phenomena described by Simon (1904), Jost (1931-1932), and others. Whether or not regeneration

complete symmetrical hexarch vascular tissue arrangement.—Fig. 7. Same root as fig. 5–6, cut in the apical region at the level of first maturation of the protophloem elements, showing the hexarch vascular tissue arrangement.—Fig. 8. Section of root in the region of transition from the triarch vascular tissue arrangement to the hexarch pattern, showing the original triarch pattern and the first mature xylem element (to the right of *a*) of a new vascular strand, the fourth strand, in the course of differentiation (Root 7, table 1).

involves the formation of a new meristem in place of the old or the initiation of lateral roots which in turn assume the function of the removed tip probably depends upon the amount of damage at the decapitation surface. If little damage occurs to the procambial tissues, a new meristem is formed; if extensive tissue damage occurs, new meristems (lateral roots) of quite different initial orientation are formed. The activity of the pericycle is largely responsible for the regeneration in either case. When more extensive tissues are excised from the growing root, lateral root formation always occurs (Torrey, 1951). Following decapitation, even of 0.5-mm. tips, apparently the root has lost, temporarily at least, the capacity to produce an organized vascular pattern in the main axis, a capacity which is restored only when the newly organized meristem is fully functioning.

Regeneration of decapitated roots in IAA medium.—In sharp contrast to the rather irregular regeneration phenomena which occur in the decapitated pea roots grown in control medium, is the type of regeneration which occurs when decapitated roots are cultured in a control medium to which has been added the natural growth hormone, indoleacetic acid, at concentrations which effectively inhibit normal root elongation.

Regeneration in the presence of indoleacetic acid at 10^{-5} M is quite precise and regular. Superficially, it resembles the first type described above in which a new meristem is rapidly organized from the procambial cylinder with cell divisions in the pericycle largely contributing to the new meristem which is oriented in the position of the old tip. Regeneration takes place at about the same rate as in the control medium. The regenerated root elongates only to a limited extent due to the inhibition of extension growth by the auxin in the medium. In no case was regeneration in the auxin medium of the second type, i.e., by lateral root formation. In 37 roots studied in detail from 8 different experiments conducted at 5 different times over a period of 3 years, essentially the same result was observed.

In describing the regeneration in auxin medium, it is most convenient to trace the course of development of the regenerating root fixed one week after decapitation, by following in serial sections the changes in the tissues from the normal triarch base of the decapitated root to the tissues of the newly regenerated root.

In the mature tissues of the decapitated root base, the usual triarch vascular pattern is evident. In sections progressively closer to the new root, but still within the old root tissues, the first evidence of response to the decapitation is a marked activity in the pericycle, especially opposite the protoxylem elements (fig. 5). The pericycle cells at these points elongate radially and then undergo a series of periclinal divisions, resulting in as many as 4 or 5 new cell layers. Progressively more distal, the wave of mitotic activity in the pericycle proceeds circumferentially in either direction from opposite the protoxylem points until the entire pericycle becomes 4-5 cell layers in thickness. During this proliferation, the endodermis lying immediately external to the pericycle is disrupted, becomes necrotic and may be crushed, especially opposite the xylem strands, where pycnotic nuclei are evident and darkly staining materials are seen in the crushed endodermal cells themselves and in the intercellular spaces between the endodermis and the pericycle. At this level the triarch arrangement of the mature vascular tissues is typical of the decapitated root itself (fig. 5), with three primary xylem strands possessing incompletely matured metaxylem tissues in the center of the root, alternating with extensive phloem bundles with mature phloem fibers.

Still more distal to the region of pericyclic proliferation, one passes almost abruptly into the new root tissues. The abnormalities in the pericycle disappear and, within a few hundred microns, one finds the strikingly different vascular pattern of the newly regenerated root. Instead of the triarch vascular arrangement of the root base, one sees a symmetrical *hexarch* vascular pattern (fig. 6). The original triarch strands are evident in essentially their original positions, but three new strands of xylem have been interposed and six phloem bundles now alternate in precise fashion with the six xylem strands. In roots maintained on the auxin medium, this hexarch pattern can be traced distally into the meristem region itself (fig. 7), where the hexarch pattern is clearly visible in the arrangement of the first protophloem elements. Thus, there has been effected a remarkable change in the vascular pattern, a doubling of the normal triarch arrangement, which is a predictable response in these regenerating roots to the auxin treatment.

The course of the striking change in vascular pattern occurs in all roots studied in essentially the same manner. Immediately distal to the region of pericyclic proliferation is a short length of root tissue, perhaps 500 μ, which appears to have the normal triarch arrangement of vascular tissues; this root length is apparently the first product of the regenerating meristem, although the fact cannot be ascertained with assurance from the sectioned material. Here, a normal pericycle is active, but phloem fibers have disappeared from the phloem bundles. The new strands arise de novo suddenly, each at a point midway between existing strands, on the inner side of each phloem area—the usual site for the initiation of the vascular cambium. In fig. 8 is seen in transverse section the earliest evidence of the changing vascular pattern: the appearance of a single mature xylem element in the new, —the fourth,—xylem strand. The single mature element is surrounded externally by primary phloem tissue and internally by phloem and/or interstitial parenchyma. Within a few hundred microns, simi-

lar strands, each consisting first of a single xylem element, are formed in each phloem area, so that a hexarch pattern becomes evident. To each strand is added by centrifugal maturation additional xylem elements that separate the phloem areas equally and establish the regular alternate and radial hexarch pattern.

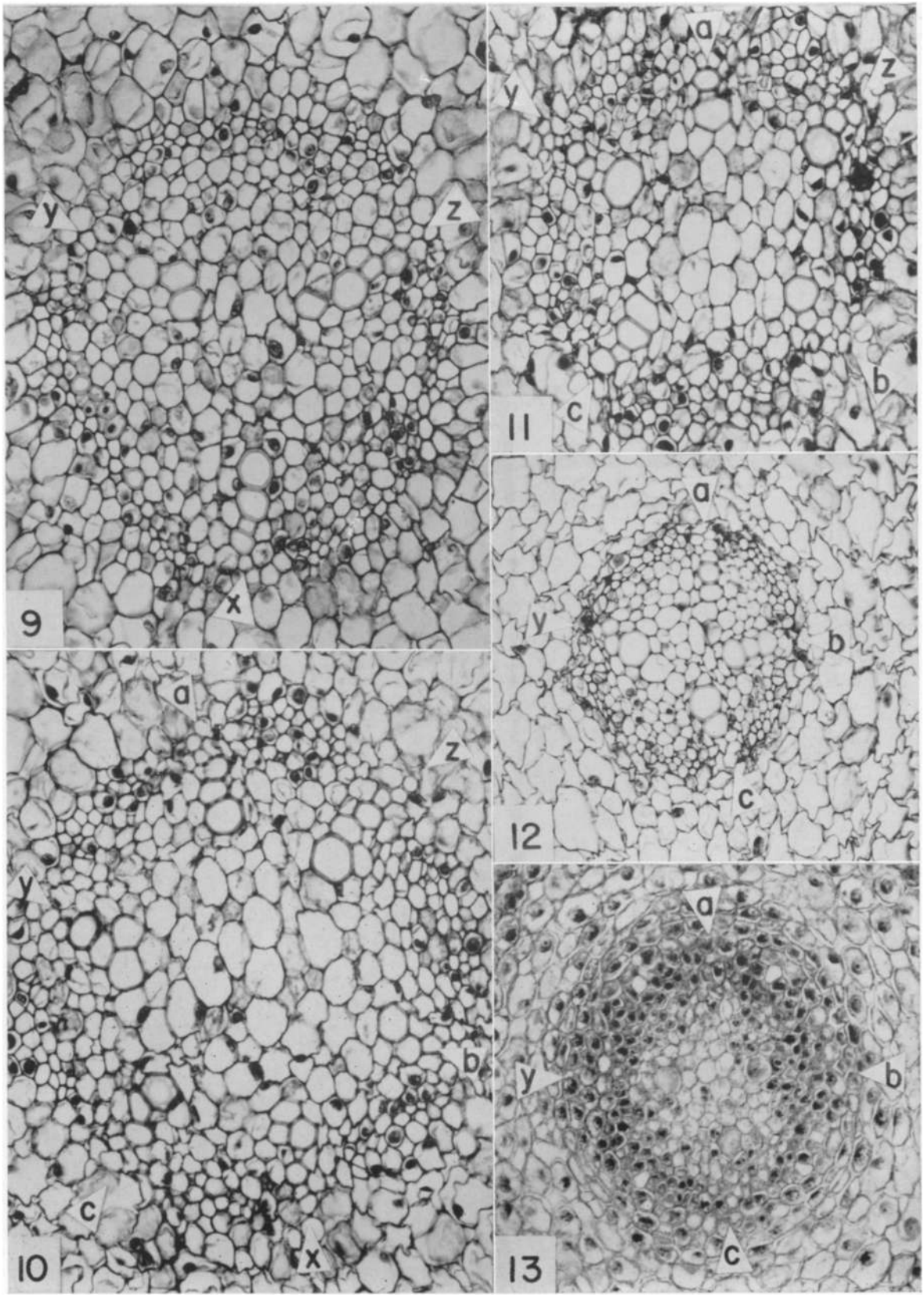
The appearance first of the new xylem in the midst of existing phloem tissue and the rearrangement of the new vascular pattern by subsequent differentiation of separate phloem bundles represents the same sequence of tissue differentiation as one finds at the meristem where, as has been pointed out by Bünning (1951), the initial orientation of the differentiating xylem elements establishes the whole subsequent pattern of vascular tissue differentiation. It should be noted that there is no mutual incompatability between xylem and phloem differentiation here, since xylem elements are not inhibited from differentiating in the midst of phloem tissue. Subsequently, however, once the new xylem strands are formed, the new phloem tissues assume a position at the usual distance from the xylem strands.

The transition from triarch to hexarch pattern occurred in different roots within a length of as short as 200 μ or as long as 2000 μ. Sometimes the new strands were composed of two rows of xylem elements (fig. 6) whereas the original strands may have been only uniseriate. It was not possible to establish whether the blocking out of the new strands, i.e., their earliest differentiation, was centrifugal, although their maturation clearly was. As in the normal xylem strands, the xylem elements of the new strands show a gradient in size from the smallest elements at the periphery to the largest elements toward the center of the root. The resulting hexarch pattern of vascular tissues is strikingly symmetrical and normal in appearance. In its overall diameter the mature hexarch root shows little or no increase over that of the triarch root base (compare fig. 5 and 6).

It should be emphasized that the meristem itself produces the hexarch pattern, as can be seen by examining sections of the meristem of the regenerated root at the site where the pattern is blocked out. Thus the meristem itself has been changed from one producing a triarch arrangement (the old meri-

TABLE 1. *The effects of duration and concentration of IAA-treatment on vascular pattern formation in regenerated roots of half-mm. decapitated pea roots grown in vitro. Roots are arranged approximately according to increasing final length. Data are taken from four separate experiments.*

Treatment				Vascular Pattern (Percent of total length)		
IAA conc. (M)	Time in Days	Root No.	Total length of root (mm.)	Hexarch	Pentarch	Tetrarch
5×10^{-6}	8	1	3.5	100	0	0
		2	4.8	100	0	0
		3	4.9	100	0	0
		4	5.2	100	0	0
		5	5.9	100	0	0
		6	6.7	100	0	0
		7	8.9	100	0	0
		8	6.7	42	14	44
5×10^{-5} trfd to	7	9	5.3	22	24	54
10^{-5}	7	10	8.2	50	0	50
10^{-5} trfd to	7	11	6.1	63	37	0
5×10^{-5}	7	12	7.3	0	23	77
10^{-4} trfd to	7	13	8.3	44	10	46
10^{-5}	7	14	9.4	41	59	0
10^{-5} trfd to 10^{-5}	7 7	15	10.1	0	53	47
10^{-5} trfd to control	7 3	16	12.9	7	9	84
		17	14.5	37	15	48
10^{-5} trfd to control	7 7	18	20.9	11	22	67
		19	23.3	0	9	91
		20	30.1	4	4	92
		21	38.3	4	0	96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stem) to one producing the hexarch pattern (the meristem regenerated in the presence of auxin).

So long as the regenerated root is allowed to continue its slow elongation in the auxin medium, it continues to be a hexarch root. Regenerated hexarch roots 6-8 mm. long have been grown routinely in the 7-8-day period following decapitation (see table 1).

In the 37 roots analyzed in serial sections following regeneration in auxin medium, 75 per cent of the roots were of the hexarch pattern as described above. Four roots were studied in which a pentarch pattern was formed, i.e., only two new strands were formed. In 3 other roots, tips were regenerated which produced the usual triarch pattern which was continuous with that of the root base. In these roots, pericycle proliferation was evident in the region of the decapitation, but the conditions leading to the usual hexarch pattern did not develop.

The effect of auxin concentration on pattern formation.—What happens to the vascular pattern when the hexarch roots are transferred to auxin-free medium and are allowed to continue their elongation? Table 1 summarizes the results of a series of experiments in which roots which had regenerated and elongated in auxin medium for 7-8 days were transferred for periods of 3 or 7 days to auxin-free medium or to medium containing auxin concentrations other than that in which regeneration had occurred.

In the auxin-free medium, root elongation becomes much more rapid than in the auxin medium. In the absence of an external supply of auxin, there is a clear tendency of the regenerated root meristem to produce fewer vascular strands. One can usually trace in serial sections the rapid or gradual dropping-out of vascular strands with a reduction to pentarch, thence to tetrarch and in one clear case to the triarch pattern typical of pea roots. Most of the roots studied, e.g., roots 8-10, 12, 13, 15-21, which were of various lengths, appeared to stabilize at the tetrarch arrangement. In fig. 9-13 in a single root (root 18, table 1) is seen the appearance of the central cylinder at different levels, beginning in the triarch base of the original root (fig. 9), the hexarch arrangement of the tip regenerated in auxin (fig. 10), then the mature pentarch (fig. 11) and tetrarch arrangements (fig. 12) resulting from subsequent growth in auxin-free medium. The meristem itself at the time of fixation of the 20-mm. root was tetrarch as is seen at the level of the first protophloem maturation (fig. 13) and had been forming a tetrarch root for 13.7 mm., a large proportion of the elongation in the auxin-free medium. All figures are at the same magnification and it is apparent judging from the mature structures, that although the hexarch arrangement shows a vascular cylinder diameter slightly larger than that of the triarch base, the mature tetrarch root shows a diameter considerably smaller than the triarch base. The size of the mature structure is no absolute guide to the vascular complexity. At each level, symmetrical vascular arrangements were formed.

From table 1 it is evident that pentarch roots likewise tend to revert toward the simpler vascular arrangement (roots 12, 15, 19). Also it is seen that hexarch roots may revert directly to the tetrarch condition by dropping out two strands (roots 10, 21).

The auxin concentration during the regeneration period appears not to be critical, so long as it is strongly inhibitory to root elongation. Thus, hexarch roots were formed when regeneration was allowed to proceed for one week either at 10^{-4}, 5×10^{-5}, 10^{-5}, or 5×10^{-6} M IAA. In other experiments, 10^{-6} M IAA or more dilute concentrations did not elicit this response; rather, roots regenerated in the random fashion described for the control medium. From table 1 it also becomes evident that so long as roots are maintained in the high auxin concentration in which regeneration has occurred, they will retain the more complex vascular arrangement. Transfer of the regenerated roots to lower auxin concentrations or to auxin-free medium permits the reduction in vascular complexity to occur. Similar roots which were placed in auxin medium following the decapitation of 1.0-mm. tips instead of 0.5-mm. tips were unable to regenerate new tips, but erratically formed lateral roots which were inhibited in their elongation.

Two important facts concerning the continuity of the vascular strands are notable. First, during regeneration in the presence of auxin the three vascular strands of the original root base are almost always perpetuated in the same position in the new root. Thus in the roots shown in fig. 5-6 and 9-10 the three original strands can be clearly distinguished in the hexarch roots. This fact implies that

Fig. 9–13. Transverse sections showing at several levels the central cylinder tissues of a 0.5–mm.-decapitated pea root allowed to regenerate a new tip for 7 days on an auxin nutrient medium (10^{-5} M IAA) and then grown on control medium for an additional 7 days (Root 18, table 1). All ×350.—Fig. 9. Section showing mature tissues of the triarch vascular tissue arrangement of the original root with xylem strands marked *x*, *y* and *z*.—Fig. 10. Section cut at 305 μ distal to fig. 9 showing symmetrical hexarch vascular tissue arrangement. New xylem strands are marked *a*, *b*, and *c* in the order of their appearance.—Fig. 11. Section cut at 2585 μ distal to fig. 9 showing symmetrical pentarch vascular tissue arrangement. Strand *x* has disappeared.—Fig. 12. Section cut at 7115 μ distal to fig. 9 showing symmetrical tetrarch vascular tissue arrangement. Strand *z* had disappeared.—Fig. 13. Section cut at 20890 μ distal to fig. 9 showing tetrarch vascular tissue arrangement in the meristem at the level of the first protophloem maturation. Strands *y*, *a*, *b*, and *c* persist.

under these conditions of regeneration the older tissues do, in fact, influence the location of the vascular tissues of the new root. Each new strand then originates in a position midway between two original strands. Thus, although an entirely new pattern is formed, the orientation reflects that of the vascular tissues in the old root base.

The second important fact concerns the disappearance of strands following the transfer of the roots to media of reduced auxin concentration. Usually one or more of the strands which drop out belong to the original triarch pattern. In the root illustrated in fig. 9-13, strands *x* and *z* (fig. 10) have disappeared in fig. 11 and fig. 12, respectively. The final tetrarch pattern includes only one strand of the original triarch pattern. In certain roots which reverted rapidly from hexarch to tetrarch, the newly formed strands are the ones which disappeared with the original strands persisting. In either case, the newly-formed hexarch pattern does not continue to determine by induction the pattern formed by the new root meristem. Pattern formation is influenced rather by the physical-chemical environment which surrounds the meristem at the time the pattern is blocked out.

DISCUSSION.—By the simple expedient of adding a single growth hormone of known chemical composition to the medium surrounding a root regenerating a new tip, it is possible to change profoundly the fundamental tissue arrangement of the root, an arrangement which seems to be normally under genetic control so constant is its pattern. What is the mechanism of action of the added auxin that can so fundamentally modify the course of development?

The auxin in the medium must affect the formation of the new meristem in some way that changes the capacities of the meristem with respect to the blocking out of the vascular pattern at the time of its inception. It is a well known fact that externally supplied auxin when applied to the roots of intact seedlings, while not changing the vascular pattern, does stimulate increased cell divisions, especially in the pericycle, resulting in radial rows of new cells (cf. Bond, 1948; Torrey, 1951). According to Levan (1939) auxin-treated roots actually show a major reorientation of the axes of cell divisions, especially in the pericycle, from the longitudinal to the radial direction. In regenerating root tips, the pericycle plays the predominant role in forming the new tip. In the presence of added auxin, the newly formed meristems are prevented from elongating rapidly; a larger proportion of cell divisions are oriented in the radial direction than without added auxin so that the meristem is proportionately larger in the radial dimension than the pre-existing meristem. The tendency toward increase in diameter is evident also in portions of the old root where pericyclic proliferation actually leads to disruption of the endodermis and crushing of tissues of the cortex.

On the assumption that auxin does in fact act to increase the proportion of cell divisions in the radial direction in the new meristem during its formation causing an increase in its radial dimensions, one can propose a theory to explain the basic mechanism for the control of vascular pattern changes such as occurred in the experiments described. Evidence has been presented (Torrey, 1955) which suggests that the complexity of the vascular pattern is directly related to the diameter of the procambial cylinder at the level at which the blocking-out of the vascular pattern occurs, i.e., with an increased procambial cylinder diameter at the level of pattern inception, there is an increase in the number of vascular strands. It is postulated that the presence of relatively high concentrations of auxin in the medium directly affects the dimensions of the regenerated apical meristem, causing an increase in the radial dimension of the meristem at the level where vascular patterns are blocked out. According to the concept of Thoday (1939), the unitary vascular strand, consisting of xylem and phloem tissue, has itself a rather stable dimension, varying within rather narrow limits. An increase in the dimensions of the procambial cylinder, under auxin control, will allow an increase in the number of vascular strands that can be initiated. The auxin apparently does not influence directly the processes controlling the vascular strand formation but affects only the rates and/or orientation of cell divisions which determine the dimensions of the pattern-forming tissue system. Thus, by simply increasing the proportion of cells dividing in the radial direction in the meristem, auxin influences all subsequent steps in pattern formation and fundamental tissue patterns are modified. About the factors determining the dimensions of the "unitary vascular strand" and the precise radial and alternate arrangement of xylem and phloem, there is no evidence from these experiments.

Direct evidence to show that the effect of auxin in the medium is to increase the relative dimensions of the procambial diameter at the site of pattern formation by affecting the orientation of cell divisions is not easily obtained. Furthermore, it is clear that absolute procambial dimensions per se do not determine the pattern, which depends rather upon the complex relationship between the diameter of the procambium and the three-dimensional conical structure of the root tip as a whole. Under the conditions of these experiments, the diameters of the procambial cylinder of hexarch roots at the level of pattern inception (e.g., 210, 215, 235, 260, and 310 μ) were consistently greater than comparable measurements of tetrarch roots (135, 155, 155, and 170 μ). This contrast is evident when comparing fig. 7 and 13. However, too few data are available from these experiments to give convincing proof on

this point. Nevertheless, the observations of the present experiments, as well as those on pattern formation in excised 0.5-mm. tips (Torrey, 1955) fit into the theory proposed. The reduction in vascular complexity observed in isolated 0.5–mm. tips in culture might be related to reduced auxin levels following excision of the tip from the root.

In intact seedling roots of peas grown under normal conditions, it is indeed remarkable that the vascular pattern is so stable since, under experimental manipulation, it can be shown that the apical meristem is an extremely plastic system with a wide range of potentialities for vascular pattern formation following surgical or chemical treatment. The triarch pattern, one might say, is "genetically-fixed," so characteristic is it of the primary root of the seedling. The "genetic" control of triarch vascular pattern formation centers, however, not in direct genic control of the determination of the pattern itself, but rather in control of the balance of the physical-chemical conditions within the root apex which in turn control the dimensions of the procambial tissues where blocking-out of the vascular pattern occurs. Thus a physiological homeostasis in intact pea roots produces a tissue system wherein the triarch pattern is normally produced. Upsetting this balance by tip excision and auxin treatment produces a new physiological condition in which a different pattern may be expressed. The stabilizing of the vascular pattern of the experimental roots discussed above at the tetrarch arrangement suggests a more or less permanent shift in the physiological system toward conditions in which the more complex pattern replaces the normal triarch pattern.

It is tempting to suggest that one major component in this physiologically balanced system is the auxin produced by the apical region of the growing root itself. About the natural formation and role of auxin within the elongating root we have regrettably little knowledge. van Overbeek (1939) has demonstrated that auxin is produced by the apical 10-mm. tips of isolated pea roots during elongation in vitro, although the site of this production in the root is not established. The terminal few millimeters of the growing root usually show the highest auxin concentration upon extraction (e.g., Thimann, 1934). It is conceivable that the relatively high concentration of endogenous auxin in the apical region influences the dimensions of the procambial cylinder and thereby acts as a controlling factor in the determination of the vascular pattern formed. Under experimental conditions in which the normal auxin gradient is upset or eliminated such as following isolation of the tip itself or removal of the tip and substitution of a different auxin gradient, the vascular pattern can be readily affected. Recently, Samantarai and Sinha (1957) observed that induced adventitious roots on isolated leaves of *Ipomoea* and several other plant species showed an increased number of vascular strands following auxin treatment and that strand number was directly correlated with increasing auxin concentration. They concluded that auxin together with certain nutrients, notably sugar and nitrogen, are involved in the control of vascular patterns in these roots. The observation of Flaskämper (1910) that removal of the cotyledons from seedlings of *Vicia faba* resulted in retarded root elongation and a reduction in vascular strand number might be interpreted in terms of reduced supply of metabolites moving to the root tip resulting in depressed auxin formation in the region of the root meristem. Similarly, in nature there may occur conditions that would affect the auxin production of the growing root tip, thereby leading to such changes in vascular pattern as have been observed occasionally (cf. Torrey, 1955). The theory presented here is subject to further experimental testing. Its establishment or rejection could be a first step in understanding vascular pattern formation in roots in specific physicochemical terms.

SUMMARY

Isolated pea roots grown in sterile nutrient medium were subjected to 0.5-mm. decapitation including root cap; the decapitated roots were allowed to regenerate new meristems and then elongate in the absence or presence of the auxin, indoleacetic acid. Regeneration of new meristems proceeded at about the same rate in the different media with regenerated tips visible 3 days after decapitation. Roots were fixed, sectioned, and histological studies were made of the regeneration responses with particular attention to vascular pattern formation. Two general types of response in the control medium were seen: (a) regeneration of a single meristem from the tissues of the central cylinder with slight or extreme discontinuity of vascular tissue pattern from the triarch base into the new root; (b) regeneration by lateral root formation with one lateral root changing orientation to substitute for the excised tip. Regeneration in auxin media at concentrations of 5×10^{-6} M or higher was strikingly different. A single meristem was always regenerated from the central cylinder tissues and complete tissue continuity with the original root structure was evident. However, the new roots showed a symmetrical *hexarch* vascular pattern so long as they continued to elongate in the auxin medium. Hexarch roots transferred to auxin-free medium reverted back toward the original triarch pattern characteristic of pea roots. Transfer of roots to intermediate auxin concentrations after regeneration produced pentarch or tetrarch roots. The origin of the hexarch condition from triarch roots and the transition back from the hexarch toward the less complex patterns are described. Usually during the transition one or more of the strands which disappeared were of the original triarch pattern. It is proposed that the auxin in the medium influences

the radial dimensions of the new meristem during the course of tip regeneration, resulting in a larger procambial cylinder at the level where the vascular tissue pattern is first blocked out. It is further suggested that in intact plants the endogenous auxin, produced in the region of the root meristem, may control the dimensions of the root meristem by its actions on cell division and cell enlargement and thereby determine indirectly the vascular tissue pattern.

DEPARTMENT OF BOTANY,
UNIVERSITY OF CALIFORNIA,
BERKELEY 4, CALIFORNIA

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SALINITY OPTIMA FOR MARINE FUNGI AFFECTED BY TEMPERATURE[1]

Don Ritchie

FUNGI HAVE been long recognized as organisms capable of thriving in such improbable and inhospitable habitats as acid baths, refrigerators, brines, and variously "poisoned" surfaces. On man and his animals, and on his food and possessions, the number of mycological free-loaders is known to be countless, but in the sea, that reservoir of biological types, the fungi are generally so inconspicuous that biologists have not generally thought of them as common components of the marine biota.

Having found that filamentous fungi are commonly present in the oceans touching the Isthmus of Panama, and having satisfied myself that the organisms cultured out were able to grow and reproduce under simulated marine conditions, I set out to find what environmental variation could do to the growth and form of these plants.

Since the organisms were isolated from the littoral zone, they could have been subjected to changes in salinity due to efflux of fresh water from streams or to drying of tidal pools. They could also be subjected to temperature changes, even though in the immediate region where they were collected, the variation could never have been great. These two variables, then, temperature and salinity, and their combined effects upon the growth rates of several marine fungi are the subject of the present communication.

MATERIALS AND METHODS.—The fungi used were obtained from pine panels submerged in the sea as previously described (Ritchie, 1954). A species of *Phoma* closely resembling *P. herbarum* West. in morphology, and one of *Pestalotia,* similar to *P. aletridis* (Pat.) Guba, were from Limon Bay, Panama. *Lulworthia medusa* var. *biscaynia* Meyers was collected by Dr. S. P. Meyers in San Juan, Puerto Rico.

The plants were grown on agar at pH 7.0–7.5, containing 0.5 per cent glucose and 0.1 per cent yeast extract, made up with either natural sea wa-

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