THE EFFECT OF CERTAIN METABOLIC INHIBITORS ON VASCULAR TISSUE DIFFERENTIATION IN ISOLATED PEA ROOTS [1]

John G. Torrey

THE SEQUENCE of events occurring during cellular differentiation may be described and, to some degree, understood in terms of manifest structural changes which the individual cell undergoes during its ontogeny. Yet little is known concerning the biochemical processes in plant tissues which are fundamentally related to these ontogenetic changes.

Isolated pea root tips, cultured in the dark in a sterile synthetic nutrient medium, grow in a reproducible manner, showing usual patterns of tissue differentiation. Inhibition of elongation in cultured roots may be produced by a variety of metabolic inhibitors which act through their effect on biochemical processes essential for normal root growth. This paper describes the effects of three inhibitors, indole-3-acetic acid, iodoacetic acid, and 2,4-dinitrophenol on primary vascular tissue differentiation in isolated pea roots which have been subjected in each case to reagent concentrations causing approximately 90 per cent inhibition of root elongation. The purpose of such a study is to attempt to relate biochemical processes to the known anatomical stages in primary vascular tissue differentiation.

Attempts to relate root elongation and cellular differentiation have been made in several studies of inhibited root elongation. As early as 1893 Pfeffer reported apparent acceleration of acropetal differentiation of vascular tissue elements in the roots of a number of plants when root elongation was inhibited mechanically by plaster of Paris encasement of the root. Using *Vicia faba* roots grown in solid gypsum, Pfeffer found acropetal differentiation of pitted vessel elements to within 1 mm. from the tip at the end of 15 days, as compared to 25–35 mm. in uninhibited roots. Pfeffer's finding has been substantiated by numerous subsequent workers (Nathansohn, 1898). More recently Kojima (1931, 1933), also using the technique of Pfeffer, reported that such mechanical pressure inhibits cell division in the root tip as early as 16 hr. after beginning treatment. In inhibited roots, xylem elements were apparent at an average distance of 1.4 mm. from the root apex at the end of one week, while in control roots, xylem was apparent first at 13.7 mm. Kojima (1931) pointed out that in terms of position, the inhibited roots appeared to show accelerated differentiation of xylem cells, but in terms of age of cells, cellular differentiation in the inhibited roots was actually hindered. He concluded that the mechanical inhibition of root elongation by solidified gypsum involved inhibition of cell elongation, cell division, and cell differentiation. He demonstrated that the high osmotic pressures of concentrated sucrose solutions caused essentially similar inhibitions of these cell processes in these roots.

Several workers have concerned themselves with the effects of chemical inhibitors on cell elongation and differentiation. Cholodny (1931), in his analysis of the action of auxin on root elongation, suggested that applied auxin so speeds up the maturation of cells that the phase of growth during which elongation usually occurs is omitted. Noirfalise (1940) reported that treatment of roots of *Vicia faba* with relatively high concentrations of indoleacetic acid (1:40,000) causes cessation of mitotic activity in the apical meristem followed by premature vacuolation of the apical initials. The rapid appearance of lignified vessels closely adjacent to the apical meristem was interpreted as an acceleration of cellular differentiation. Barghoorn (1942) was unable to demonstrate any effect of applied l-proline in stimulating protoxylem differentiation in the roots of beans or cotton. Hayward and Blair (1942) illustrated an interesting case of naturally occurring inhibition of root elongation in the dormant roots of Valencia orange. In the dormant condition, the apical meristem becomes relatively inactive and reduced. Continued differentiation of vascular tissues results in the appearance of mature elements closely adjacent to the apical region. The environmental conditions normally inducing this dormant root condition were reproduced experimentally by treatment of the roots of Valencia orange seedlings with high chloride solutions at a high pH. Heimsch (1951) reported that in rapidly elongating barley roots, maturation of vascular elements occurs at a greater distance from the meristem than in slowly elongating roots.

In his studies of mineral nutrition of wheat roots, Burström (1947) showed that high phosphate enhances cell multiplication. Under conditions of low phosphate (1/10,000 M) for 5 days, the number of cell divisions in the apical meristem is sufficiently reduced to result ultimately in the disappearance of the meristem as the cells are "used up" by elongation and differentiation. In this case, the meristem disappears due to an unfavorable balance among cell division, elongation, and differentiation.

Inhibition of root elongation has been produced by X-irradiation (Smith and Kersten, 1942) and recently by radiation from P^{32} (Mackie et al., 1952). In each case an upset in normal differentiation processes has also been observed. Ac-

[1] Received for publication January 30, 1953.

This investigation was supported in part by a research grant from the National Institutes of Health, Public Health Service.

The writer is indebted to Dr. Tova Arzee for her competent technical assistance.

cording to Smith and Kersten, dry seeds of *Vicia faba* pretreated with soft X-rays showed markedly inhibited root elongation upon germination. Pitted vessel elements were observed closely adjacent to the apical meristem in all treated roots at the end of 5 days. The authors concluded that in the absence of elongation, only pitted vessel elements are formed.

In all the cases cited above, inhibition of root elongation, whether by mechanical or chemical means, resulted in the ultimate appearance of mature vascular elements much closer to the apical meristem than in untreated roots. Apparently, in all cases in which cell divisions in the meristem ceased, resulting ultimately in cessation of root elongation, the differentiation processes continued uninterrupted in an acropetal direction. In none of these cases, however, is there conclusive evidence for either acceleration or inhibition of vascular tissue differentiation under conditions of root inhibition.

MATERIALS AND METHODS.—Throughout all experiments, sterile root tips excised from 48-hr. germinated seeds of the garden pea, *Pisum sativum*, variety Alaska (Asgrow strain No. 44007) were cultured in pea root medium (Bonner and Devirian, 1939) in Petri dishes in the dark at 26°C. as previously reported (Torrey, 1950). Five or ten mm. tips were excised aseptically and transferred immediately to liquid medium to which appropriate additions of the inhibitor had been made. One ml. additions of the inhibitor solutions, sterilized by Seitz-filtration, were made to 100 ml. amounts of the nutrient medium and the pH was adjusted with sterile 0.05N NaOH to the appropriate pH noted below for each inhibitor. At the end of the treatment period, root tips were killed in formalin-acetic acid-alcohol with aspiration, dehydrated in tertiary butyl alcohol series, and embedded in "Tissue-mat." Serial sections were cut at 8μ and stained with Heidenhain's hematoxylin and safranin. Measurements of sectioned roots were made by an actual count of sections multiplied by section thickness. For measurements of longitudinal sections a suitably calibrated ocular micrometer was utilized.

As has been reported earlier (Torrey, 1951), isolated pea roots growing in a synthetic nutrient medium exhibit the typical sequence of primary vascular tissue differentiation found in the roots of a number of dicotyledonous plants (Esau, 1943; Williams, 1947). Immediately proximal to the apical initials, the enlargement and vacuolation of procambial cells progressively "blocks out" or delimits the future triarch arrangement of xylem tissue, according to the pattern described by Wetmore (1947). The differentiation of primary phloem and xylem proceeds acropetally, with mature protophloem elements becoming apparent before the complete xylem pattern has been blocked out. The first mature protoxylem elements are seen at the outermost poles of the xylem strands, usually at a considerable distance proximal to the first mature phloem elements. Subsequent centripetal differentiation of the primary xylem progresses without interruption from these outer small protoxylem elements until the complete triarch xylem pattern is formed. The level of the first mature protophloem and protoxylem elements can be quite accurately determined by careful study of serial transverse sections. Using the criteria adopted by Heimsch (1951), mature protophloem elements are those identified by scarcity of stainable contents and lack of nucleus; mature protoxylem elements are designated as those containing no nucleus. Throughout these discussions, the term "differentiation" includes all stages in the ontogeny of the cell from its initial formation at the apical meristem to its ultimate maturation into a characteristic anatomical cell type. Each stage of differentiation may in itself comprise a sequence of separate related events.

RESULTS.—The effectiveness of indoleacetic acid as an inhibitor of root elongation was recognized by Kögl et al. (1934) soon after its chemical isolation. Lane (1936), using *Avena* seedlings, showed that the inhibition of root elongation is proportional to auxin concentration. Subsequent workers have studied extensively the auxin inhibition of root elongation, but no clear understanding of the mechanism of its action has been achieved. The inhibitory action of iodoacetic acid on root elongation was shown by Albaum and Commoner (1941) who reported consistent inhibition of root elongation in *Avena* seedlings grown in 10^{-4} M solutions of iodoacetic acid. Kandler (1950) recently reported the inhibition by 2,4-dinitrophenol of root elongation in isolated corn roots grown in synthetic nutrient medium containing 10^{-4} M concentration of the inhibitor.

In order to study primary tissue differentiation under conditions of inhibited root elongation, it was necessary to establish suitable inhibitor concentrations which would produce comparable inhibition of pea root elongation. Serial dilutions of each of the inhibitors were tested in liquid nutrient medium for effective inhibition of root elongation. In fig. 1 are plotted representative data for inhibition of pea root elongation by indoleacetic acid (IAA), iodoacetic acid (IODOAC), and 2,4-dinitrophenol (DNP). The minimal concentration of each inhibitor producing approximately 90 per cent inhibition of elongation was established as the concentration to be used in these experiments.

Root elongation and primary vascular tissue differentiation in control medium.—Five mm. root tips grown in control medium elongate to an average length of 50 mm. in one week. Representative tips were fixed at daily intervals, embedded and sectioned. Exact levels of the first mature phloem and mature xylem elements were determined by microscopic examination of serial cross-sections. Average figures for each day were calculated, using a minimum of five roots for each figure. In fig. 2

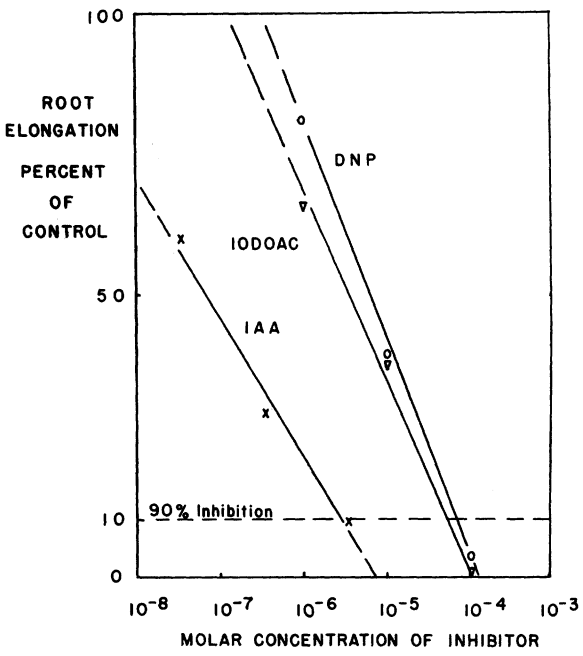

Fig. 1. The inhibition of elongation of isolated pea roots grown in nutrient medium with different growth inhibitors. Five-mm. root tips excised from germinating seeds and grown in the dark at 25°C. for 3 days. Each point represents average of 20 roots. All solutions adjusted to pH 5.0 with 0.05 N NaOH.

are presented typical measurements of root elongation and the location of mature vascular tissue in roots grown in the control medium. Distances to the first mature vascular tissue are presented throughout in microns along the length of the root from the apical meristem (exclusive of the root cap). During the course of root elongation, the positions of the first mature phloem and the first mature xylem, relative to the apical meristem, remain fairly constant. First mature phloem occurs between 330–550μ from the apical initials, whereas the first xylem, showing wider variation, is apparent from 4250–5800μ. Changes in the rate of root elongation which occur during the period are accompanied by corresponding changes in the rate of tissue maturation, so that the relative position of mature primary vascular elements in the elongating root is maintained.

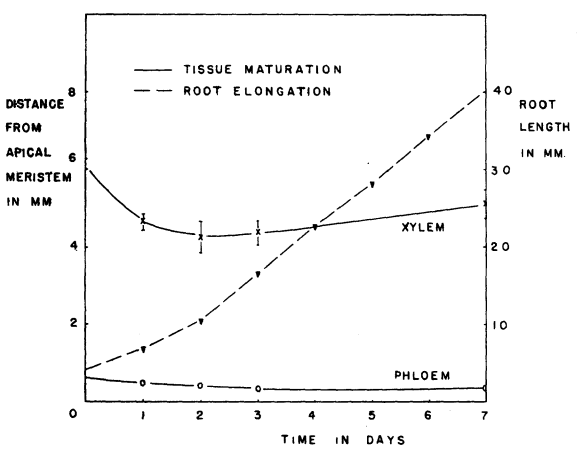

Fig. 2. Root elongation and primary vascular tissue maturation of 5-mm. pea root tips grown in control nutrient medium for 7 days. Tissue maturation represents the average level at which the first mature tissue can be distinguished.

Root elongation and tissue maturation during inhibition of root elongation: Inhibition by IAA.— Among the most effective inhibitors of root elongation are the auxins. At pH 5.0, 1 p.p.m. (5.7×10^{-6} M) IAA inhibits the elongation of pea roots about 90 per cent. During the treatment, secondary effects, including an increase in root diameter and lateral root initiation, may be noted. The striking effect of IAA on vascular tissue maturation becomes evident very early in treatment when microscopic examination of the tissues is made. In fig. 3 are presented data on root elongation and primary vascular tissue maturation in IAA-treated roots during the first 3 days of treatment. Data for control roots (untreated) are also included so that exact comparisons may be made. The effective inhibi-

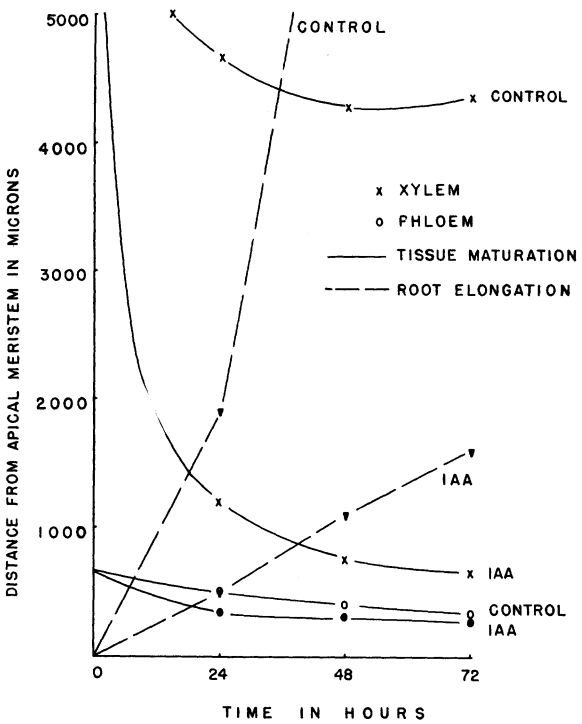

Fig. 3. Primary vascular tissue maturation and increase in root length of isolated 5-mm. pea roots grown in nutrient medium with and without 5.7×10^{-6} M indoleacetic acid during 3 days. Averages were determined from 2 separate experiments. Root elongation curves represent the increase in root length in microns during the treatment period. Curves for control roots are equivalent to those in fig. 2.

tion of root elongation is clearly evident. Little difference in the levels of first mature phloem in treated and untreated roots can be noted. Very striking, however, is the decrease in the distance of the first mature xylem from the apical meristem in the IAA-treated roots.

The auxin effect is most striking during the initial 24-hr. period of IAA treatment. In table 1 is presented the detailed analysis of root elongation and tissue maturation in one representative experiment with auxin-treated and untreated roots. It is evident from the figures presented that the accelerated xylem maturation in the 24-hr. period cannot be accounted for alone by the decreased root elongation, as was shown to be the case for mechanical inhibition over longer experimental periods by Kojima (1931). During the initial 24-hr. period, xylem maturation in the treated roots progressed a distance of over 5 mm. (5240μ) compared to 1940μ in control roots. Root elongation, however, was inhibited only to the extent of about 600μ in comparison to control roots. The discrepancy of about 3300μ cannot be attributed to differences in root elongation, but such difference is apparently due to the specific effect of IAA in increasing the rate of xylem maturation.

In the last column of table 1 are presented for each 24-hr. period the average distances over which xylem maturation progressed. In the control roots, it is clear that the rate of xylem maturation closely reflects the rate of root elongation. Thus, during the third 24-hr. period, primary xylem elements achieved a mature state along a length of root almost 6½ mm. long. In the auxin-treated root, despite almost complete inhibition of root elongation, nearly this same rate was achieved during the first 24-hr. period of treatment. No comparable acceleration effect of auxin on the rate of phloem maturation was evident, nor was there any apparent effect of auxin on the initial delimitation of the xylem pattern by procambial cell enlargement and vacuolation. Longitudinal sections of typical control and auxin-treated roots are seen in fig. 4 and 5 respectively. In the latter figure, the proximity of mature primary xylem to the apical meristem is strikingly evident. A comparison of relative maturation in control and IAA-treated roots may be made by comparing fig. 6 and 7, transverse sections cut in each case at a level of 1840μ proximal to the apical meristem.

A similar study of the effect of IAA on 10-mm. tips excised from initial tips grown one week in control medium, then excised and treated with auxin for similar periods gave essentially the same results as above. Thus, an auxin concentration which prevents elongation of the root accelerates the rate of maturation of primary xylem elements. It is interesting to note that this same auxin concentration is about optimal for maximum elongation of pea *stem* sections (Galston and Hand, 1949).

In fig. 10, the spatial relationships between the primary vascular tissues within the 24-hr. IAA-treated root are diagrammatically represented, showing the position of first mature xylem elements acropetally differentiated to within about 1 mm. of the apical meristem. The protoxylem elements which are produced under these conditions are typically short, reticulately-pitted elements (fig. 5), characteristic of xylem elements differentiated under conditions of inhibited growth (Smith and Kersten, 1942). Although the centrifugal delimitation of the xylem, which proceeds in the procambium immediately proximal to the apical meristem, is apparently unaffected by the auxin treat-

TABLE 1. *Root elongation and distance of mature primary vascular tissue from the apical meristem in isolated pea roots grown in nutrient medium with and without IAA. Initial root length was 5.0 mm.*

Hr.	Distance from apical meristem, μ		Average increase in root length, μ	Increment of matured tissue in μ/day	
	1st mature phloem	1st mature xylem		Phloem	Xylem
Roots grown in control medium at pH 5.0					
	Av.	Av.			
0	664, 640 } 650	6156, 6224 } 6190	-----	-----	-----
24	592, 608 } 600	5130, 5180 } 5150	900	950	1940
48	490, 408 } 450	4690, 5408 } 5050	4600	4750	4700
72	384, 286 } 340	5130, 4696 } 4910	6200	6310	6340
Roots treated with 5.7×10^{-6} M IAA at pH 4.8					
0	650	6190	-----	-----	-----
24	330, 432 } 380	1183, 1320 } 1250	300	570	5240
48	320, 320 } 320	750, 896 } 820	400	460	830
72	258, 286 } 270	645, 856 } 750	400	450	470

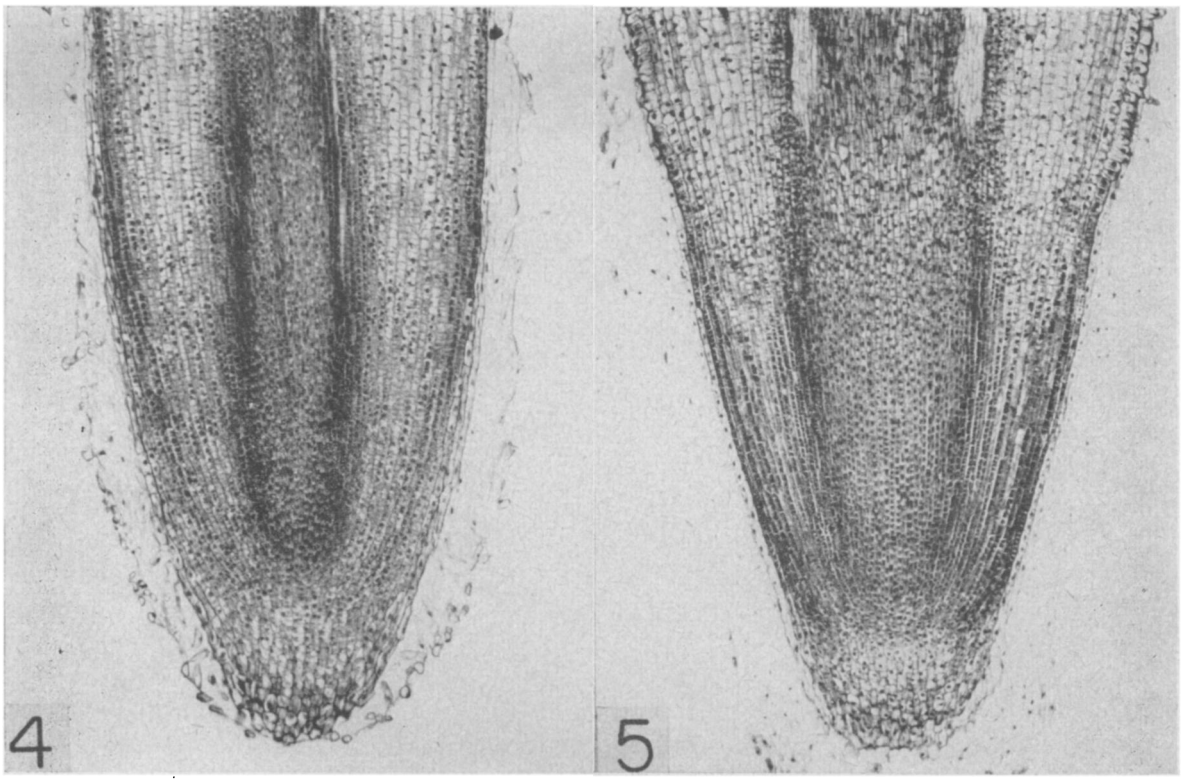

Fig. 4–5.—Fig. 4. Longitudinal section of isolated pea root grown in control nutrient medium for 48 hr. Total length of root in figure equals about 1350μ. Note lack of mature primary xylem. ×70.—Fig. 5. Longitudinal section of isolated pea root grown in nutrient medium containing 5.7×10^{-6} M IAA for 72 hr. Total length in figure equals about 1350μ. Note mature primary xylem strands approaching the apical meristem. In this root, the first primary xylem occurred lateral to this section at 645μ from the apical meristem. The enlargement of the cortical cells at the proximal end of the root is a characteristic response to auxin treatment. ×70.

ment (fig. 10), the rate of centripetal maturation of these xylem elements in the xylem strands *is* accelerated by the treatment with IAA.

Inhibition by iodoacetic acid.—Iodoacetic acid is reported to inhibit respiration by inactivating -SH groups. The inhibitor is considered to be a nonspecific inhibitor of sulfhydryl enzymes and is especially effective on certain enzymes of aerobic respiration. At pH 5.0, a concentration of 10^{-4} M iodoacetate almost completely inhibits pea root elongation (fig. 1). Machlis (1944) reported a greater than 60 per cent inhibition of aerobic respiration in barley roots treated with 5×10^{-5} M iodoacetate at pH 5.0. Salt accumulation was correspondingly reduced in the presence of the inhibitor. Laties (1949) also reported the strong inhibitory effect of iodoacetic acid on root tissue respiration. Morphologically, no macroscopic changes are apparent in the treated roots, which remain white, turgid and normal in appearance.

In table 2 are summarized the results of measurements made on roots treated 48–72 hr. with 10^{-4} M iodoacetate at pH 4.9, in the nutrient medium. It is evident that there is an apparent acceleration of xylem tissue maturation, similar to that produced by IAA treatment, although less marked. Progressive centripetal maturation of xylem elements is not as rapid as in IAA-treated roots (fig. 8). In all the roots treated with iodoacetate, no mitotic divisions in the apical meristem nor along the length of the root were observed.

Secondary wall formation in these roots was abnormal in that xylem elements, especially newly formed protoxylem, had very thin, dark-staining cell walls, showing little or no lignification. The mature state of such cells was evident only by the loss of the cell contents. Maturation of sieve-tube elements of the phloem appeared to be normal, but in mature regions of the root phloem fibers, like the elements of the xylem, showed only slight secondary wall thickening. The inhibition of usual secondary wall thickening must be attributable to some direct or indirect effect of the inhibitor on cellulose deposition by these cells with the result that this normal phase of xylem maturation is omitted.

Inhibition by 2,4-dinitrophenol.—This reagent has been shown to be active in animal and plant tissues (Loomis and Lipmann, 1948) in uncoupling aerobic respiration from the phosphorylative system, producing a concomitant stimulation of oxygen

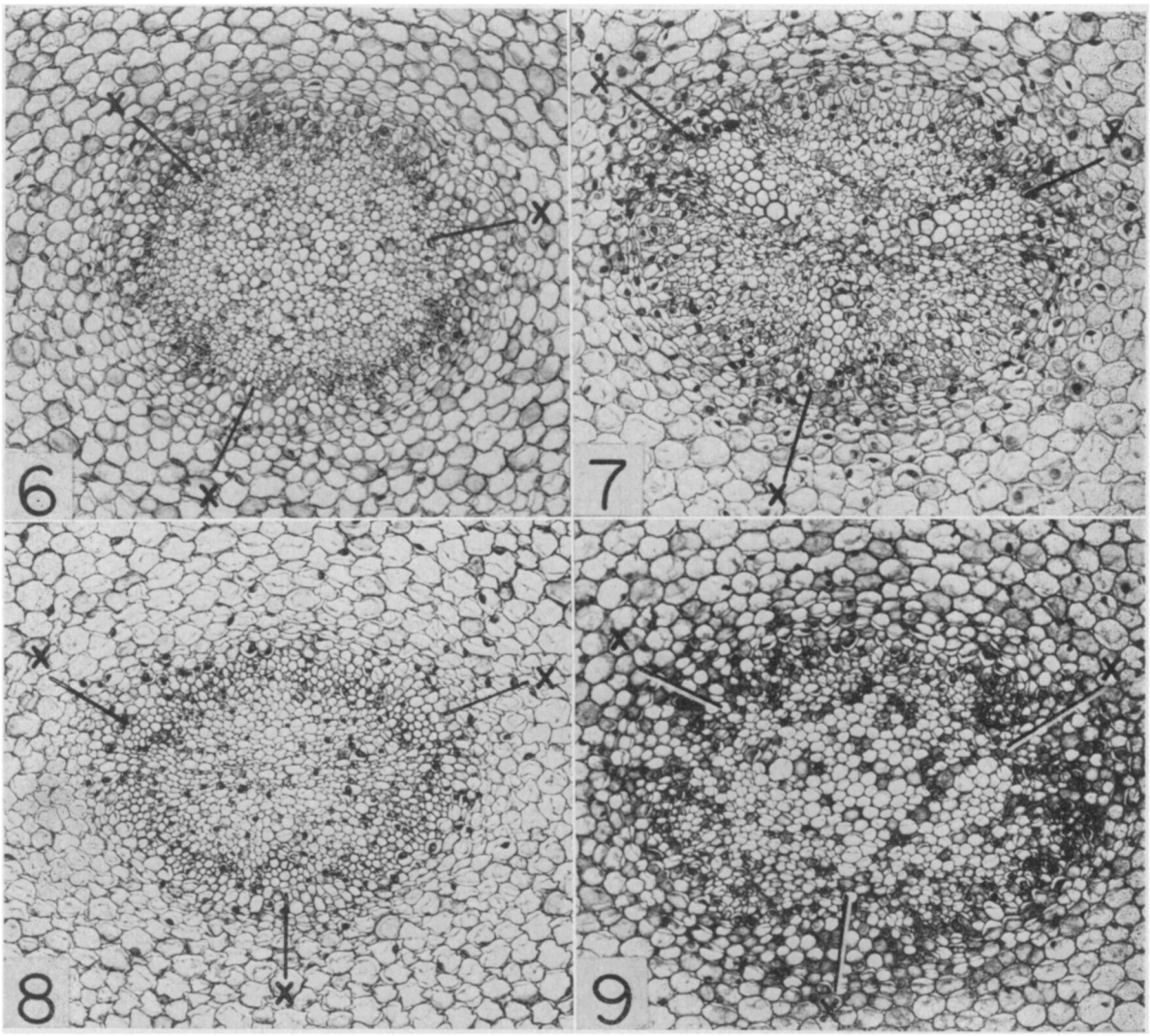

Fig. 6–9.—Fig. 6. Transverse section of isolated pea root grown for 48 hr. in control nutrient medium, cut at 1840μ proximal to the apical meristem, showing tissues of the central cylinder. Note delimitation by cellular enlargement and vacuolation of the triarch pattern of the future primary xylem tissue (protoxylem poles of future xylem strands indicated by *X*). Mature primary phloem elements are evident in the periphery of the cylinder at alternate radii with the xylem points. Level of section comparable to that in fig. 6-8. ×144.—Fig. 7. Transverse section of isolated pea root grown for 48 hr. in nutrient medium containing 5.7×10^{-6} M IAA, showing central cylinder of root. Section cut at 1840μ proximal to the apical meristem. Note maturation of primary xylem elements (*X*) in triarch xylem tissue. ×144. —Fig. 8. Transverse section of isolated pea root grown for 48 hr. in nutrient medium containing 10^{-4} M iodoacetic acid, showing central cylinder of the root. Section cut at 1840μ proximal to the apical meristem. Note beginning of primary xylem maturation at each of the three protoxylem points (*X*). ×144. Fig. 9. Transverse section of isolated pea root grown for 48 hr. in nutrient medium containing 10^{-4} M 2,4-dinitrophenol, showing central cylinder of the root. Section cut at 1840μ proximal to the apical meristem. Note the triarch pattern of the future primary xylem (*X*) evident in the enlarged central procambial cells. Mature primary phloem elements may be seen at alternate radii with the future xylem poles, but no maturation of primary xylem is evident. ×144.

consumption. Bonner (1949) has shown that DNP strongly inhibits the elongation of *Avena* coleoptiles. Stenlid (1949) demonstrated that DNP inhibited absorption of glucose by wheat roots while at the same time increasing O_2 consumption. Strong inhibition of pea root elongation by relatively low concentrations of DNP is shown in fig. 1.

In table 2 are presented typical measurements from roots treated with 10^{-4} M DNP at pH 5.0 in nutrient medium. From these data it is apparent that primary vascular tissue maturation was markedly affected. The inhibitor completely stopped xylem differentiation (fig. 9). The distance of mature xylem from the apical meristem in the treated

TABLE 2. *Root elongation and distance of mature primary vascular tissue from the apical meristem in isolated pea roots treated in nutrient medium with growth inhibitors.*

Hr.	Average root length in mm.	Distance from apical meristem, μ: 1st mature phloem		1st mature xylem	
Roots grown in control medium at pH 5.0					
0	5.0	650		>5000[a]	
24	5.8	640		>4820	
48	9.3	380		>5000	
72	15.9	340		>4500	
Roots treated with 10^{-4} M Iodoacetate at pH 4.9					
			Av.		Av.
24	5.2	-----		-------	
48	5.3	340	340	1840	1710
		410		1260	
		260		2030	
72	5.4	190	180	1220	910
		150		710	
		190		800	
Roots treated with 10^{-4} M 2,4-dinitrophenol at pH 5.0					
		1020	980	>5000	
		1080		>5000	
24	5.0	850		>5000	
48	5.3	1250	1130	>5000	
		1010		>5000	
72	5.5	1280	1190	>5000	
		1100		>5000	

[a] Indicates first mature xylem appears at a distance greater than the length of the root sectioned.

roots was unchanged during the treatment period. DNP inhibited those phases of xylem maturation which include secondary wall formation, lignification and the subsequent loss of cell contents. It is not possible to state from these studies whether DNP also prevents the earlier stages of xylem differentiation which give rise to the early delimitation of the pattern of the primary xylem tissue. In none of the DNP-treated roots was cellular division observed.

The differentiation of phloem tissue is also influenced by this growth inhibitor. During DNP treatment, the position of the first mature phloem elements was progressively more distant from the apical meristem—even more distant than at the start of the experiment. This anomalous condition can be explained in terms of the limited cell elongation which occurred under these conditions. It is clear that no maturation of phloem tissue occurred after the onset of inhibitor treatment. That the first mature elements became progressively somewhat more distant from the apical meristem indicates that the limited cell elongation which occurred in the treated roots must have occurred in the cells located between the apical initials and the first mature phloem. The important conclusion to note, however, is that DNP inhibits root elongation and prevents differentiation of both xylem and phloem elements.

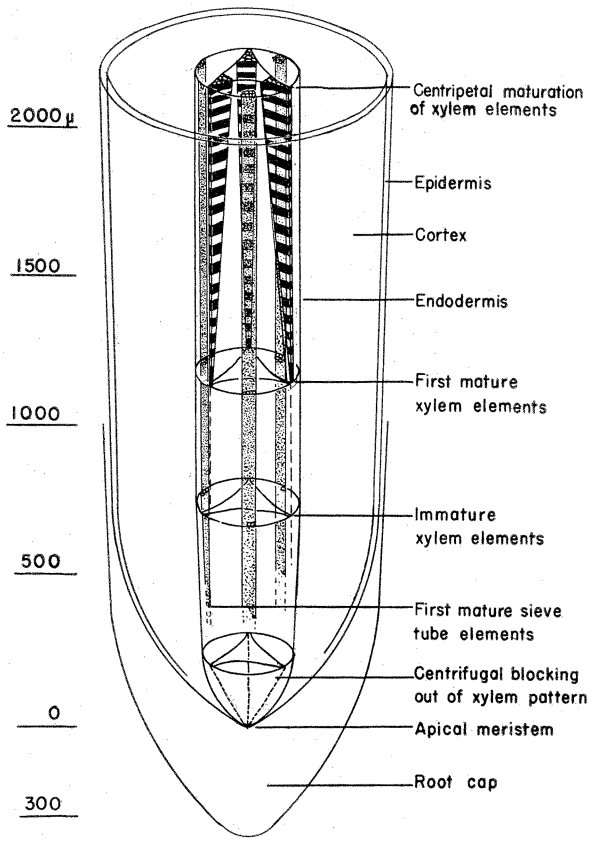

Fig. 10. Diagrammatic representation of isolated pea root grown for 24 hr. in nutrient medium containing 5.7×10^{-6} M IAA, showing spatial relationship between primary tissues in inhibited root. First mature sieve-tube elements of phloem appear about 350μ proximal to apical meristem. First mature xylem elements appear about 1250μ proximal to apical meristem. The early centrifugal blocking out or delimitation of the triarch primary xylem is contrasted to the subsequent centripetal maturation of these primary xylem tissues. Based on the type of diagram devised by Esau (1941).

DISCUSSION.—The differentiation of any cell must involve a large number of biochemical reactions, which act together in such a direction as to lead ultimately to the formation of a given mature cellular component. These reactions underlying cellular differentiation are modified and controlled for each cell by the physical and chemical environment established locally by all adjacent differentiating cells. For different cell types, the complexity of the differentiation pattern might be expected to differ—from relatively simple to extremely complex patterns. Modification of any single aspect of the chemical environment may well modify and change the course of the differentiation pattern.

By treatment of elongating roots with chemical inhibitors of growth, it has been shown that the sequence of differentiation of the vascular tissue may be modified, i.e., accelerated in all its aspects,

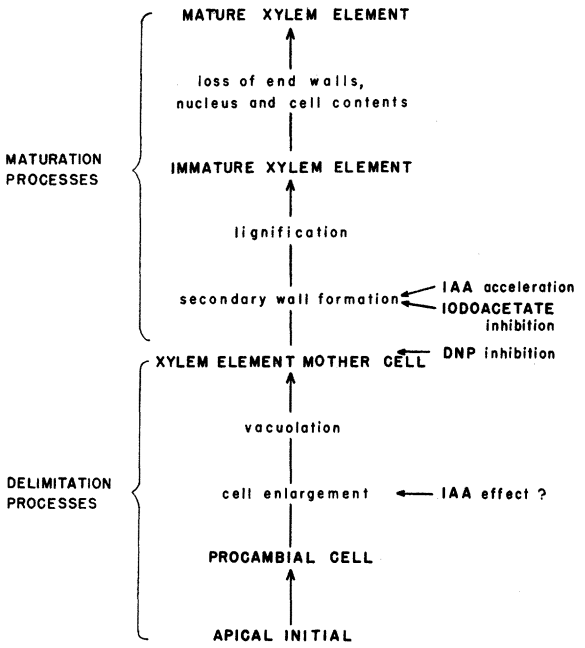

Fig. 11. Ontogenetic steps in the differentiation of a vessel element in the primary xylem of a pea root, from its origin at the apical meristem to the formation of the mature xylem element. Probable site of inhibitor action is indicated.

accelerated in only certain phases, or prevented entirely. From the known activity of the growth inhibitors used in this study, one might make some interesting speculations concerning the biochemical processes involved in primary xylem differentiation —or at least certain aspects of that differentiation.

Steps involved in the differentiation of a particular cell type may be outlined somewhat arbitrarily in histogenetic terms and each step then subjected to analysis and study. Only by such a step-wise analysis can one hope to approach an understanding of the over-all process. In fig. 11 is presented in summary fashion a statement of the ontogeny of a vessel element in the pea root. Each arrow indicates a series of chemical reactions which must occur in the direction of differentiation. In this scheme are indicated the steps at which the chemical inhibitors studied most probably exert their influence.

No evidence is given in the present work concerning the role of IAA in cell enlargement. There is evidence to indicate that auxin is involved actively in the maturation of primary xylem elements—i.e., the formation of the secondary wall, its lignification and the subsequent loss of cell contents. All of these phases of differentiation are markedly accelerated by IAA. Whether this effect is direct or indirect cannot be decided from the evidence presented here. Recently, Jacobs (1952) has shown auxin to be the factor limiting xylem regeneration in wounded stem of *Coleus*.

The phases of xylem element maturation considered here are intimately tied to the carbohydrate metabolism of the tissue. Secondary wall formation involves a considerable deposition of cellulosic materials at the expense of the available carbohydrates in the metabolizing cell. That iodoacetic acid does not prevent maturation suggests that these processes are not completely dependent upon the aerobic respiration of the tissue. On the other hand, DNP, which interferes with the phosphorylative system, prevents the maturation steps. Although practically nothing is known concerning the enzyme systems involved in cellulose synthesis, it is reasonable to expect that phosphorylated intermediates are involved and that inhibition of cellulose synthesis by depleting the supply of phosphorylated substrates might well interfere with the maturation process. Conclusive evidence concerning the specific nature of action of each of these inhibitors in cell differentiation must await the development of more critical methods of attacking this problem.

SUMMARY

Five mm. Alaska pea roots excised from 48-hr. germinated seed and grown in a synthetic nutrient medium in Petri dishes in the dark were treated with the known chemical inhibitors of root elongation, indoleacetic acid, iodoacetic acid and 2,4-dinitrophenol, at concentrations causing approximately 90 per cent inhibition of root elongation. A detailed histological study was made of these inhibited roots, using serial transverse and longitudinal sections, in an attempt to relate biochemical processes of the root tissues to the differentiation of primary vascular tissues. At a concentration of 5.7×10^{-6} M (1 mg./l.), indoleacetic acid almost completely inhibits root elongation but markedly accelerates the maturation of primary xylem elements, while having little effect on the differentiation of primary phloem. Iodoacetic acid at 10^{-4} M at pH 4.9, a concentration reported to inhibit strongly aerobic respiration in root tissues, causes slight acceleration of primary xylem maturation while inhibiting root elongation. Secondary wall formation of the primary xylem elements was abnormal under iodoacetate treatment, with protoxylem elements showing reduced secondary wall thickening and lignification. 2,4-dinitrophenol, an inhibitor which has been shown to uncouple aerobic respiration from the phosphorylative system, at a concentration of 10^{-4} M at pH 5.0, completely stops primary vascular tissue differentiation as well as root elongation. It is pointed out that the action of all three inhibitors centers around cellular processes associated with carbohydrate metabolism. Thus, by use of chemical inhibitors, it has been possible to modify the usual sequence of primary vascular tissue differentiation in these roots.

Department of Botany,
University of California,
Berkeley 4, California

LITERATURE CITED

ALBAUM, H. G., AND B. COMMONER. 1941. The relation between the four-carbon acids and the growth of oat seedlings. Biol. Bull. 80: 314-323.

BARGHOORN, E. S. 1942. The effects of l-proline on proliferation of cells and differentiation of protoxylem in roots of cotton and bean. Growth 6: 23-31.

BONNER, J. 1949. Limiting factors and growth inhibitors in the growth of the *Avena* coleoptile. Amer. Jour. Bot. 36: 323-332.

———, AND P. S. DEVIRIAN. 1939. Growth factor requirements of four species of isolated roots. Amer. Jour. Bot. 26: 661-665.

BURSTRÖM, H. 1947. A preliminary study on mineral nutrition and cell elongation of roots. Kungl. Fysiograf. Sällskap. Lund Förhandl. 17: 3-13.

CHOLODNY, N. 1931. Zur Physiologie des pflanzlichen Wuchshormons. Planta 14: 207-216.

ESAU, KATHERINE. 1941. Phloem anatomy of tobacco affected with curly top and mosaic. Hilgardia 13: 437-490.

———. 1943. Origin and development of primary vascular tissues in seed plants. Bot. Rev. 9: 125-206.

GALSTON, A. W., AND MARGERY E. HAND. 1949. Studies on the physiology of light action. I. Auxin and the light inhibition of growth. Amer. Jour. Bot. 36: 85-94.

HAYWARD, H. E., AND W. M. BLAIR. 1942. Some responses of Valencia orange seedlings to varying concentrations of chloride and hydrogen ions. Amer. Jour. Bot. 29: 148-155.

HEIMSCH, C. 1951. Development of vascular tissues in barley roots. Amer. Jour. Bot. 38: 523-537.

JACOBS, W. P. 1952. The role of auxin in differentiation of xylem around a wound. Amer. Jour. Bot. 39: 301-309.

KANDLER, O. 1950. Über den Einfluss von 2,4-Dinitrophenol auf Atmung und Wachstum in vitro kultivierter Maiswurzeln. Zeitschr. Naturf. 5b: 338-344.

KÖGL, F., A. J. HAAGEN-SMIT, AND H. ERXLEBEN. 1934. Über den Einfluss der Auxine auf das Wurzelwachstum und über die chemische Natur des Auxins der Graskoleoptilen. Zeitschr. Physiol. Chem. 228: 104-112.

KOJIMA, H. 1931. Division, growth and differentiation of cells in the root of *Vicia Faba* artificially inhibited from further elongation. Jour. Dept. Agric. Kyushu Imp. Univ. 3: 121-147.

———. 1933. Cell division and elongation in the decapitated roots of *Vicia faba* L. Jour. Dept. Agric. Kyushu Imp. Univ. 3: 269-301.

LANE, R. H. 1936. The inhibition of roots by growth hormone. Amer. Jour. Bot. 23: 532-535.

LATIES, G. G. 1949. The role of pyruvate in the aerobic respiration of barley roots. Arch. Biochem. 20: 284-299.

LOOMIS, W. F., AND F. LIPMANN. 1948. Reversible inhibition of the coupling between phosphorylation and oxidation. Jour. Biol. Chem. 173: 807-809.

MACHLIS, L. 1944. The influence of some respiratory inhibitors and intermediates on respiration and salt accumulation of excised barley roots. Amer. Jour. Bot. 31: 183-192.

MACKIE, RUTH W., J. M. BLUME, AND C. E. HAGEN. 1952. Histological changes induced in barley plants by radiation from P^{32}. Amer. Jour. Bot. 39: 229-237.

NATHANSOHN, A. 1898. Beiträge zur Kenntniss des Wachsthums der trachealen Elemente. Jahrb. Wiss. Bot. 32: 671-686.

NOIRFALISE, A. 1940. Recherches sur le développement des racines de *Vicia faba* traitées à l'hétéroauxine. Cellule 48: 307-333.

PFEFFER, W. 1893. Druck- und Arbeitsleistung durch Wachsende Pflanzen. S. Hirzel. Leipzig.

SMITH, G. F., AND H. KERSTEN. 1942. The relation between xylem thickenings in primary roots of *Vicia Faba* seedlings and elongation, as shown by soft X-ray irradiation. Bull. Torrey Bot. Club. 69: 221-234.

STENLID, G. 1949. The effect of 2,4-dinitrophenol upon oxygen consumption and glucose uptake in young wheat roots. Physiol. Plant. 2: 350-371.

TORREY, J. G. 1950. The induction of lateral roots by indoleacetic acid and root decapitation. Amer. Jour. Bot. 37: 257-264.

———. 1951. Cambial formation in isolated pea roots following decapitation. Amer. Jour. Bot. 38: 596-604.

WETMORE, R. H. 1947. The differentiation of primary vascular tissues in the vascular plants. (Abstract) Amer. Jour. Bot. 34: 591.

WILLIAMS, B. C. 1947. The structure of the meristematic root tip and origin of the primary tissues in the roots of vascular plants. Amer. Jour. Bot. 34: 455-462.