BOT. GAZ. 130(1):62–69. 1969.

FACTORS LIMITING THE STIMULATION OF POLYPLOID MITOSES IN INTACT PEA ROOTS AND EXCISED ROOT SEGMENTS[1]

ANN G. MATTHYSSE[2] AND JOHN G. TORREY
Biological Laboratories, Harvard University, Cambridge, Massachusetts 02138

ABSTRACT

Although polyploid mitoses can be elicited in pea root segments 1 mm in length cultured on a defined medium, the same medium failed to elicit any polyploid mitoses when applied to the roots of intact pea plants. Auxin treatments, which are effective in eliciting polyploid mitoses in the roots of other plants, were also ineffective when applied to pea roots. Polyploid mitoses were not obtained when excised whole pea roots were cultured on a medium which effectively induced such mitoses in 1-mm segments, nor were polyploid mitoses found when the roots were fed basally with this medium. Polyploid mitoses were observed in 1-mm root segments split in half along the axis of the vascular cylinder and cultured with the cut surface against the surface of the medium so that the vascular cylinder was oriented parallel to the surface of the medium. If these segments were turned so that the vascular cylinder was still oriented parallel to the surface of the medium, but so that the cut surface was up and the intact epidermis in contact with the culture medium, no polyploid mitoses were observed. Thus wounding alone does not initiate polyploid mitoses. The appearance of polyploid mitoses requires the presence of a cut surface in contact with the culture medium.

Root segments 3 mm in length were cultured with one cut surface against the surface of the culture medium and the vascular cylinder oriented perpendicular to the culture medium. After 74 hr in culture the segments were cut into three 1-mm pieces—top, middle, and bottom. A significant number of diploid mitoses was observed in all three pieces, although there were fewer mitoses in the top piece than in the bottom. A significant number of polyploid mitoses was observed only in the bottom piece. The orientation of the 3-mm segments (basal or apical end down) had little effect on the results. Thus not only does the appearance of polyploid mitoses require that a cut surface of the root be in contact with the medium but polyploid mitoses are also elicited only within 1 mm of this cut surface. These facts make the failures to elicit polyploid mitoses in excised roots and roots of whole plants easily understandable; that is, the necessary stimulatory substances presumably never reached the root tissues. Wounding of the intact root by passing a needle through it stimulates mitoses of both diploid and polyploid cells. We suggest that interference of the normal flow of hormonal materials, probably of a cytokinin, from the root tip toward the root base (shoot) is responsible for the local accumulation of stimuli leading to polyploid mitoses.

Introduction

Polyploid nuclei are known to occur in the somatic cells of many diploid plants and particularly in plant roots. The existence of cells with polyploid nuclei can be demonstrated by microspectrophotometric absorption measurements of interphase nuclei (McLEISH and SUNDERLAND, 1961), by the observation of occasional spontaneous polyploid mitoses (e.g., STOMPS, 1911; TSCHERMAK-WOESS and DOLEŽAL, 1953), and by the artificial stimulation of polyploid mitoses by treatments with plant hormones (LEVAN, 1939; D'AMATO, 1952*b*) or by wounding (D'AMATO, 1948). In general, polyploid cells are not found in the apical meristem of the root. The frequency of polyploid cells and the degree of ploidy of these cells have been observed to increase with increasing distance from the root tip (D'AMATO, 1952*b*; DEELEY, DAVIES, and CHAYEN, 1957). This observation had led to the postulation of a connection between polyploidy and differentiation (e.g., D'AMATO, 1964). However, this connection, if it exists, cannot be an essential one since plants are known in which no polyploid cells occur. For example, only diploid cells are reported to occur in tissues of *Helianthus tuberosus* (PARTANEN, 1959, 1963*b*) and *Crepis capillaris* (REINERT and KÜSTER, 1966).

Microspectrophotometric absorption measurements show that pea roots contain polyploid cells (McLEISH and SUNDERLAND, 1961). Spontaneous polyploid mitoses have been observed occasionally in pea roots, usually in the cortex near a lateral root (WIPF and COOPER, 1940; OINUMA, 1948; TSCHERMAK-WOESS and DOLEŽAL, 1953). Polyploid mitoses are also associated with the formation of root nodules in peas (WIPF, 1939; WIPF and COOPER, 1938). Nevertheless, attempts to stimulate polyploid mitoses in intact pea roots have been largely unsuccessful.

MELETTI (1950) treated roots of pea seedlings with 10 ppm 2,4-dichlorophenoxyacetic acid (2,4-D) at 12–15 C. He observed diploid mitoses in the roots after three, four, six, and nine days; but he was unable to find polyploid mitoses in the roots at any of these times. He also germinated peas in 10 ppm 2,4-D. This treatment produced no polyploid mitoses in the hypocotyl after nine days. HOLZER (1952) dipped the roots of 4-day-old seedlings into 20 ppm indole-3-acetic acid (IAA) for 4 hr and then placed the plants in water for a recovery period of up to 48 hr. He observed no polyploid mitoses in the roots after this auxin treatment. The treatment with IAA may have failed because pea roots possess an active

[1] This research was supported in part by grants from the National Institutes of Health, U.S. Public Health Service, GM-22, 257 to A. G. M. and GM-08145 to J. G. T. The technical assistance of Gary Gardner is gratefully acknowledged.

[2] Present address: Division of Biology, California Institute of Technology, Pasadena, California 91109.

IAA oxidase (TANG and BONNER, 1947), but this fact does not explain the failure of the 2,4-D treatments. MÜHLING *et al.* (1960) illustrated a polyploid division figure from pea seedling roots treated with α-(2,4-dichlorophenoxy)-n-butyric acid. This report is the single case found of chemically induced polyploid mitoses observed in roots of intact pea plants.

Polyploid mitoses can be stimulated in excised pea root segments grown on a sterile culture medium (TORREY, 1961). In such pea root segments polyploid mitoses were observed only when the culture medium contained salts, sucrose, vitamins, auxins, and kinetin (TORREY, 1961; MATTHYSSE and TORREY, 1967*a*, 1967*b*). This suggested that kinetin as well as auxin might be necessary for the initiation of polyploid mitoses in intact pea roots. NAGL (1963) treated 5- to 12-day-old pea roots with kinetin (10, 20, 30, and 40 ppm each). He fixed the roots after 6 hr of treatment and after 24 hr. He also gave roots which had had 24 hr of treatment a recovery period of 12, 24, and 96 hr in water before fixing them. No combination of these treatments, treatment times, and recovery times stimulated polyploid mitoses in the roots. Thus, although polyploid cells are known to exist in pea roots, and although cells which show polyploid mitoses can be stimulated to divide in pea root segments in culture, it has not been possible to stimulate polyploid mitoses in intact pea roots. In this paper additional attempts to stimulate polyploid mitoses in intact roots are reported.

Material and methods

PREPARATION OF MEDIA.—The standard medium used was the S-2 medium with 1 mg/l kinetin (MATTHYSSE and TORREY, 1967*a*). This medium contains salts, auxins, and amino acids. Both liquid medium and solid agar (0.7%) medium were used. In some experiments S-1 medium, which is the S-2 medium without the mixture of amino acids, was used. For experiments with whole plants, solutions of the auxins or double-distilled water (sterilized by autoclaving) were used as well. For experiments involving basal feeding of roots, the standard S-2 medium with 1 mg/l kinetin and 0.7% agar was poured into small 1-dr vials (35 mm × 10 mm). The vials were then autoclaved in a covered crystallizing dish and, after the medium had solidified, aseptically placed horizontally on a sterile petri plate containing solid agar medium.

PREPARATION OF CULTURES.—Seeds of the garden pea, *Pisum sativum* L., variety Alaska, were surface sterilized with 0.1% $HgCl_2$ or a filtered solution of sodium hypochlorite (5% Pittchlor, Pittsburgh Plate Glass Co., Pittsburgh, Pa.). They were germinated in sterile double-distilled water in the dark at 25 C for 60 hr. The details of these procedures are described in MATTHYSSE and TORREY (1967*a*).

In experiments using whole plants, the young plant (60 hr old) was transferred aseptically to a rack of stainless steel wire mesh (approximately 10 mm × 15 mm × 50 mm, with mesh of about 10 wires per inch), which had been placed inside a sterile test tube (25 mm × 150 mm). The rack had a hole approximately 5 mm in diameter in the center, and the root was placed through this hole. Sufficient sterile liquid medium was poured into the tube to cover the tip of the root. The level of the medium was below the rack on which the cotyledons rested. These plants were grown in the dark at 25 C for 72 hr.

In the wounding experiments, pea roots of the variety "Little Marvel" were used. After aseptic germination for 66 hr, seedlings were suspended on wire racks in test tubes and the roots provided with one-quarter-strength Hoagland's solution. Plants were wounded by passing a no. 27 hypodermic needle transversely through the center of the root axis about 10–11 mm behind the root apex. Plants in tubes were grown under fluorescent light (16-hr day), and whole root samples were fixed at 24, 48, and 72 hr. Root segments 1 mm on either side of the wound were cut, squashed, and stained; their cytological behavior was compared to that of segments cut from the corresponding position on unwounded roots.

In experiments using isolated roots or root segments, the root or segment was excised aseptically and placed on a sterile culture medium. Solid agar medium was used in petri plates. Liquid medium was used in liquid filter paper cultures (MATTHYSSE and TORREY, 1967*a*). A rectangular piece of filter paper was inserted into a tube. The tube was autoclaved, and 5 ml of sterile medium were added. The root or root segment was placed on the filter paper. In general, unless otherwise specified, the root segments were placed with the cut surface down on the agar medium or filter paper. The standard used for these experiments was a 1-mm segment cut from the tenth to the eleventh millimeter behind the root tip and placed with the cut surface on the surface of the agar medium or on the filter paper so that the vascular cylinder of the root was oriented vertically. The orientation of the segments with respect to apical or basal end down was random unless otherwise specified. The standard medium was the S-2 medium with 1 mg/l kinetin.

In experiments using basal feeding of roots, the root was cut from the plant aseptically and placed horizontally on the surface of the agar medium in a petri plate with the cut basal end pushed a few millimeters into a horizontal vial containing agar S-2 medium with 1 mg/l kinetin. This method of culture was described by RAGGIO and RAGGIO (1956).

All segment or excised root cultures were grown in the dark at 25 C for 72–74 hr.

FIXATION AND STAINING.—The procedures used for fixation and staining are described in detail in MATTHYSSE and TORREY (1967*a*). Briefly, the root segments or pieces of the whole roots were fixed in a mixture of propionic acid, formaldehyde, isopropanol, and distilled water—10:13:150:150, volume: volume—at room temperature for about 24 hr. They were then stored in 35% isopropanol. The segments were hydrolyzed in 1 N HCl at 60 C, stained with Schiff's reagent, and squashed on slides. One segment was placed on each slide. The slides were dehydrated and mounted in permount.

SCORING OF SLIDES.—The slides were scored for the frequency of diploid and polyploid mitoses. The ploidy was determined by chromosome counts. Pea has a diploid chromosome number of 14, so diploid and polyploid mitotic figures were easily distinguished. In most experiments the mitotic frequencies were compared using Student's *t*-test with a standard which was run at the same time. The results are reported as none, no significant difference from none, greater than none, less than standard, no significant difference from standard, and greater than standard. Each experimental treatment involved about five segments and was repeated twice. The separate repetitions were each compared with the standard run at the same time, and the results of the comparison were combined using Fisher's method of combining significance tests (MOSTELLER and BUSH, 1954). It was considered that the experimental material showed no significant difference from the standard if the probability of obtaining the difference observed between the experimental and the standard was greater than 10%. In the experiments on wounding roots of intact plants only relative frequencies of mitotic figures are reported.

Results and discussion

ATTEMPTS TO STIMULATE POLYPLOID MITOSES IN ROOTS OF WHOLE PLANTS.—Whole pea plants were grown aseptically in tubes on wire racks as described above. The roots were in sterile double-distilled water. After 72 hr in culture, 1-mm segments 10, 20, 25, 30, 35, and 40 mm behind the root tip were cut out, fixed, stained, squashed, and scored for mitoses. Approximately the same frequency of diploid mitoses (less than standard but greater than none) was observed in all the segments. There was a slight, but not significant, tendency for the frequency of diploid mitoses to decrease with increasing distance from the root tip. No polyploid mitoses were observed in any of the segments.

If, instead of water, a solution of 30 mg/l IAA (*p*H unadjusted), 30 mg/l IAA (*p*H 4.5), 5 or 25 mg/l 2,4-D (*p*H unadjusted), 25 mg/l 2,4-D (buffered at *p*H 5.5 with 0.1 M phosphate citrate buffer), S-1 (*p*H 5.5), or S-2 with 1 mg/l kinetin (*p*H 5.5) was used, no polyploid mitoses were observed in the tenth millimeter behind the root tip, cut out and fixed after 72 hr. Diploid mitoses were observed in the tenth millimeter with all the treatments except the buffered 2,4-D.

Whole plants were grown aseptically with the roots in 30 mg/l IAA or 25 mg/l 2,4-D for 8 hr, followed by 64 hr in double-distilled water. Then the tenth millimeter was excised and fixed. Diploid mitoses were observed in these segments, but no polyploid mitoses were seen. An attempt was made to repeat the observation of MÜHLING *et al.* (1960). Seedling roots were immersed in a solution of 60 ppm 2,4-dichlorophenoxybutyric acid for 24 hr and sampled immediately or after 1, 2, or 3 days recovery in auxin-free solution. Tip segments (0–1 mm, 1–2 mm) or segments taken 10–11 mm behind the root tip all showed frequent diploid mitoses, but no polyploid mitoses were observed.

All of the treatments used on whole plants resulted in some stunting of root growth when compared with the growth in double-distilled water. Some of the treatments produced thickenings or bulges on the roots just behind the tip. Such treatments included S-1, S-2, S-2 with 1 mg/l kinetin, 30 mg/l IAA for 8 hr followed by 64 hr in water, and 25 mg/l 2,4-D for 8 hr followed by 64 hr in water. These bulges were excised, fixed, stained, squashed, and scored for mitoses. All were found to contain a large number of diploid mitoses and no polyploid mitoses. LEVAN (1939) studied such bulges produced in onion roots by treatment with IAA. In onion he observed that these bulges contained many polyploid mitoses.

The treatments used on intact pea roots were similar to those in other plants. D'AMATO (1952*a*) observed polyploid mitoses in cells in intact roots of many species of plants after a 3-day treatment in 10, 20, or 50 mg/l 2,4-D. Polyploid mitoses have been observed in intact onion roots after treating the roots for 8 hr with 30 ppm 2,4-D and allowing them to recover in water for 3 days (LEVAN, 1939; PARTANEN, 1963*a*). The fact that the treatments reported above were unsuccessful on intact pea roots agrees with the findings of MELETTI (1950), HOLZER (1952), and NAGL (1963), who were also unable to initiate polyploid mitoses in intact pea roots with auxin (IAA or 2,4-D) or auxin and kinetin treatments. This failure is not due to a lack of capacity of the cells to respond to such treatments. The S-2 medium with 1 mg/l kinetin initiated polyploid mitoses in excised pea root segments in culture after 3 days (TORREY, 1961), but intact pea roots treated

with the same medium did not show any polyploid mitoses. These results suggested that either (*a*) there was an inhibitory influence of the rest of the plant (stem, cotyledons, and/or root tip) on the initiation of polyploid mitoses in the root, (*b*) there was inadequate penetration of the required hormones into the intact plant, (*c*) a wound was required for the initiation of polyploid mitoses, or (*d*) more than one of these influences was involved. Experiments using whole roots (without the rest of the plant attached), wounding of roots or root parts, basal feeding of excised roots, and treatment of root segments in a variety of orientations were carried out to determine which of the possible explanations was correct.

ATTEMPTS TO STIMULATE POLYPLOID MITOSES IN ISOLATED WHOLE ROOTS.—Whole roots, 15–20 mm in length, were cut off aseptically from the hypocotyl of 60-hr seedlings and placed horizontally on the surface of agar medium or on the filter paper of liquid filter paper cultures so that the uninjured epidermis was in contact with the medium. The medium used was S-2 with 1 mg/l kinetin. Diploid mitoses, but no polyploid mitoses, were observed in the tenth millimeter behind the tip of such roots fixed at 72 hr.

Basal feeding was also used to attempt to stimulate polyploid mitoses in whole roots. One-dram vials were filled with about 4 ml of 0.7% agar S-2 medium with 1 mg/l kinetin and autoclaved. These vials were placed horizontally on the surface of sterile petri plates containing 0.7% agar, agar S-2 medium, or agar S-2 medium with 1 mg/l kinetin. The cut end of a root 15–20 mm in length excised from a 60-hr seedling was placed aseptically in the medium in the vial. The root was oriented horizontally so that one side of it touched the agar medium of the petri plate. The tenth millimeter of such roots was excised after 72 hr and fixed. No polyploid mitoses were observed in these pieces.

Thus, although polyploid mitoses were stimulated in 1-mm pea root segments by culturing them on S-2 medium with 1 mg/l kinetin, this same medium did not stimulate polyploid mitoses in either the roots of the intact plant, the excised whole root placed on the medium, or roots basally fed with this medium.

ATTEMPTS TO STIMULATE POLYPLOID MITOSES BY WOUNDING ROOTS OF INTACT PLANTS.—The stimulation of polyploid mitoses in intact roots by wounding is due to stimuli of unknown source. As early as 1905 NEMEC reported observing mitotic figures with high chromosome number in wounded roots. More recent reports include those of GRAFL (1939) and D'AMATO (1948). Stimulation by wounding is complex to interpret, since wounding may involve an increased access of cells to the surrounding solution, the release of substances from the wound which may stimulate mitotic activity, or interference with normal transport within the organ.

Roots of seedling pea plants grown in dilute inorganic solution and wounded by passing a needle through the mature tissues of the root were sampled from the wound site at 24, 48, and 72 hr after wounding. The occurrence of diploid and polyploid mitoses was compared with samples taken from intact roots of the same age. Table 1 shows relative frequencies of mitoses in roots sampled from such a time sequence.

It is clear from these results that wounding initiated mitoses in both diploid and polyploid cells of seedling roots which showed no polyploid mitoses when unwounded. Polyploid mitoses were observed at all three sampling times and were most frequent at 48 hr. They were much less frequent, however, than diploid mitoses at any of the sampling times. Chromosome counts indicated that the polyploid cells were mostly tetraploid. These results raised the question of the importance of wound stimulation per se in the mitotic activity of root segments excised and cultured in vitro as well as the position of the wound relative to the root axis. Therefore, experiments were designed to test the effect of wounding at cut surfaces.

TABLE 1

RELATIVE MITOTIC ACTIVITY IN INTACT AND WOUNDED PEA SEEDLING ROOTS

TIME AFTER TREATMENT (HR)	MITOSES[a]	
	Diploid	Polyploid
Intact roots:		
24	+	0
48	+	0
72	+	0
Wounded roots:		
24	+++	++
48	+++	++
72	+++	+

[a] Mitotic frequencies are reported as follows (based on number of mitotic figures counted): 0 = none; + = 1–10; ++ = 11–20; +++ = >20 mitoses counted per slide.

EFFECT OF THE POSITION OF THE CUT SURFACE.—Segments of 1 mm were cut from the tenth millimeter behind the root tip of pea seedlings germinated for 60 hr. In the standard treatment these segments were placed with one cut surface down on the surface of solid agar S-2 medium with 1 mg/l kinetin so that the vascular cylinder was oriented vertically (fig. 1*a*). When these 1-mm segments were cut in half along the vascular cylinder and placed with one cut surface down on the same medium so that the cut vascular cylinder was oriented horizontally (fig. 1*b*), then the frequencies of diploid and polyploid mitoses after 74 hr were the same as those of the standard.

Thus the horizontal cut and the horizontal, as opposed to the vertical, orientation of the segment had no effect on the frequency of diploid and polyploid mitoses.

In additional experiments, 1-mm segments were cut in half longitudinally along the vascular cylinder, and both halves were cultured on 0.7% agar S-2 medium with 1 mg/l kinetin. One half was placed with the longitudinal cut surface down on the medium (fig. 1*b*), and the other half was placed with the intact epidermis down on the medium and the longitudinal cut surface up (fig. 1*d*). The segments were cultured for 72 hr and then fixed. The frequencies of diploid and of polyploid mitoses observed in the segments with the longitudinal cut surface down were the same as those observed in the 1-mm vertical segments. The frequency of diploid mitoses in segments with the cut surface up was slightly less than the standard, but a significant number of diploid mitoses was still observed. Very few polyploid mitoses were observed in these segments. The results of this experiment are shown in table 2.

These observations would indicate that the presence of a wounded surface alone is not sufficient to elicit polyploid mitoses. Indeed, the S-2 medium with 1 mg/l kinetin only stimulates polyploid mitoses in root segments if a cut surface of the segment touches the medium. Thus, the stimulatory substances evidently are unable to pass through the intact epidermis of the root. This observation would explain all of the failures to stimulate polyploid mitoses in intact roots by chemical treatment.

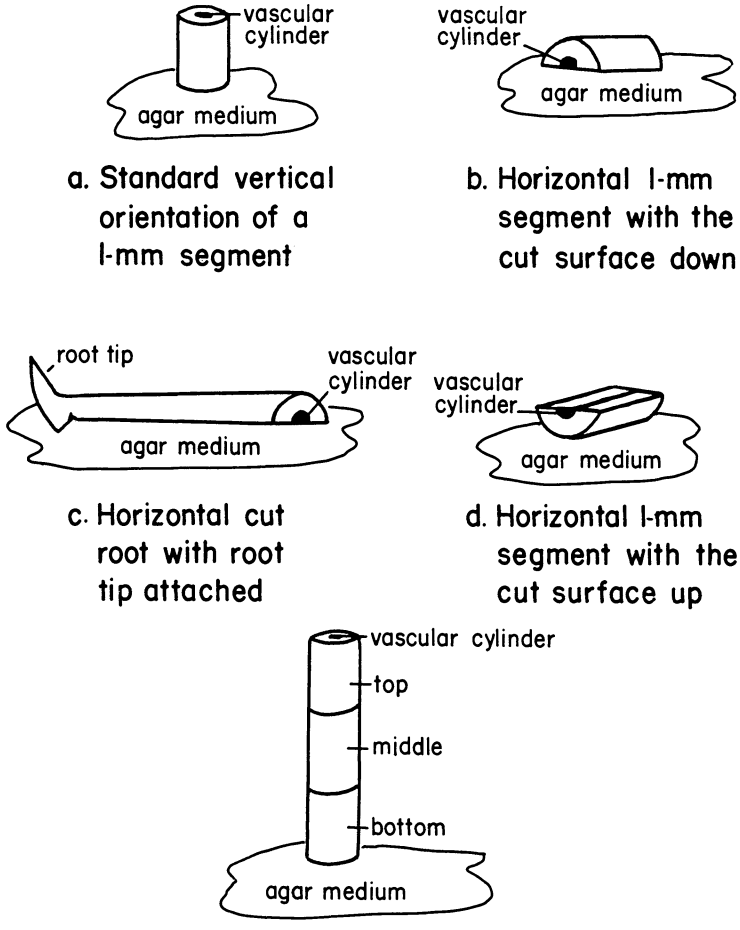

FIG. 1.—Orientations of root segments in tissue culture

EFFECT OF THE ROOT TIP.—Experiments were carried out to test whether the presence of the root tip had an effect on the initiation of polyploid mitoses in treated roots. Roots 10–15 mm in length were excised from seedlings 60 hr old. The roots were split in half longitudinally from the second millimeter behind the tip to the basal end. This procedure gave two pieces from each root, one with an attached

intact root tip and the other without the root tip. These pieces were placed with the longitudinal cut surface down on 0.7% agar S-2 medium with 1 mg/l kinetin (fig. 1*c*). The tenth millimeter behind the root tip was excised and fixed after 72 hr in culture. The frequencies of diploid and polyploid mitoses were the same in pieces with and without the root tip. This result suggests that the root tip has no effect on polyploid mitoses 10 mm behind the tip and that the failure to induce polyploid mitoses in whole intact roots is not due to an inhibition of polyploid mitoses by the root tip. However, this conclusion must be tentative, since the cuts which were made to produce these root pieces injured the vascular tissue. This damage could prevent inhibitory substances from the root tip from reaching the tenth millimeter behind the tip in the cut root pieces.

EFFECT OF USING 1-MM SEGMENTS FROM VARIOUS PARTS OF THE ROOT.—Segments of 1 mm were excised from 0–1 mm, 2–3 mm, 4–5 mm, 6–7 mm, 8–9 mm, 10–11 mm, 12–13 mm, and 14–15 mm behind the root tip of 60-hr-old pea seedlings. These segments were cultured on the S-2 medium with 1 mg/l kinetin in liquid filter paper cultures and fixed after 72 hr. Approximately the same frequency of diploid mitoses was observed in all segments. No polyploid mitoses were observed in the first segment (0–1 mm). Approximately the same frequency of polyploid mitoses was observed in all other segments.

In another experiment whole pea seedlings germinated for 60 hr were placed aseptically in wire racks in tubes with the root dipping into sterile double-distilled water. After 72 hr, 1-mm segments were excised 10, 20, 25, 30, 35, and 40 mm behind the root tip. Some of these segments were fixed at once. The others were placed aseptically in liquid filter paper culture tubes with S-2 medium with 1 mg/l kinetin. After 72 hr in culture they were fixed.

Some diploid mitoses were observed in all the segments fixed immediately after excision. No polyploid mitoses were observed in any of these segments. An increased frequency of diploid mitoses was observed in all segments after 72 hr in culture on S-2 medium with 1 mg/l kinetin. The frequencies of diploid mitoses in the various segments were approximately the same. Polyploid mitoses were also observed in all segments after 72 hr in culture. There were no significant differences in their frequencies in different segments.

Thus no polyploid mitoses were observed in untreated roots, although a few diploid mitoses were observed as far as 40 mm behind the root tip. Polyploid mitoses were observed in treated 1-mm segments excised 2–40 mm behind the root tip. No polyploid mitoses were seen in the first millimeter of the root tip. These results can be compared with the data of MCLEISH and SUNDERLAND (1961) on the occurrence of cells with DNA values above the diploid level in pea roots as determined by microspectrophotometric absorption measurements. They found only cells with the diploid amount of DNA in the first two millimeters of the pea root and an increasing frequency of cells with larger amounts of DNA 2–8 mm behind the root tip. Thus the polyploid mitoses which can be stimulated in excised pea root segments could be mitoses of pre-existing polyploid cells.

EXPERIMENTS USING 3-MM ROOT SEGMENTS.—Segments 3 mm in length were excised 10–13 mm behind the root tip of 60-hr-old pea seedlings. These segments were cultured on agar S-2 medium with 1 mg/l kinetin. They were placed with one of the cut surfaces down on the surface of the agar medium so that the vascular cylinder was oriented vertically (fig. 1*e*). It was noted whether the apical (toward the root apex) or the basal end of the root segment was placed in contact with the medium. After 74 hr in culture those segments which had fallen over were discarded. The segments which had remained upright were cut into three 1-mm pieces, that is, top, middle, and bottom. These pieces were fixed, squashed, and scored separately. The results of these experiments are shown in table 3. The standard used was 1-mm segments placed with the vascular cylinder vertically on the same agar S-2 medium with 1 mg/l kinetin. A significant number of diploid mitoses were observed in all three pieces whether the apical or the basal end of the long segment had been placed down on the surface of the medium. There were fewer diploid mitoses in the top piece than in the bottom piece with either orientation. A significant number of polyploid mitoses were observed only in

TABLE 2

EFFECT OF THE POSITION OF THE CUT SURFACE IN ROOT SEGMENTS SPLIT ALONG THE AXIS AND PLACED HORIZONTALLY ON THE MEDIUM

POSITION	MITOSES[a]	
	Diploid	Polyploid
Cut surface down.....	*S*	*S*
Cut surface up.....	*S*−*	+/−
	+*	.....

[a] Mitotic frequencies are reported as follows: 0 = none; +/− = no significant difference from none; + = greater than none; *S*− = less than standard; *S* = no significant difference from standard. For a standard, 1-mm segments were placed vertically on the S-2 medium with 1 mg/l kinetin.

* These results are significant at the .01 level. The statistical methods used are described in the text.

the bottom piece. This observation suggests that some substance or substances from the medium which are necessary for the stimulation of polyploid mitoses do not penetrate more than about 1 mm into the tissue. The lack of effect of the orientation of the segments (apical or basal end down) suggests that this substance is not transported but that cells at either end of the 3-mm segment can be induced to divide as polyploid cells if exposed directly to both

TABLE 3

EFFECT OF ORIENTATION AND DISTANCE FROM SURFACE OF THE CULTURE MEDIUM IN THREE-MILLIMETER ROOT SEGMENTS

Orientation and piece	Mitoses[a]	
	Diploid	Polyploid
Apical end down:		
Top	$S-$*	$+/-$
	$+$*	
Middle	$S-$	0
	$+$*	
Bottom	S	$S-$*
		$+$*
Basal end down:		
Top	$S-$*	$+/-$
	$+$*	
Middle	S	$+/-$
Bottom	S	S

[a] Mitotic frequencies are reported as follows: 0 = none; $+/-$ = no significant difference from none; $+$ = greater than none; $S-$ = less than standard; S = no significant difference from standard. For a standard, 1-mm segments were placed vertically on the S-2 medium with 1 mg/l kinetin.

* These results are significant at the .01 level. The statistical methods used are described in the text.

auxins and kinetin. However, it should be noted that the frequencies of both diploid and polyploid mitoses tended to be slightly higher in pieces of segments oriented with the basal end down. This may be due to a slightly better penetration of the stimulatory substances into the root tissue or to the fact that segments with the basal end down are more stable and make better contact with the medium than segments with the apical end down, since, in general, the basal end is larger than the apical end.

The identity of the substance which is required for the initiation of polyploid mitoses, and which does not penetrate more than about 1 mm into the pea root tissue, is not known. It is known that the appearance of polyploid mitoses is observed in segments cultured in the presence of auxin, kinetin, salts, sucrose, and vitamins in the culture medium (TORREY, 1961; MATTHYSSE and TORREY, 1967*a*). Since there is no reason to suppose that the penetration of sucrose, salts, or vitamins should be difficult in this tissue, the most probable limiting substance from this list of requirements seems to be either the auxins, 2,4-D and IAA, or kinetin. Kinetin transport rather than auxin transport might be expected to be the limiting factor, however, since where kinetin transport has been studied, it has been found generally to be rather low (SETH, DAVIES, and WAREING, 1966), whereas BONNETT (1964) found that there was auxin (IAA) transport in *Convolvulus* root segments excised from the region of the root used in these studies (10–20 mm behind the root apex).

Cells of intact roots are not stimulated to divide by externally provided hormonal substances because they are unable to penetrate the root epidermis in the region of the mature tissues.

Since the wounding produced by cutting root segments for culture on S-2 medium does not elicit polyploid mitoses, one must conclude that the stimulation of polyploid mitoses by wounding of intact seedling roots involves an effect of the needle wound on transport of materials which can elicit polyploid mitoses. Synthesis of cytokinins in roots seems now well established, and their movement from root tips to the shoot via the vascular system has been demonstrated (see review by KENDE and SITTON, 1967). It seems probable that the wound caused by the needle actually blocks the normal flow of substances in the vascular tissue. A localized increase in concentration of these substances would result in mitoses of polyploid cells.

One is led to suggest that, in pea, endogenous cytokinin produced in the root tip and transported from the root to the shoot is the material whose transport is blocked by the wound and whose accumulation leads to polyploid mitoses at the site of the wound.

LITERATURE CITED

BONNETT, H. T. 1964. Hormonal control of endogenous bud and root formation in *Convolvulus* roots. Ph.D. thesis. Harvard University.

D'AMATO, F. 1948. Cytological consequences of decapitation in onion roots. Experientia **4**:388.

———. 1952*a*. New evidence on endopolyploidy in differentiated plant tissues. Caryologia **4**:121–144.

———. 1952*b*. Polyploidy in the differentiation and function of tissues and cells in plants. *Ibid.* **4**:311–358.

———. 1964. Endopolyploidy as a factor in plant development. Caryologia **17**:41–52.

DEELEY, E. M., H. G. DAVIES, and J. CHAYEN. 1957. The DNA content of cells in the root of *Vicia faba*. Exp. Cell Res. **12**:582–591.

GRAFL, I. 1939. Kernwachstum durch Chromosomenvermehrung als regelmässiger Vorgang bei pflanzlichen Gewebedifferenzierung. Chromosoma **1**:265–275.

HOLZER, K. 1952. Untersuchungen zur karyologischen Anatomie der Wurzel. Österreichische Bot. Z. **99**:118–155.

KENDE, H., and D. SITTON. 1967. The physiological significance of kinetin- and gibberellin-like root hormones. Ann. N.Y. Acad. Sci. **144**:235–243.

LEVAN, A. 1939. Cytological phenomena connected with the root swelling caused by growth substances. Hereditas **25**: 87–96.

MCLEISH, J., and N. SUNDERLAND. 1961. Measurements of deoxyribonucleic acid (DNA) in higher plants by Feulgen photometry and chemical methods. Exp. Cell Res. **24**: 527–540.

MATTHYSSE, A. G., and J. G. TORREY. 1967*a*. Nutritional requirements for polyploid mitoses in cultured pea root segments. Physiol. Plantarum **20**:661–672.

———, and ———. 1967*b*. DNA synthesis in relation to polyploid mitoses in excised pea root segments cultured in vitro. Exp. Cell Res. **48**:484–498.

MELETTI, P. 1950. Reazioni cito-istologische ed effetti rizogeni in plantule di alcune leguminose trattate con 2,4-D. Nuovo Giornale Bot. Ital. (n.s.) **57**:499–514.

MOSTELLER, F., and R. R. BUSH. 1954. Selected quantitative techniques. Pp. 328–331 *in* G. LINDZEY [ed.], Handbook of social psychology. Addison-Wesley, Cambridge, Mass.

MÜHLING, G. N., J. VAN'T HOF, G. B. WILSON, and B. H. GRIGSBY. 1960. Cytological effects of herbicidal substituted phenols. Weeds **8**: 173–181.

NAGL, W. 1963. Versuche zur Mitoseauslösung in der Wurzelrinde von Pisum sativum mit Hilfe von Kinetin. Österreichische Bot. Z. **110**:371–372.

NEMEC, B. 1905. Studien über die Regeneration. Gebrüder Borntraeger, Berlin.

OINUMA, T. 1948. Cytological and morphological studies on root nodules of garden peas, *Pisum sativum* L. Seibutu **3**:155–161.

PARTANEN, C. R. 1959. Quantitative chromosomal changes and differentiation in plants. Pp. 21–45 *in* D. RUDNICK [ed.], Developmental cytology. Ronald, New York.

———. 1963*a*. The validity of auxin-induced divisions in plants in evidence of endopolyploidy. Exp. Cell Res. **31**: 597–599.

———. 1963*b*. Plant tissue culture in relation to developmental cytology. Int. Rev. Cytol. **15**:215–243.

RAGGIO, M., and N. RAGGIO. 1956. A new method for the cultivation of isolated roots. Physiol. Plantarum **9**:466–469.

REINERT, J., and H. J. KÜSTER. 1966. Diploide, chlorophyllhaltige Gewebekulturen aus Blättern von *Crepis capillaris* (L). Wallr. Z. Pflanzenphysiol. **54**:213–222.

SETH, A. K., C. P. DAVIES, and P. F. WAREING. 1966. Auxin effects on the mobility of kinetin in the plant. Science **151**:587–588.

STOMPS, T. J. 1911. Kernteilung und Synapsis bei *Spinacia oleracea*. Biol. Centralblatt **31**:257–309.

TANG, Y. W., and J. BONNER. 1947. The enzymatic inactivation of indoleacetic acid. I. Some characteristics of the enzymes contained in pea seedlings. Arch. Biochem. **13**: 11–25.

TORREY, J. G. 1961. Kinetin as trigger for mitosis in mature endomitotic plant cells. Exp. Cell Res. **23**:281–299.

TSCHERMAK-WOESS, E., and R. DOLEŽAL. 1953. Durch Seitenwurzelbildung induzierte und spontane Mitosen in den Dauergeweben der Wurzel. Österreichische Bot. Z. **100**: 358–402.

WIPF, L. 1939. Chromosome numbers in root nodules and root tips of certain Leguminosae. BOT. GAZ. **101**:51–67.

WIPF, L., and D. C. COOPER. 1938. Chromosome numbers in nodules and roots of red clover, common vetch, and garden pea. Nat. Acad. Sci. (U.S.), Proc., **24**:87–91.

———, and ———. 1940. Somatic doubling of chromosomes and nodular infection in certain Leguminosae. Amer. J. Bot. **27**:821–824.