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OBSERVATIONS ON THE PROLIFERATION OF STEM PITH PARENCHYMA IN VITRO

II. THE INTERNAL STRUCTURE OF STEM PITH CYLINDERS OF *HELIANTHUS TUBEROSUS* L. CULTIVATED IN VITRO

BY

P. SEVENSTER AND W. K. H. KARSTENS
(*Botanical Laboratory, Leiden, The Netherlands*)

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INTRODUCTION

The stem of the Jerusalem Artichoke, a typical short day plant, exhibits an intense vegetative growth during summer and early autumn. Flowerbuds in this country do not initiate before the end of September.

During the vegetative period the more robust stems show at their apical end living medullar parenchyma over a length of five to ten inches.

MATERIAL AND METHODS

The medullar parenchyma from stem tops sterilized on the outside can be easily punched out by means of sterilizable copper borers as described in Part I (KARSTENS, 1955). In this way cylinders of about 1.5 cm long and 2.5 mm in diameter could be obtained. They were cultivated at a temperature of 23° C in diffuse daylight in culture tubes with a nutrient medium of the following constitution: Knop's solution diluted by half with an addition of 1 gm/l KCl and 10 drops of a modified solution of Berthelot (GAUTHERET, 1942), glucose 20 gms, agar 10 gms, aneurin 1 mg, Ca-pantothenate 1 mg, inositol 100 mg, biotin .01 mg, cystein 10 mg, and α-naphtylacetic acid .5 mg per l. Later it was apparent that very simple culture media suffice for obtaining proliferation.

A certain number of cylinders were stuck into the medium, others were laid down upon the surface of the medium. In both cases a distinct polarity of the original material became clearly visible. Cylinders with their basal end (in relation to their original position in the intact stem) stuck into the medium showed proliferation only in the parts in direct contact with the culture medium or in its immediate neighbourhood. The top end remained unaltered.

When, however, the pith cylinders are stuck into the medium with their apical end, proliferation takes place over the whole length (Plate 1a). Dependent on the age of the explanted pith longitudinal growth may occur to a greater or lesser degree.

Plate 1b shows cylinders laid down flat upon the agar after five weeks of cultivation. If by irregular longitudinal growth the top end of the cylinder is lifted from the medium it will not show any proliferation at all and a smooth "tail" results. If, however, the basal part of the cylinder happens to be lifted from the medium, proliferation over the whole length results.

Cylinders were fixed directly after being punched out from the stem and at the end of five weeks of cultivation. As a fixative CRAF, consisting of a mixture of equal parts of chromic acid, glacial acetic acid and 10 % formol was used. After treatment with 70 % aethanol (three times during fifteen minutes) the material was dehydrated in four steps with butanol and embedded in paraffine (melting point 49° C). Sections 10 μ thick were cut, the paraffine was dissolved by means of xylene, and transferred to aethanol 50 %. The sections were stained in 1 % saffranin in 50 % aethanol during three hours, rinsed with aethanol 50 %, counterstained with a 1 % solution of fast green in an aethanol-xylene mixture 1 : 3. After rinsing with xylene the sections were mounted in balsam.

RESULTS

The untreated cylinders consist of longitudinal rows of parenchyma cells of uniform size, measuring around 100 μ in diameter and 50 μ in height. The outer surface shows the action of the borer; the cells are more or less damaged and have collapsed. In these cells the cell walls will show suberization in due time. Small intercellular spaces are present.

Details about the internal structure of one of the cylinders that has been cultivated during five weeks may follow here. In this case contact with the nutritive medium has been retained by means of basal part (Plate 1a). The basal part shows heavy proliferation slowing down to the smooth apical part. The total length of the explantate amounts to about one inch.

A short description of the anatomical structure of twelve equidistant transverse sections from a series of sections through part I of the explantate are given here (see Fig. 1).

Section 1. Sections through the smooth tail-end show no difference from the original pattern.

Section 2. At some distance from the end of the explantate the first symptoms of activity of the medullar cells become apparent. Some cells of the subsurface layer have divided by the formation of tangential cell walls. The division has taken place only in cells from that side of the explantate that was at some time in contact with the nutritive medium.

Section 3. The activity described above is perhaps somewhat greater in this section.

Section 4. Plate 2a shows that here the activity is much greater. Cells have divided by a repeated formation of tangential walls, thus forming a sort of meristematic zone. Later on cell walls in other directions have been formed. In this manner groups of cells are formed. Their joint origin from one and the same medullary cell is apparent. Some of the newly formed cells show a reticulate lignified cell wall. These cells are comparable with those described by SINNOTT and BLOCH (1945) and might be called tracheids. As before, the described activity is limited to that side of the explantate that was at some time in contact with the culture medium.

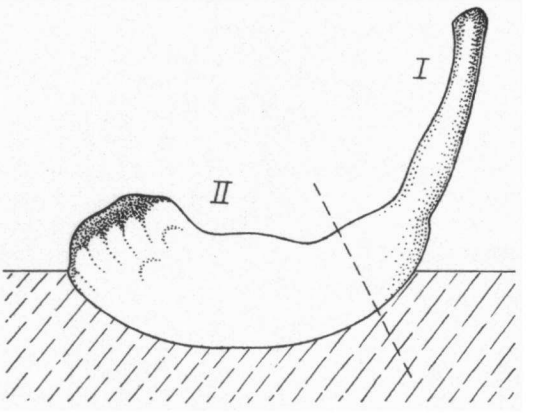

Fig. 1. Explanation see text.

The extreme surface layer consists mostly of cells that do not show any activity. At two points the suberized surface layer has been broken by small proliferations originating from cells of the sub-surface layers. The greater part of the section shows the original pattern, i.e. isodiametric cells with small intercellular spaces.

Section 5. The zone of activity extends along the surface. A few more tracheids are present. A new proliferation has broken the suberized surface layer.

Section 6. The extension of the zone of activity along the surface is much greater. Some groups of 6–9 tracheids or vessels are present, sometimes in transverse section, sometimes in longitudinal section, illustrating the bizarre course of these formations. On the distal side of the groups of tracheids some phloem elements can be found.

Section 7. The zone of activity has extended much more, in fact there is a nearly closed circular band of meristematic tissue present. A number of proliferations have broken through the surface layer and have partly merged into each other. In these areas the original borderlayer of collapsed cells lies amidst meristematic tissue. In the proliferations outside the original borderlayer scattered groups of vascular

elements are present. Some scattered cells on the outmost border possess lignified reticulate cell walls (Plate 2b). In the meristematic band, present in a circle within the original borderlayer large groups of xylem and phloem elements have been differentiated. In general the phloem is orientated towards the outside of the explantate. The composing elements of these groups exhibit radial arrangement by the activity of a cambiumlike meristem (Plate 3a). The course, however, is, as has been mentioned, very irregular. The xylem elements when seen in longitudinal direction show beautiful perforation plates and may be called vessels. Within the just-described zone a core of non-modified medullary cells is still present. Similar cells, sometimes much enlarged, can be found between the vascular groups.

Section 8 and 9. The ring of vascular groups has lost something of its striking regularity by unequal development of its component groups.

Section 10. Many of the vascular groups undergo a continued radial expansion caused by cambial activity. The original borderlayer of collapsed cells is broken up more and more by the expansion of the underlying tissues. The cells of the sub-surface layer exhibit cell division by the formation of series of tangential walls, resulting in a second meristematic zone within the old margin of the explantate. Fig. 2 represents this stage.

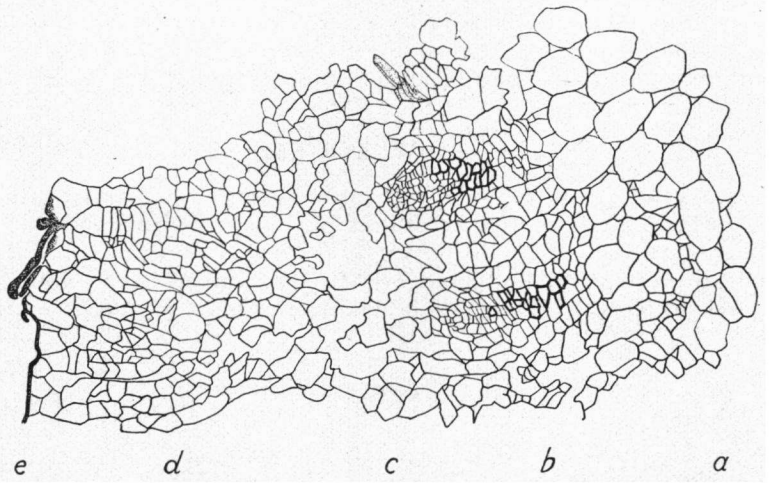

Fig. 2. *Helianthus tuberosus* L. *a*. Unchanged pith cells. *b*. zone with vascular groups. *c*. lacunae. *d*. meristematic zone. *e*. suberized margin. 65 ×.

Section 11 and 12 show the phenomena described in section 10 in a more pronounced way. The peripheral parts within the original borderlayer exhibit a great activity. The zone with the vascular groups undergoes distortion as the vascular groups expand in an irregular way. In the neighbourhood of these groups lacunae are formed by

enlargement of intercellular spaces. Already in section 10 a few of such lacunae are to be found (Fig. 2).

The internal structure of Part II of the explantate (Fig. 1) is essentially very much alike that seen in section 12 of Part I. However, the situation distal to Part I becomes more and more chaotic. The vascular groups no longer show predominantly external phloem. They often have a very bizarre course (Plate 3b). In the centre of the explantate unaltered parenchyma cells are present in small scattered groups, the rest of them having undergone cell division.

DISCUSSION

From the above mentioned results it is obvious that the original hope that the medullar tissue from the stem might proliferate thereby producing a homogeneous tissue culture was not realised.

Pith cells prove able to dedifferentiate (BUVAT, 1944, 1945, 1951). The process of dedifferentiation is accompanied by active cell division after which differentiation, one might call it redifferentiation, in different directions takes place. Vascular groups consisting of xylem and phloem elements are formed. A cambiumlike meristem is present.

At first a pronounced tendency for external phloem exists, later on this regularity is destroyed by irregular growth and very strange vascular groups and strands result.

Apart from the head-tail polarity a periphery-centre polarity appears to act which might be responsible for the observed phloem-xylem orientation. Further experiments are contemplated to elucidate this point.

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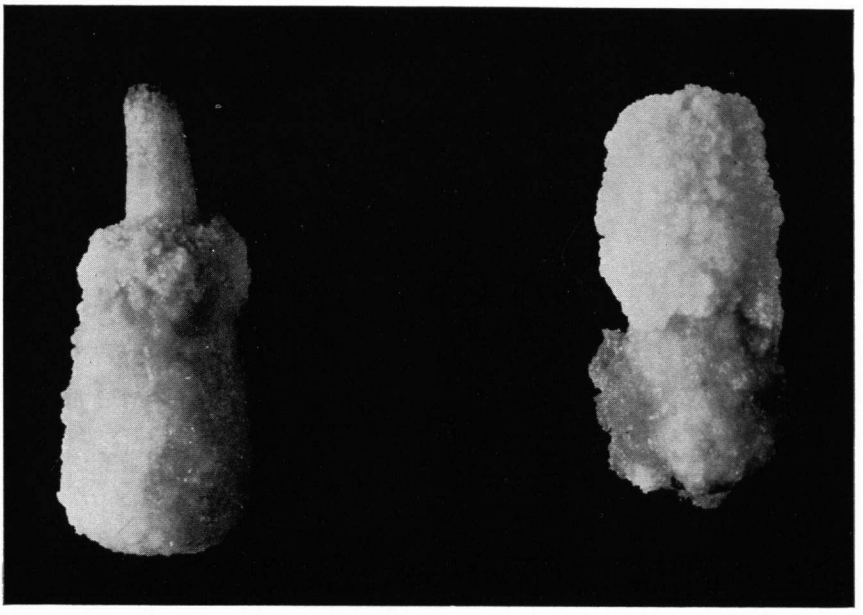

Plate 1a. *Helianthus tuberosus* L. Cylinders of stem pith after five weeks cultivation in vitro. Left: cultivated with basal end stuck into nutritive medium. Right: the same for the apical end. 3 ×.

Plate 1b. *Helianthus tuberosus* L. Cylinders of stem pith after five weeks cultivation in vitro. Left: cultivated with apical end in contact with nutritive medium. Right: the same for the basal end. 3 ×.

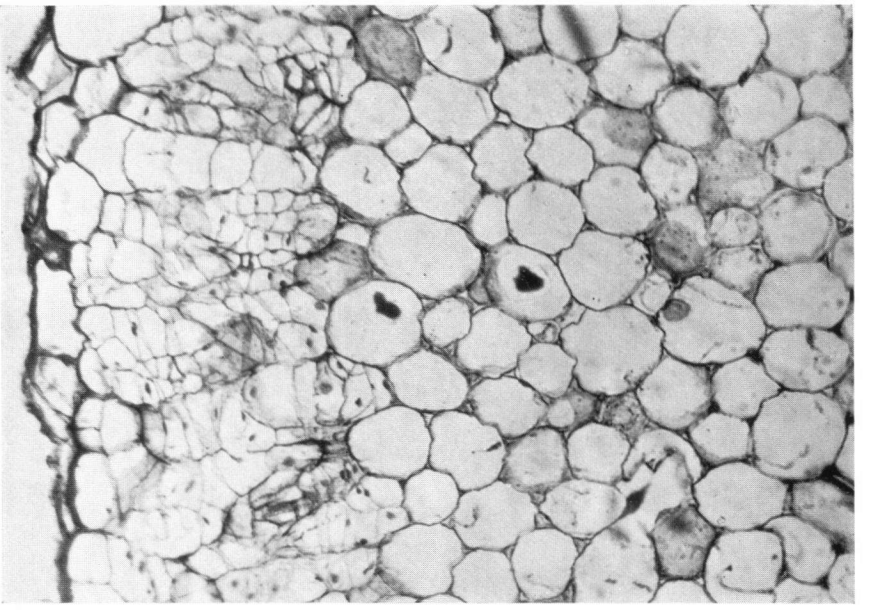

Plate 2a. *Helianthus tuberosus* L. Formation of a meristematic zone by the formation of tangential cell walls. The origin of the cell groups from medullary cells is obvious. A few tracheids are visible. Margin suberized. Considerable core of non-modified cells. 120 ×.

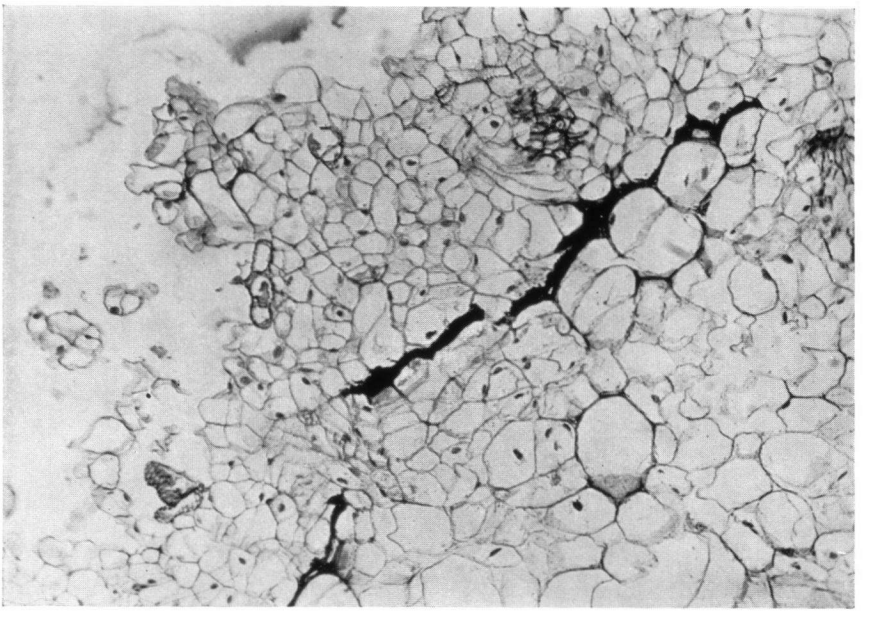

Plate 2b. *Helianthus tuberosus* L. The suberized margin is ruptured by proliferating cells, some of them are lignified. 100 ×.

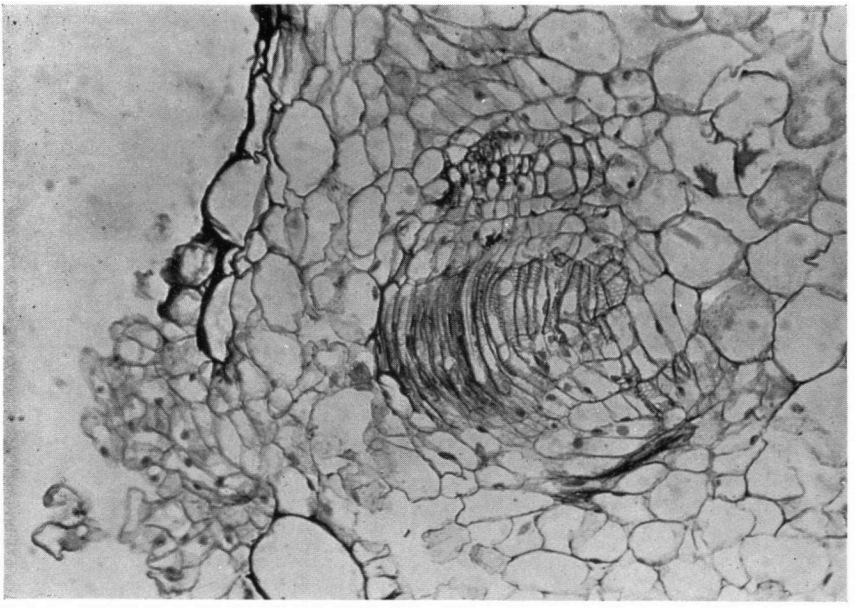

Plate 3a. *Helianthus tuberosus* L. A vascular group with external phloem and internal xylem, partly in longitudinal, partly in transverse section. Radial arrangement of the composing elements. Perforation plates in the xylem elements. 120 ×.

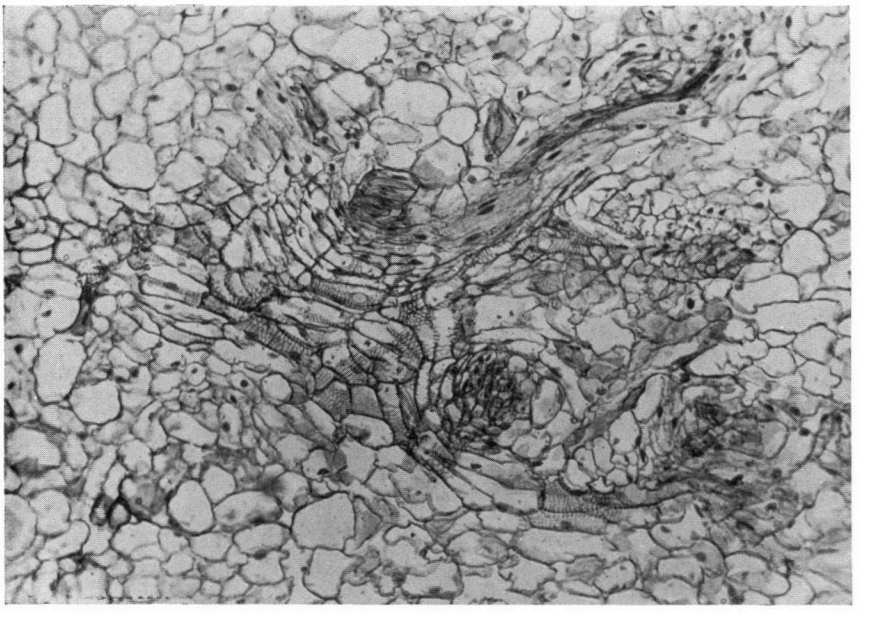

Plate 3b. *Helianthus tuberosus* L. Vascular groups and strands. Perforation plates. Remnants of the original medullary tissue still clearly visible. 65 ×.