Division, Growth And Differentiation Of Cells In The Root Of Vicia Faba Artificially Inhibited From Further Elongation

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DIVISION, GROWTH AND DIFFERENTIATION OF CELLS IN THE ROOT OF *VICIA FABA* ARTIFICIALLY INHIBITED FROM FURTHER ELONGATION[1]

Hitoshi KOJIMA

I. INTRODUCTION

The growth of a plant body is the result of the growth of the cells of which it is composed. The growth of the cells must be considered in two ways, i. e. increase in cell number and increase in cell size. If the elongation of the root were inhibited partially or absolutely by external pressure, what would be the results in the processes of increase in the number and length of the cells? One is grateful to several authors[2] who have already published their information concerning this question. Yet there remain several points that must be more critically discussed. This paper is the record of the writer's investigations regarding this subject during several years, and though they are not yet complete, he hopes that it may contribute to a better understanding concerning this question.

The writer wishes to acknowledge his gratitude and indebtedness to Professor Dr. R. KÔKETSU, who suggested the problem, for his encouragement and interest during the progress of the investigation.

II. EXPERIMENT ON THE ROOT EMBEDDED IN GYPSUM

Materials and methods

As material for the experiment seeds of *Vicia Faba*, nearly equal

[1] Contributions from the Botanical Laboratory, Kyushu Imperial University, No. 37.

[2] PFEFFER (1893), HERING (1896), KNY, (1896, 1902), POPOVICI (1900), HOTTES (1901, 1929), HALLBAUER (1909), STÅLFELT (1921) etc.

in size and form were selected. The seeds were soaked in tap-water for 23½ to 50 hours in order to make the seed-coat absorb sufficient water and swell; then sown in a box containing moist saw-dust, loosely packed. The box was placed in an incubator of constant temperature electrically regulated, and the seeds were allowed to germinate. Seedlings of as equal a root length as possible were chosen. The roots were embedded in gypsum as applied by PFEFFER (1893) and others. A paste of gypsum, obtained by adding water to plaster of Paris (gypsum ustum), was spread one or two cm thick on a glass plate, and the young roots of the chosen seedlings were laid on the paste so that the cotyledons were never embedded, then the roots were embedded by the addition of another layer of paste of gypsum over them and covered with another glass plate. The gypsum paste soon became stiff. The materials thus embedded were buried vertically in moist saw-dust and placed in an incubator of constant temperature. The gypsum thus hardened is referred to hereafter in this paper as "hard gypsum" for convenience; and on the contrary gypsum treated in the following manner is described as "soft gypsum." The soft paste of gypsum was procured by mixing sufficient water with plaster of Paris, kneading and watering it in a mortar. The soft gypsum never harden so long as it is kept in moist saw-dust. In this soft gypsum the roots of the seedlings were embedded and the materials were placed vertically in the moist saw-dust in the incubator. After a definite period of time, the blocks of gypsum were broken carefully with a hammer and knife, and the roots were freed.

The seedlings used as controls for comparison were placed without treatment with gypsum in a vertical position in the same saw-dust.

The elongation of the root during the research period was measured by reading the variation of the total length of the root, from the attached point of the cotyledons to the root apex. In case of need, roots were marked with Indian ink[1] at points 5 mm and 10 mm distant from the apex[2] before the experiment, and the degree of

[1] PFEFFER failed in his observation on the elongation of roots embedded in gelatine plaster, because marks of Indian ink glided on the root surface (PFEFFER, 1895 and HERING, 1896). In our case even when the root was embedded in soft gypsum the marks were not removed, but always adhered to the roots; however in one or two cases of rapid elongation of the root the lines of the marks spread and became useless.

[2] In *Vicia Faba* the boundary of the root proper and root cup is not clear (HABERLANDT, p. 84) so the writer measured the distance from the root apex.

elongation of each space at the end of the experiment was carefully measured.

To prepare them for the microscope the roots of the materials above mentioned were fixed in FLEMMING's weaker solution (CHAMBERLAIN, p. 27), and paraffin sections of 10 μ thick were made parallel to the long axis of the root, then stained with HEIDENHAIN's iron haematoxylin. ZEISS' microscope with AA × K8 or DD × K8, tube-length 160 mm, was used. The intensity of cell division was expressed, as described in the author's previous paper (KOJIMA, p. 80), by the relative number denoted by the quotient $\frac{N}{A}$; where N is the total number of mitotic figures found on five sections of root tip, sectioned parallel to the axis of the root and passing as near as possible to the axis, within the space of 3 mm from the root apex, and A is the relative value of the area examined, which is represented by the sum of the numbers indicating the diameters measured on the five sections above mentioned at distances of respectively 1, 2 and 3 mm from the root apex. The length of the cells was measured with the aid of an ocular micrometer on the sections (usually five sections for each root), which were made through the portion near the central axis of the root. The cells in resting stage were chosen for the purpose of measuring their length from the outer portion of the cortex of the root; and with regard to the cells in mitosis, because they were relatively rare, all the mitotic figures found in the cortex as well as in the central cylinder, within about 3 mm from the root apex, were counted and measured.

The differentiation of tissue in the root was determined by the existence of the tracheal element; and as the measure of the grade of this phenomenon the distance from the root apex to the point where the tracheal element began to appear was used. The lignification was also observed by the colour reaction produced by adding phloroglucin solution and hydrochloric acid to the preparations obtained by removing the paraffin from the paraffin sections. The grade of lignification of the tissue was denoted by the distance from the root apex to the point where the reaction of lignification appeared.

Results and considerations

1) The total elongation of the root.

a) The elongation of the root in a hard gypsum block was in-

variably very little. The average amounts of the total elongation of the roots embedded in hard gypsum for 4, $7\frac{1}{2}$, 12, 16, $22\frac{1}{2}$, 26, $36\frac{1}{2}$, 56, 74, $78\frac{1}{2}$, 99, 114 and $170\frac{1}{2}$ hours are given in table 1. Only on some occasions, especially when the duration of embedment was long, it was seen that the root stretched some mm. With reference to this fact, it is probable that some of the roots lengthened through cracks in the gypsum block, which were probably crazed by the increasing turgor pressure of the root as described by PFEFFER (1893); and, e.g., in the case of the roots embedded for $170\frac{1}{2}$ hours in the gypsum block (the elongation of the roots was 5 mm in one case and 2 mm in another), it was really seen that there were cracks created about the root tip. HOTTES (1929) mentioned that when a root which had suffered under the pressure of the surrounding gypsum block was brought away from the gypsum, the root grew in length to some extent because of the diminution of external pressure. The writer measured the length of the root as soon as it was freed from the block, the error of measurement caused as HOTTES suggested is therefore probably not considerable, even if any. In addition to this, the writer frequently discovered that the gypsum block in contact with the root tip was softened; such portion of the gypsum would allow the root to elongate more or less. Unless such exceptional conditions occured the hard gypsum would in all cases inhibit the elongation of the root; it may be said, therefore, that the hard gypsum block by its pressure does not permit the root to grow in length.

Table 1. Elongation of the root embedded in hard gypsum

Duration of embedment (in hours)	No. of roots observed	Mean of total elongation (in mm)	Mean of elongation per hour (in mm)	Duration of embedment (in hours)	No. of roots observed	Mean of total elongation (in mm)	Mean of elongation per hour (in mm)
4	8	0.3	0.1	56	5	2.5	nearly 0
$7\frac{1}{2}$	6	1.0	0.1	74	2	0.0	0
12	3	0.3	0.1	$78\frac{1}{2}$	5	2.0	nearly 0
16	5	0.1	nearly 0	99	4	0.6	nearly 0
$22\frac{1}{2}$	4	0.0	0	114	2	2.0	nearly 0
26	4	0.5	nearly 0	$170\frac{1}{2}$	2	3.5	nearly 0
$36\frac{1}{2}$	3	0.5	nearly 0				

b) The roots in soft gypsum elongated to some extent as seen in table 2.

Table 2. Elongation of the root embedded in soft gypsum

Duration of embedment (in hours)	No. of roots observed	Mean of total elongation (in mm)	Mean of elongation per hour (in mm)
4	7	4.9	1.2
$7\frac{1}{2}$	8	8.4	1.1
16	10	12.2	0.8

c) In table 3 the elongation of normal roots (control materials) is given. The normal roots elongated, as expected, more quickly in comparison with the roots of two groups above mentioned; for the averages of elongation per hour of the normal roots were from 1.4 to 1.6 mm, while those of the roots in hard gypsum were nearly zero and those of the roots in soft gypsum were from 0.8 to 1.2 mm.

Table 3. Elongation of the normal root (control)

Duration of leaving in saw-dust as control (in hours)	No. of roots observed	Mean of total elongation (in mm)	Mean of elongation per hour (in mm)
4	7	6.1	1.5
$7\frac{1}{2}$	8	10.6	1 4
16	10	24.3	1.6
$22\frac{1}{2}$	4	30.8	1.4

2) The intensity of cell division.

a) The intensity of cell division of the roots in a hard gypsum block is given in table 4. The roots embedded in a hard gypsum block for a shorter period had intensity of cell division to a certain degree, but those embedded longer (more than 16 hours) had very small intensity, excepting the roots which had a period of 114 hours and $170\frac{1}{2}$ hours, which showed somewhat larger intensity (in these cases

the external pressure was weakened as already mentioned above, and the cell, therefore, regained its ability for division).

Table 4. Intensity of cell division[1] of the roots embedded in hard gypsum

Duration of embedment (in hours)	No. of roots observed	No. of mitotic figures observed	Average intensity of cell division	Duration of embedment (in hours)	No. of roots observed	No. of mitotic figures observed	Average intensity of cell division
4	7	1078	14.2	$36\frac{1}{2}$	3	16	0.6
$7\frac{1}{2}$	6	521	7.9	56	2	7	0.3
12	2	43	2.1	$78\frac{1}{2}$	3	11	0.3
16	11	38	0.3	99	1	0	0
$22\frac{1}{2}$	4	9	0.2	114	2	111[2]	3.9[2]
26	3	10	0.3	$170\frac{1}{2}$	2	75[2]	2.9[2]

[1] Intensity of cell division is indicated by the quotient $\frac{N}{A}$ (%), which is explained in the text.

[2] See the explanation in the text.

b) The intensity of cell division in the roots embedded in soft gypsum is given in table 5.

Table 5. Intensity of cell division of the roots embedded in soft gypsum

Duration of embedment (in hours)	No. of roots observed	No. of mitotic figures observed	Average intensity of cell division
4	7	2282	28.7
$7\frac{1}{2}$	8	4948	54.1
16	10	2270	22.8

c) The intensity of cell division of the normal roots (controls) is seen in table 6.

Table 6. Intensity of cell division of the normal roots (controls)

Duration of leaving in saw dust as control (in hours)	No. of roots observed	No. of mitotic figures observed	Average intensity of cell division
4	7	3222	43.8
$7\frac{1}{2}$	8	6338	69.3
16	10	5138	53.2
$22\frac{1}{2}$	3	1142	38.2

d) In comparing the amount of intensity of cell division in these three groups of material we get the ratio as seen in table 7. The amounts of intensity of cell division in soft gypsum always show a smaller number in comparison with those of the control roots; and the roots in hard gypsum show extremely small amount, moreover it is to be remarked that the amount in question diminishes as the duration of embedment increases. It is presumable from these facts that the mechanical pressure caused by embedment in gypsum inhibits the cell division of the root tip, and this is in agreement with the opinion of PFEFFER (1893). HOTTES (1929) also acknowledged the inhibitory action of mechanical pressure upon cell division in the root, but he described in his paper that the cells near the tip continue to divide mitotically for approximately four days. In our case, however, the cell division in the root in hard gypsum almost ceased within 16 hours.

Table 7. Comparison of intensity of cell division found in the roots embedded in hard and soft gypsum with that found in the normal roots (control = 100)

Duration of embedment or leaving in saw dust (in hours)	Roots in hard gypsum	Roots in soft gypsum	Normal roots (controls)
4	32	66	100
$7\frac{1}{2}$	11	78	100
16	0.6	43	100
$22\frac{1}{2}$	0.5	—	100

3) The relation between the elongation of root and the intensity of cell division.

Considering the cell division together with the elongation of root we find that there is some relation between them, as seen in table 8, namely that the roots elongated in a small degree have but a slight intensity of cell division. The materials embedded in hard gypsum for 4 and $7\frac{1}{2}$ hours, in spite of little elongation, showed somewhat larger intensity of cell division. The reason for this discrepancy may be that the duration of embedment was too short to exert mechanical pressure sufficient to inhibit cell division. The comparison with the results found in materials embedded in hard gypsum for 114 and $170\frac{1}{2}$ hours is omitted in this table for the reason explained already in the previous paragraph.

Table 8. Relation between the elongation of root and the intensity of cell division

Duration of embedment or leaving in saw dust (in hours)	In hard gypsum		In soft gypsum		Control	
	Average elongation of root per hour	Average intensity of cell division	Average elongation of root per hour	Average intensity of cell division	Average elongation of root per hour	Average intensity of cell division
4	0.1	14.2	1.2	26.7	1.5	43.8
$7\frac{1}{2}$	0.1	7.9	1.1	54.1	1.4	69.3
12	0.1	2.1	—	—	—	—
16	0.1	0.3	0.8	22.8	1.6	53.2
$22\frac{1}{2}$	0	0.2	—	—	1.4	38.2
26	0	0.3	—	—	—	—
$36\frac{1}{2}$	0	0.6	—	—	—	—
56	0	0.3	—	—	—	—
$70\frac{1}{2}$	0	0.3	—	—	—	—
99	0	0	—	—	—	—

4) The length of cells in mitosis.

a) The length of cells in mitosis found in roots embedded in hard gypsum is seen in table 9, where the average lengths of cells embedded for 4, $7\frac{1}{2}$ and 16 hours are respectively 31.0, 27.5 and 26.9 μ.

Table 9. Length of cell in mitosis in the root embedded in hard gypsum

Duration of embedment (in hours)	No. of roots observed	No. of cells observed	Average length of cells (in μ)
4	5	1673	31.0
$7\frac{1}{2}$	6	492	27.5
16	9	68	26.9

b) The length of cells in mitosis in roots embedded in soft gypsum is given in table 10, where the average lengths of cells embedded for 4, $7\frac{1}{2}$ and 16 hours are respectively 27.8, 27.2 and 26.0 μ.

Table 10. Length of cell in mitosis in the root embedded in soft gypsum

Duration of embedment (in hours)	No. of roots observed	No. of cells observed	Average length of cells (in μ)
4	5	2725	27.8
$7\frac{1}{2}$	7	4021	27.2
16	10	2290	26.0

c) The length of the cells in question found in normal roots is shown in table 11. The average lengths of cells in roots which were left in saw-dust for 4, $7\frac{1}{2}$ and 16 hours as controls are respectively 30.2, 27.8 and 27.9 μ.

Table 11. Length of cell in mitosis in the normal root (control)

Duration of leaving in saw dust as control (in hours)	No. of roots observed	No. of cells observed	Average length of cells (in μ)
4	5	3301	30.2
$7\frac{1}{2}$	7	4950	27.8
16	10	5220	27.9

d) Comparison of the average lengths of cells in mitosis of roots treated with hard and soft gypsum with those of normal roots is given in table 12. Compared with the cell length of the normal root, that of the root in soft gypsum is a little smaller, and that of the root embedded in hard gypsum for 16 hours is also somewhat smaller, while that of the root embedded in hard gypsum for 4 hours is slightly larger; but on the whole there is little difference between the three kinds of material.

Table 12. Comparison of the length of cells with mitotic figures in the normal root with those in roots embedded in hard and soft gypsum

Duration of embedment or leaving in saw dust (in hours)	Relative lengths (control = 100)		
	Normal roots (controls)	Roots embedded in soft gypsum	Roots embedded in hard gypsum
4	100	92	103
7½	100	98	99
16	100	93	96

5) The length of cell with resting nucleus.

a) The length of resting cells, in roots embedded in hard gypsum, is shown in table 13.

Table 13. Length of resting cell in the root embedded in hard gypsum

Duration of embedment (in hours)	No. of roots observed	Average length[1] of resting cells (and number of cells observed given in brackets) situated at the distance from the root apex of :					
		1 mm	2 mm	3 mm	4 mm	5 mm	6 mm
4	5	22.1 (611)	18.9 (700)	28.7 (516)	52.3 (462)	74.4 (468)	94.4 (358)
7½	6	20.1 (696)	19.6 (676)	31.5 (418)	50.0 (367)	68.8 (507)	89.7 (343)
16	9	20.8 (879)	19.0 (853)	31.9 (751)	50.5 (667)	66.8 (566)	87.4 (500)

[1] The unit of length is μ.

b) The length of resting cells, in roots embedded in soft gypsum, is given in table 14.

Table 14. Length of resting cell in the root embedded in soft gypsum

Duration of embedment (in hours)	No. of roots observed	Average length (in μ) of resting cells (and number of cells observed given in brackets) situated at the distance from the root apex of:					
		1 mm	2 mm	3 mm	4 mm	5 mm	6 mm
4	5	20.3 (577)	18.5 (551)	30.1 (486)	52.6 (486)	82.0 (485)	107.4 (293)
7½	8	20.7 (542)	18.1 (906)	27.9 (489)	48.2 (466)	74.7 (566)	103.1 (431)
16	10	20.1 (954)	16.6 (1218)	28.7 (544)	51.7 (419)	80.5 (556)	100.8 (464)

c) The length of resting cells in the normal (control) roots is indicated in table 15.

Table 15. Length of resting cell of the normal root (control)

Duration of leaving in saw dust as control (in hours)	No. of roots observed	Average length (in μ) of resting cells (and number of cells observed given in brackets) situated at the distance from the root apex of:					
		1 mm	2 mm	3 mm	4 mm	5 mm	6 mm
4	5	24.0 (516)	19.2 (623)	26.5 (574)	46.5 (503)	71.1 (443)	99.1 (283)
7½	7	21.5 (509)	18.9 (827)	30.9 (501)	50.9 (453)	70.5 (547)	95.6 (345)
16	10	21.4 (841)	18.8 (1276)	28.2 (620)	53.9 (548)	83.8 (559)	114.1 (430)

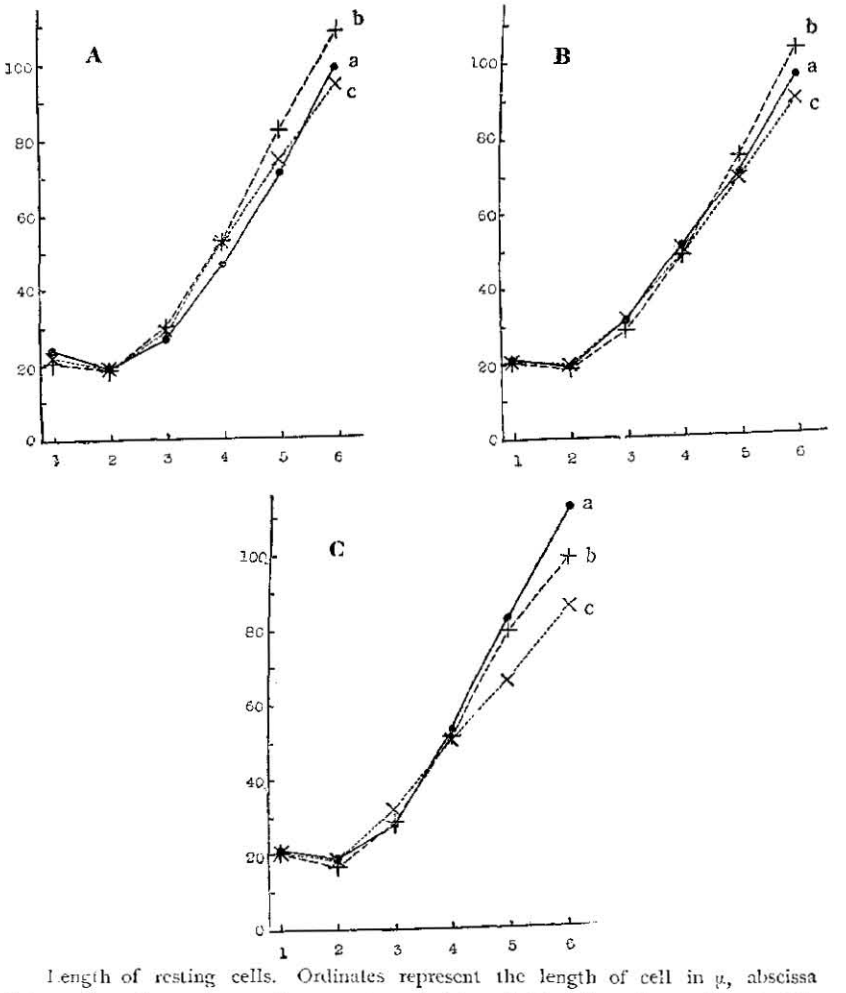
d) To compare the cell length of the three different kinds of materials the relative amounts are given in table 16 and the real amounts are plotted in fig. 1, in three separate groups according to the duration of embedment.

Table 16. Comparison of the length of resting cells in the normal root with those in roots embedded in hard and soft gypsum

Duration of embedment or leaving in saw dust (in hours)	Kind of materials	Relative lengths of resting cells situated at the distance from the root apex of (control = 100):					
		1 mm	2 mm	3 mm	4 mm	5 mm	6 mm
4	Normal roots (control)	100.0	100.0	100.0	100.0	100.0	100.0
"	Roots in soft gypsum	84.5	96.3	113.5	113.1	115.3	108.3
"	Roots in hard gypsum	92.0	98.4	108.3	112.4	104.6	95.2
$7\frac{1}{2}$	Normal roots (control)	100.0	100.0	100.0	100.0	100.0	100.0
"	Roots in soft gypsum	96.2	95.7	90.2	94.6	105.9	107.8
"	Roots in hard gypsum	93.4	103.7	101.9	98.2	97.5	93.8
16	Normal roots (control)	100.0	100.0	100.0	100.0	100.0	100.0
"	Roots in soft gypsum	93.9	88.2	101.7	95.9	96.0	88.3
"	Roots in hard gypsum	97.1	101.0	113.1	93.6	79.7	76.5

In all cases the length of the cells at a distance of 2 mm from the root apex is shortest; considering this fact together with the fact that this point almost coincides with the growing point of the root, it is presumable that the daughter cells newly formed by cell divisions may accumulate most abundantly about this point. And under all circumstances in the three groups the length of the cells at a distance of 2 mm from the apex in the roots embedded in soft gypsum is shorter than the corresponding length of the other two, though in a slight degree. As a whole, in all cases the three curves run parallel with each other, but the curves show that the cell length at a distance of 6 mm from the apex in the roots in hard gypsum is always less than those of the other two. The parallelism of the curves in fig. 1 might be caused by the fact that, though the speed of elongation of cells may differ according to the external pressure and may cause differences of cell length, yet the differences are not appreciable, because the ages of the cells observed at the

Fig. 1



Length of resting cells. Ordinates represent the length of cell in μ, abscissa distance from the root apex in mm. A, materials embedded for 4 hours; B, materials embedded for $7\frac{1}{2}$ hours; C, materials embedded for 16 hours. Curve a, roots in hard gypsum; b, roots in soft gypsum; c, control roots.

same distance from the root apex differ from one another. In other words a cell at a given point in a root embedded in gypsum is not the same age as a cell at the same distance from the apex of the normal root. The former cell may elongate somewhat more slowly than the latter, but the age or length of time needed to elongate is greater than that of the latter. As regards the cell length

at the distance of 6 mm from the apex of the root embedded in hard gypsum, especially in the cases of longer duration of embedment, it may perhaps be explained as follows :— The cells situated at a greater distance from the root apex must have passed through the larger part of their elongation, and the differences of the length of cells differently treated may appear more clearly in this case. This might be at least one reason among other factors bearing upon our problem.

e) On the other hand, the fact that the average lengths of cells at the same distance from the apex in the roots, treated under different conditions, are similar to each other, must show that the cells of materials, which elongate slowly in the gypsum, are shorter than those of the same age in the control materials, in which the cells are far more basipetal than the cells of the same age in the former roots, for the reason above mentioned.

6) Relation of cell division and cell length.

The length of cells in mitosis as well as of resting cells at the growing point of the roots embedded in hard gypsum is nearly equal to that in the normal root ; neverthless, the intensity of cell division in the former is very little compared with that in the latter. On the other hand, the cell length of a root embedded in soft gypsum, in which cell division is somewhat abundant, is a little less than the normal root and that in hard gypsum. In other words there is little difference in the cell length of the three different kinds of material, though the difference of the intensity of cell division is remarkable among them. It can not, therefore, be said that the small size of the cell is strictly related to the scantiness of the cell division, rather it may be thought that the pressure itself caused by the gypsum treatment inhibits the cell division in the materials treated with gypsum. And it may also be mentioned that the smallness itself of the size of the cell does not cause the inhibition of the cell division in the cases under consideration. These results coincide with the conclusions of HAMMETT (1929) and RICHARDS (1928) about the relationship of cell division and cell size.

7) The differentiation of cell.

a) The fact that the tracheal elements appear nearer to the apex of the root, when embedded in a hard gypsum block, has already been described by PFEFFER (1893), HERING (1896), NATHANSOHN (1898), HALLBAUER (1909) and HOTTES (1929), and the results in our case are shown in table 17, column A. And with the increase of the duration

of embedment, the tracheal elements appear nearer to the apex, namely the average distance in question is found to be 5.0–1.4 mm, while the average amount of distance found in five normal roots as control is 13.7 mm.[1]

The lignification of the cell wall of tracheal elements also moved in an acropetal direction as shown in table 17, column B, the average distance of the first point of lignification from the root apex being namely 4.5–1.5 mm, while the lignification in the control material advanced to the point 26.0 mm (average amount of five roots) from the apex. Thus it may be said that the progress of cell differentiation in the root embedded in hard gypsum is more rapid than that in the normal root, though the cell division and cell elongation in the former root is practically inhibited.

Table 17. Average distances from root apex to the points, where the tracheal elements begin to appear or the tracheal elements begin to show lignification, in the roots embedded in hard gypsum

Duration of embedment (in hours)	No. of roots observed	A Average distance from root apex to the point where the tracheal elements begin to appear (in mm)	B Average distance from root apex to the point where the tracheal elements begin to show lignification (in mm)
12	3	3.8	—
16	5	5.0	—
24	2	4.0	4.5
26	4	3.7	—
36½	3	2.6	—
49	5	3.1	3.5
56	4	2.6	—
73	2	2.7	3.3
114	2	1.6	2.5
170½	2	1.4	1.5

[1] PFEFFER (1893) noticed that the tracheae appear at the position 25–35 mm from the apex in a normal *Vicia*-root, and according to KOERNICKE (1905) the tracheal elements begin first about 20 mm from the apex, and according to PEKAREK (1927) the tracheae begin to appear from 18–22 mm from the apex.

b) The above mentioned explanation is derived from the consideration of the facts of the distance from the root apex; but by considering the age of the individual cell, we may come to another conclusion. The root in a gypsum block is hardly elongated at all; so the age of the cells even at the point of 5 mm from the apex, e.g, in the case of embedment for 16 hours, is older than 16 hours. On the other hand the normal root elongates 24 mm during 16 hours, so the age of the cells at 13.7 mm from the apex, where the tracheal element begins to appear, must be far less than 16 hours. And the same remark can be made upon the lignification of the cell wall. It may be said, therefore, that the progress in regard to speed of cell differentiation in the root embedded in hard gypsum is hindered to a certain extent by gypsum pressure. For this reason one must say that the cell differentiation in the root tip treated with gypsum is not absolutely, but only in a relative sense, emphasized.

III. EXPERIMENT ON THE ROOT IN CANE-SUGAR SOLUTION

Materials and methods

After making marks on the root with Indian ink at 5 mm and 10 mm from the root apex, the seedling, produced in the same way as described in the former chapter, is fixed by a pin to a wooden frame in a glass cylinder, containing cane-sugar solution of a certain concentration, so that the root is immersed in the solution in a vertical position, and the cotyledons are above the surface of the solution. The top of the glass cylinder was covered with a piece of paraffin paper to prevent excessive evaporation from solution and cotyledons. The methods of observation on cell division, cell length and cell differentiation are the same as in chapter II.

Results and considerations

1) The total elongation of the root.

Elongation of the roots immersed for $22\frac{1}{2}$, 24, 25 and $25\frac{1}{2}$–28 hours respectively in sugar solutions, tap water and distilled water is seen in table 18; though there are some differences amongst the absolute amounts found in those four kinds of materials, yet it will be found that the elongation in a more concentrated solution is less than that in a more diluted solution, tap water or distilled water, as would be

Table 18. Elongation of the roots immersed in sugar solutions and water

Solution	Duration of immersion (in hours)	No. of roots observed	Average total elongation of roots (in mm)	Average elongation per hour (in μ)
Experiment I				
20 % sugar solution	22½	2	nearly 0	nearly 0
10 % sugar solution	22½	2	18.8	817
Tap water	22½	2	21.0	933
Experiment II				
15 % sugar solution	25	3	0.2	8
12.5 % sugar solution	25	3	3.3	132
10 % sugar solution	25	3	7.0	280
5 % sugar solution	25	3	16.7	668
Tap water	25	3	16.3	652
Experiment III				
13.6 % sugar solution	24	12	5.0	263
11.5 % sugar solution	24	6	11.0	458
9.4 % sugar solution	24	12	12.0	500
7.1 % sugar solution	24	8	14.0	583
4.8 % sugar solution	24	11	15.0	625
2.4 % sugar solution	24	6	15.0	625
1 % sugar solution	24	12	18.0	750
Distilled water	24	12	15.0	625
Experiment IV				
20 % sugar solution	25½	9	−1.3	−51
15 % sugar solution	25½	10	−0.5	−20
10 % sugar solution	27½	9	4.8	175
5 % sugar solution	28	9	19.3	689
Tap water	27	5	22.2	822

expected. POPOVICI (1900) tried to inhibit the elongation of the root, by use of KNO_3-solution, diminishing the turgor pressure of it. POPOVICI used 0.5 % solution[1] and found no effect produced upon the elongation of root of *Vicia Faba*; but could see that 1 % and 1.5 % solutions[2] inhibit the elongation to a certain degree. In the present investigation it was observed that the sugar solutions, stronger than 15 %, inhibited the elongation of the root perfectly or almost perfectly.

2) The intensity of cell division.

The average amount of the intensity of cell division, immersed in solutions, is seen in table 19; as a whole, roots in solutions of higher concentration show lower amount and the roots in diluted solutions or

Table 19. Intensity of cell division in roots immersed $22\frac{1}{2}$, 25 and 24 hours long in solutions of several concentration

Solution	Duration of immersion (in hours)	No. of roots observed	Average intensity of cell division
Experiment I			
20 % sugar solution	$22\frac{1}{2}$	2	0.5
10 % sugar solution	$22\frac{1}{2}$	2	19.5
Tap water	$22\frac{1}{2}$	2	37.5
Experiment II			
15 % sugar solution	25	3	0.6
12.5 % sugar solution	25	3	4.8
10 % sugar solution	25	3	8.9
5 % sugar solution	25	3	35.1
Tap water	25	3	26.4
Experiment III*			
13.6 % sugar solution	24	4	1.5
9.4 % sugar solution	24	4	5.4
7.1 % sugar solution	24	3	28.2

[1] The pressure of 0.5 % KNO_3-solution is nearly 1.75 atm. p., and approximately equivalent to the pressure of 2.5 % cane-sugar solution (PFEFFER, 1900, p. 146).

[2] 1 % KNO_3-solution is approximately equivalent to 5.1 % cane-sugar solution; 1.5 % KNO_3-solution is approximately equivalent to 7.6 % cane-sugar solution.

4.8 % sugar solution	24	3	24.8
2.4 % sugar solution	24	4	47.2
1 % sugar solution	24	4	49.4
Distilled water	24	4	51.1

* Only the roots selected as follows were observed :—roots elongated shorter from materials immersed in higher concentrated solutions ; roots elongated longer from those in lower concentrated solutions and water; and roots elongated medially from those in medial concentrated solutions.

water show higher amount, though there were some variations among the results found in the experiments of the three different series.

3) The relation between the elongation of root and intensity of cell divison.

Regarding the intensity of cell division, the roots immersed in solutions of more than 15 % concentration showed us only a slight degree of intensity or practically none, in the same way as in the case of the elongation of the root. STÅLFELT (1921) recorded that the osmotic pressure of a normal root of *Vicia Faba* is equivalent to that of $\frac{25}{100}$ n KNO_3 -solution (nearly equal to that of 12.8 % solution of cane-sugar). It is possible that in the case under consideration the 15 % and 20 % cane-sugar solutions were hypertonic for the root, and it is reasonable that the roots immersed in solutions of more than 15 % concentration should hardly show any cell division or elongation. And further, in cases of roots in diluted solutions or water, comparing the elongation of the root and the intensity of cell division, we find that there is an intimate correlation between those two phenomena, namely the roots of rapid elongation show large amount of intensity of cell division and that of slower elongation show small amount.

4) The length of cell.

a) The length of a resting cell at 5 mm from the apex of the root immersed for $22\frac{1}{2}$ hours is shown in table 20, where it is seen that the length of the cell in the root in 20 % sugar solution is shortest and that of the root in water is longest. And the length of the cells in the resting stage at 1 mm, 2 mm, 5 mm and 10 mm from the apex of the root immersed in sugar solutions and distilled water for 24 hours, is seen in table 21, column A. Generally speaking there is no remarkable regular variation in the length of cells at 1 or 2 mm from the

Table 20. Length of resting cell of the root immersed for $22\frac{1}{2}$ hours in sugar solution or water

Solution	No. of roots observed	Average length[1] of cells at 5 mm apart from the apex at the end of immersion (no. of cells observed given in brackets)
20 % sugar solution	2	101 (234)
10 % sugar solution	2	195 (146)
Tap water	2	230 (197)

1) The unit of length is μ.

Table 21. Length of resting cells in the root immersed for 24 hours in sugar solution or distilled water

Solution	No. of roots observed	A Average length[1] of cells at different distances from root apex, at the end of immersion, namely of distances:—				B Average length[1], measured at the end of immersion, of cells situated at the points, a and b, corresponding respectively to the points 5 and 10 mm apart from the apex of the root at the beginning of immersion. Namely at the points :—	
		1 mm	2 mm	5 mm	10 mm	a	b
13.6 % sugar solution	4	23 (433)[2]	23 (410)	76 (369)	162 (327)	76 (369)[2]	162 (327)
11.5 % sugar solution	2	24 (208)	25 (220)	70 (216)	151 (212)	100 (213)	201 (182)
9.4 % sugar solution	4	25 (467)	26 (433)	81 (409)	163 (409)	125 (418)	205 (383)
7.1 % sugar solution	3	24 (303)	28 (300)	115 (276)	146 (299)	158 (301)	216 (281)
4.8 % sugar solution	3	21 (306)	25 (302)	118 (298)	180 (300)	172 (308)	191 (299)
2.4 % sugar solution	4	25 (405)	26 (422)	98 (402)	173 (405)	199 (412)	223 (403)
1 % sugar solution	4	22 (414)	24 (438)	124 (216)	213 (242)	253 (347)	263 (315)
Distilled water	4	21 (406)	21 (463)	122 (267)	188 (203)	215 (386)	238 (393)

1) The unit of length is μ.

2) Figures in the brackets are number of cells observed.

apex of roots due to the concentration of the solution, though there is a certain inclination for the cell at 5 and 10 mm from the apex of the root in diluted solution or water to be longer than that of the root in stronger solution. The above mentioned observation was made with equidistant measurements at the end of the experiment. As the rate of elongation varies with the concentration of the solution in which the roots are immersed, the age of the cells at the same distance from the root apex must differ according to the kind of material. Though, however, the individual cell of the root in a concentrated solution might elongate more slowly than that in a diluted solution, the final result obtained by the equidistant observation showed no considerable difference between their cell length, because the duration of time needed for the elongation of the former cell is greater than that of the latter.

b) In table 22 the length—measured at the end of immersion—of a resting cell situated at points corresponding to the points 5 and 10 mm from the root apex at the beginning of the immersion, is shown. The cell length of the root in the concentrated solution is clearly less than that in the diluted solution or water. And the difference between the cells of different kinds of materials is more remarkable at the distance

Table 22. Average length, measured at the end of immersion, of resting cells situated at the positions corresponding to the points 5 mm and 10 mm from the root apex at the beginning of the immersion; the duration of the immersion is 25 hours.

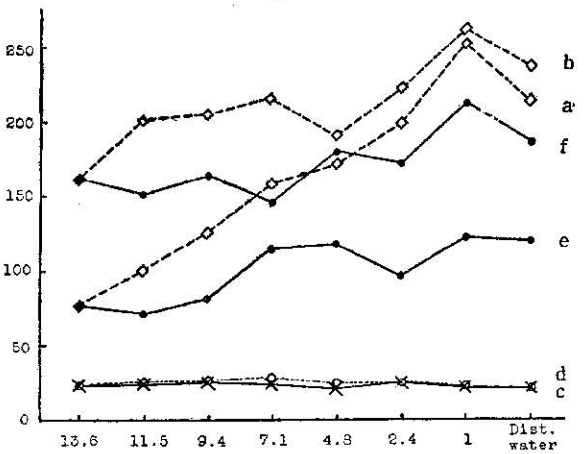
Solution	No. of roots observed	Average length[1] of cells at the positions corresponding to the different points apart from the root apex at the beginning of immersion respectively:	
		5 mm	10 mm
15 % sugar solution	3	94 (288)[2]	169 (152)[2]
12.5 % sugar solution	3	91 (249)	181 (150)
10 % sugar solution	3	126 (211)	179 (140)
5 % sugar solution	3	173 (193)	202 (97)
Tap water	3	215 (191)	248 (151)

1) The unit of length is μ.

2) The number of cells observed is given in the brackets.

of 5 mm from the root apex than at the distance of 10 mm from the apex. This fact may be explained as follows :—cells at the point 10 mm from the apex are more advanced in growth than those at 5 mm from the apex, so the remaining duration of the growth, to reach the full grown stage, is shorter in the former cells than in the latter. The retardation in the cell elongation caused by the action of the solution might, therefore, become less visible in the former material than in the latter. A similar result was also obtained from another experiment indicated by table 21, column B, where the length—measured at the end of the immersion in different solutions—of cells situated at the points corresponding to the points 5 mm and 10 mm from the apex of the root, at the beginning of the immersion. The above mentioned observations reveal the fact that, difference in the length of resting cells observed at the same distance from the root apex in the different kinds

Fig. 2



Comparison of length of cells—measured at the end of experiment—at the points 1, 2, 5 and 10 mm apart from the root apex at the end of the immersion and at the points, a and b, corresponding respectively to the points 5 and 10 mm from the root apex at the beginning of the immersion. The duration of immersion is 24 hours. The ordinates give the length of the cells in μ, the abscissa the solutions (the figures indicate the amount of sugar in per cent.) or water, in which the root immersed. Curve a, cells at the point a; b, cells at the point b; curves c (×), d (○), e and f represent respectively the cells at the points 1, 2, 5 and 10 mm from the root apex, at the end of the immersion.

of roots treated with solutions of different concentration are small, and on the contrary the length of cells of the same or nearly the same ages in those roots is clearly different. This comparison is also shown in fig. 2, where the relation of columns A and B in table 21 is graphically given. The inclination of curves (c, d, e and f in fig. 2) for cells at the points, at the end of immersion, respectively 1, 2, 5 and 10 mm apart from the apex is gently-sloping, as a whole, though there are some zig-zags; while the curve (b) for cells at the point corresponding to the point 10 mm from the apex at the beginning of the immersion is more rapidly sloping, and the curve (a) for cells at the point corresponding to the point 5 mm from the apex at the beginning of the immersion is the most steep.

c) With regard to the length of the cell in mitosis, there is little difference between the different materials. Yet in detail as a whole the amounts for roots in concentrated solutions are somewhat higher than those for roots in diluted solutions though there are some irregular variations. Therefore at least it is impossible to say that the former shows smaller amount than the latter (see table 23).

Table 23. Length of cell in mitosis in the root immersed in solution for 24 hours

Solution	No. of roots observed	No. of cells observed	Average length of cells in mitosis (in μ)
13.6 % sugar solution	4	42	32.1
11.5 % sugar solution	2	135	30.7
9.4 % sugar solution	4	120	36.2
7.1 % sugar solution	3	246	31.8
4.8 % sugar solution	3	294	29.3
2.4 % sugar solution	4	507	31.8
1 % sugar solution	4	457	29.5
Distilled water	4	560	28.3

4) Relation of cell division and cell length.

The increase in the concentration of the solution is followed by the diminution in the amount of the intensity of cell division, neverth-

less, the length of cells, with mitotic figure, in the materials treated with solutions of different concentration, is rather alike; and also there is no remarkable difference of length between resting cells at the point 2 mm from the apex of root (that point nearly coincides with the growing point of the root) in concentrated solution, and those in diluted solutions or water. Just as was mentioned in the case of materials treated with gypsum, so, it can hardly be said that the cell division is inhibited by the smallness of the cell. In this case it may be said that the osmotic high pressure itself, caused by strong solutions, inhibit the cell division, though probably in an indirect way.

5) The differentiation of the cell.

The distance from the apex to the point where the tracheal elements begin to appear, in the roots immersed in sugar solution or water, is shown in table 24, column A. Though it is not remarkable, yet there is an inclination for the average distance gradually to become shorter with the increase of the concentration of the solution, excepting in the cases of solutions of more than 13.6 % concentration, in which the distance is larger than that in more diluted solutions. The lignification of the cell wall of tracheae takes a similar progress,

Table 24. Differentiation of cell in the root immersed in sugar solution or water

Solution	Duration of immersion (in hours)	A		B	
		No. of roots observed	Average distance from root apex to the point where the tracheal elements begin to appear (in mm)	No. of roots observed	Average distance from root apex to the point where the tracheal elements begin to lignify (in mm)
Experiment I					
13.6 % sugar solution	24	4	10.5	2	11.5
11.5 % sugar solution	24	2	5.2	2	9.5
9.4 % sugar solution	24	4	8.6	3	16.0
7.1 % sugar solution	24	3	7.8	1	11.7
4.8 % sugar solution	24	3	10.0	2	20.8
2.4 % sugar solution	24	4	9.4	4	22.8
1 % sugar solution	24	4	13.2	4	25.0
Distilled water	24	4	9.2	4	26.0

Experiment II[1]					
20 % sugar solution	25½	9	16.6	9	18.8
15 % sugar solution	25½	10	16.0	10	19.2
10 % sugar solution	27½	9	8.1	9	12.8
5 % sugar solution	28	9	13.9	9	17.5
Tap water	27	6	14.2	6	23.3
Control[2]	—	10	15.1	10	20.5

1) Observation was made on hand-sections.
2) Brother seedlings to the materials before the experiment.

as seen in table 24, column B. In experiment II in table 24, the advancement of tracheæ as well as lignification in the root in 20 % or 15 % solution, where the elongation of the roots is almost completely inhibited, is nearly equal to that of the normal root, i. e. of the control material examined before the experiment. In other words, in these cases the appearance of the tracheal element or lignification did not advance during 25½ hours of treatment in the solutions. Within the limits of hypotonic pressure, the progression of the differentiation of the cell seems relatively more rapid under heavier osmotic pressure than under less pressure. And as to the time required the conclusion is similar to the case of treatment with gypsum :— the osmotic pressure rather retards the advancement of cell differentiation.

IV. GENERAL CONCLUSION

The gypsum mould surrounding the root tip inhibits further elongation of the root, neverthless the individual cells which compose the root tissues tend to elongate; this force appears as pressure in the root, consequently, at the same time, the external pressure caused by the resistance of the surrounding mould acts against the root. Amongst the causes which produced the effects upon the materials treated with gypsum mass, we must consider, besides the mechanical pressure, also other factors, namely want of the supply of oxygen and water in the mass and chemical actions which may be introduced by the use of gypsum. But according to PFEFFER (1893, p. 244–246) those factors, with the exception of the mechanical pressure, are negligible for materials treated with gypsum. The experiments with gypsum treatment

in our work were designed as nearly as possible so that only mechanical pressure acted upon the materials. On the other hand the phenomena which were observed in the materials treated with sugar solutions may be considered as the action of the osmotic pressure of the solution.

In the present experiments we see that the cells of roots embedded in gypsum are inhibited both for elongation and for division, and in the case of the cells of roots immersed in sugar solution, similar phenomena are seen; we can say, therefore, that the pressure, mechanical as well as osmotic, retards or inhibits the elongation of individual cells and cell division, partially or perfectly, according to the degree of the pressure.

In the case either of the root embedded in gypsum or of that immersed in a concentrated sugar solution, it is seen that the length of the cells in the materials under heavy pressure—in which mitotic figures are very rare—is never less than that of the materials under light pressure or normal condition—in which the mitotic figures are abundant; we may, therefore, conclude that the inhibition is not attributable to the small size of the cell, and that the pressure itself might inhibit the cell division, though probably indirectly.

The differentiation of cells in the roots under pressure, caused either by embedding in gypsum or by immersing in sugar solution, proceeds nearer to the apex than on the normal root; yet in view of the time required the progress of differentiation of the cell is really delayed owing to the influence of the pressure, namely the differentiation is not "absolutely," but only "relatively" emphasized by the pressure.

V. SUMMARY

The author treated the roots of *Vicia Faba* by embedding them in gypsum or immersing them in cane-sugar solution, to inhibit their elongation, and reached the following conclusions:—

Both the elongation of the individual cell and the cell division are inhibited by the pressure, mechanical or osmotic; the degree of inhibition is connected with the degree of pressure. This inhibitory action is caused by the pressure itself, and is not to be attributed to the small size of the cell.

The differentiation of the cell in the root under pressure advances nearer to the apex than in the normal root, but this phenomenon is not accelerated absolutely but only relatively, because its progress is in reality delayed more or less.

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