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OBSERVATIONS ON THE PROLIFERATION OF STEM PITH PARENCHYMA IN VITRO

III. GROWTH AND DEVELOPMENT OF EXCISED STEM PITH CYLINDERS OF *SAMBUCUS NIGRA* L. VAR. *PENDULA* DIPP.

BY

C. A. CLAUSING (NÉE MELCHIOR) AND W. K. H. KARSTENS

(*Botanical Laboratory, Leiden, The Netherlands*)

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INTRODUCTION

Apart from some introductory remarks it was announced in the first publication of this series (KARSTENS, 1955) that a.o. the proliferation of stem pith in vitro of the variety *pendula* Dipp. of *Sambucus nigra* L. would be described.

Although the anatomy of the untreated pith is very much alike that of the stem of *Helianthus tuberosus* L. the type of proliferation of *Sambucus* pith differs greatly from that of the Jerusalem Artichoke (SEVENSTER and KARSTENS, 1955).

MATERIAL AND METHODS

An old plant of the weeping variety of the Elder growing against the conservatory in the Botanic Garden furnished the material used in our experiments. Each spring the plant is cut back onto the old wood resulting in the growth of drooping suckers several yards long.

The material consisting of tops of suckers containing green active pith was treated as described before (KARSTENS, 1955). Pith cylinders of 1.5 cm length and with a diameter of 3 mm could be easily obtained and were put horizontally on nutritive agar. The constitution of the culture medium and further treatment are the same as described before (SEVENSTER and KARSTENS, 1955). This is also the case with fixation and sectioning of the material. For staining a combination of Delafield's haematoxylin, saffranin and fast green were used.

DESCRIPTION OF THE RESULTS

Seen in transverse section the freshly excised pith of *Sambucus* consists of more or less irregularly arranged multiangular parenchyma

cells with small intercellular spaces. The longitudinal section shows, according to expectation, cells arranged in longitudinal rows. In the phase studied the cells measure 36–90 μ in width and 20–116 μ in length.

Plate 1a, first of all, illustrates proliferation of cylinders laid horizontally upon the culture medium and seen from above. A freshly excised cylinder is shown in (d), while (a) and (b) represent cylinders after about 20 and 40 days of cultivation resp.: (c), finally, shows a cylinder with the basal end stuck into the nutritive medium after 40 days cultivation.

After 20 days cultivation the upper surface of the excised cylinder is covered with very fine downy flocks, merging into a thin more or less continuous layer along the sides of the explantate and terminating in a shallow ridge on a level with the agar medium. Later on the whole surface of the cylinder becomes covered with a greenish white layer formed by fusion of individual proliferations. This process, however, proceeds at a different pace from one point to the other. Plate 1a (b) especially the top half of the figure illustrates this clearly. In the central part of the upper surface the process is strikingly quicker than at its extremities. It stands to reason that in the photograph the border of new tissue along the lines of contact with the nutritive medium is not visible. The cut ends, finally, show cushion-shaped masses of newly formed tissue.

As is the case with the excised pith of *Helianthus tuberosus* (SEVENSTER and KARSTENS, 1955) a very distincy polarity manifests itself. Plate 1a (c) represents an excised pith cylinder with the basal end stuck into the nutritive medium. Transport of stimulants from the medium only has taken place over a short distance. As with *Helianthus* a smooth non-proliferating "tail" results.

So far the difference with *Helianthus* does not seem very important. The study of the microscopical changes, however, reveals a fundamental difference.

While the pith parenchyma of the Jerusalem Artichoke becomes active through and through on cultivation, the pith cylinder of *Sambucus* shows a very limited activity in the interior, ending in necrosis. The relatively poorly developed proliferations on the outside of the Jerusalem Artichoke cylinders, furthermore, are in striking contrast with the huge quantity of new tissue in the case of *Sambucus*. Thus, for subcultures the whole mass of tissue can be used in the case of *Helianthus* while for *Sambucus* the new outgrowth has to be taken.

Some more information regarding the internal structure of pith explantates after different periods of cultivation can be given.

Fig. 1 illustrates the line along which the development takes place. In a schematical way transverse sections are presented which show a number of zones in a series of explantates cultivated for a longer or shorter time. No I represents the situation of a freshly excised cylinder in contact with the nutritive medium. Nos IIa and b represent schematical drawings of transverse sections of one of the extremities and of the central portion of an explantate cultivated during 25 days. Nos

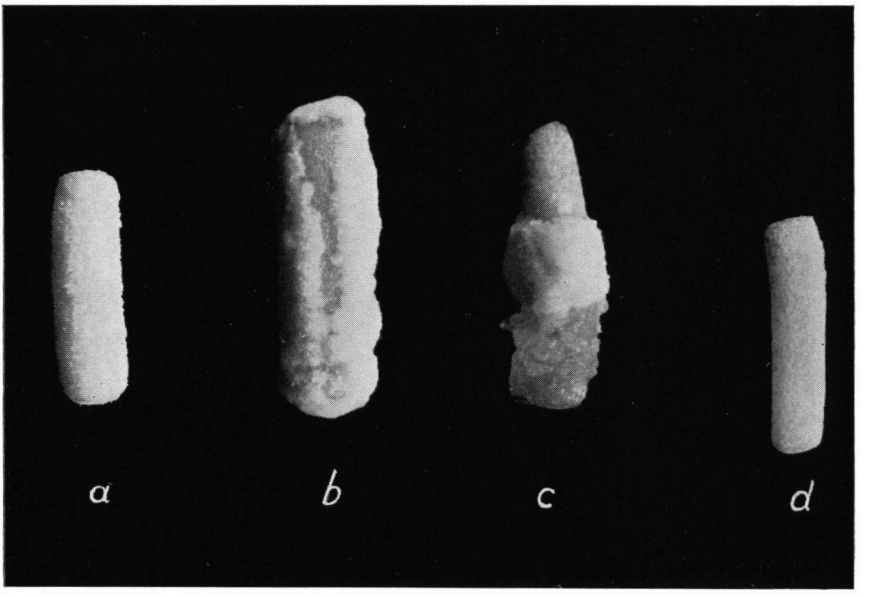

Plate 1a. *Sambucus nigra* var. *pendula*. Excised pith cylinders cultivated in vitro. (a) and (b) placed horizontally, 20 and 40 days cultivation resp., (c) stuck into culture medium with basal end after 40 days cultivation, (d) freshly excised cylinder. 1.7 ×.

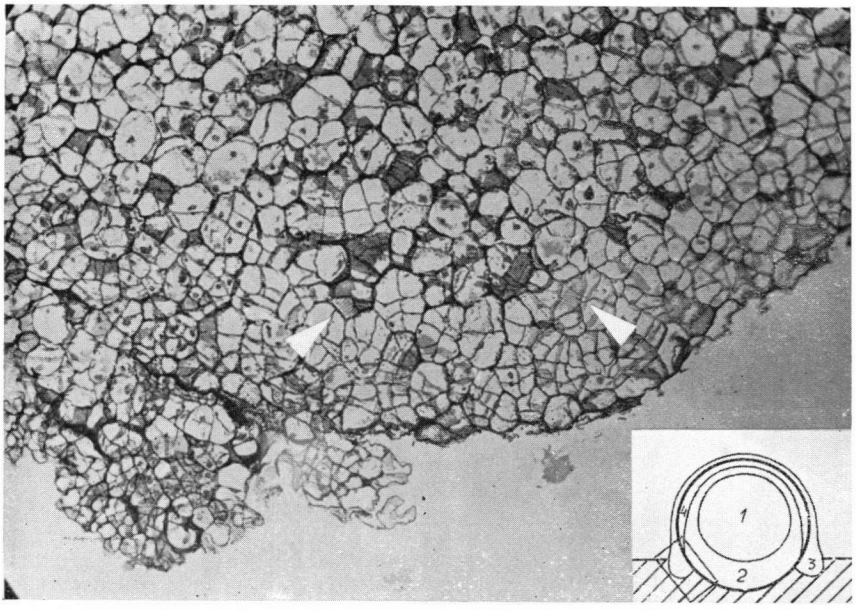

Plate 1b. *Sambucus nigra* var. *pendula*. Most medullary cells have divided more or less (2), the suberized borderline of the explantate is clearly visible as well as the new outgrowth (3). Some lignified cells are indicated by arrows. 50 ×.

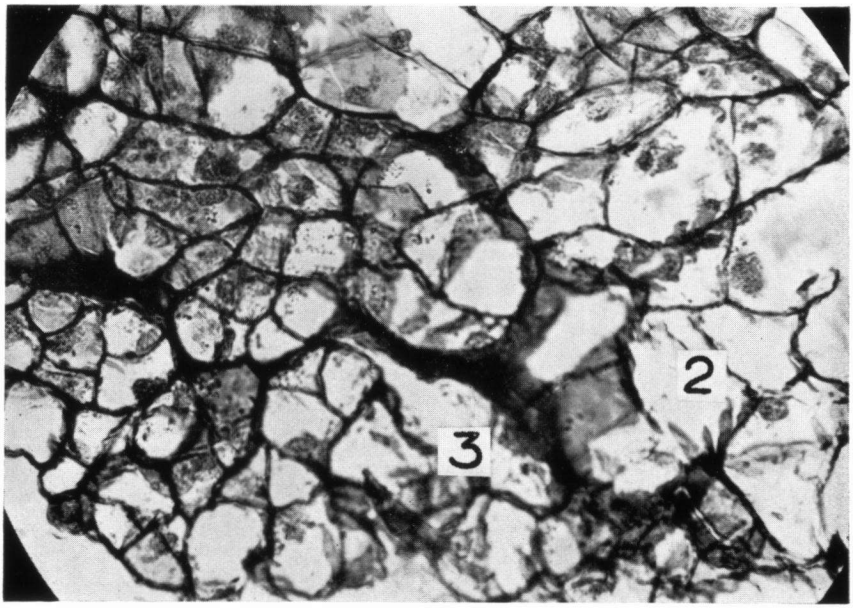

Plate 2a. *Sambucus nigra* var. *pendula*. Marginal groups of cells of zone 2 rupture the suberized border layer to contribute to the formation of zone 3. 200 ×.

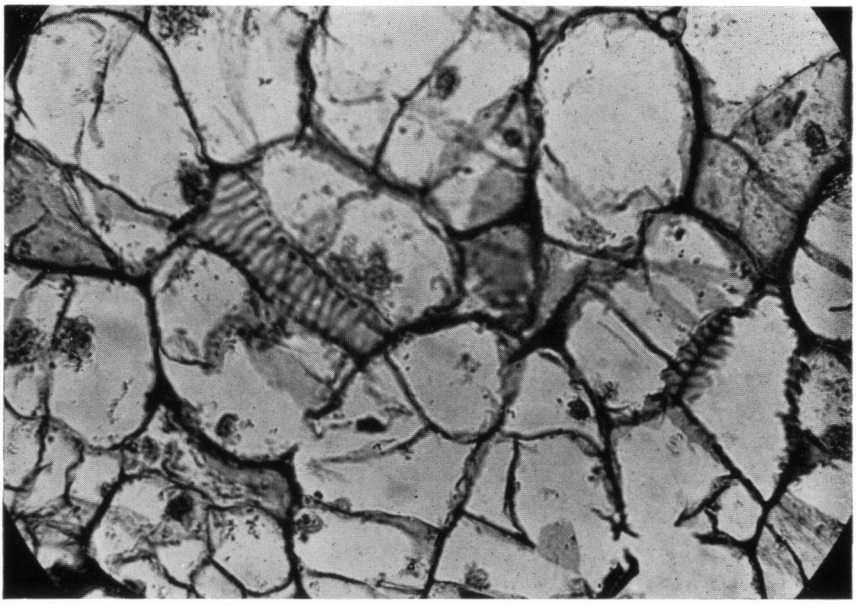

Plate 2b. *Sambucus nigra* var. *pendula*. Detail of zone 2. Medullary cells without losing the original shape have divided repeatedly. Some of the division products exhibit heavily lignified scalariform thickening of the cell wall. 225 ×.

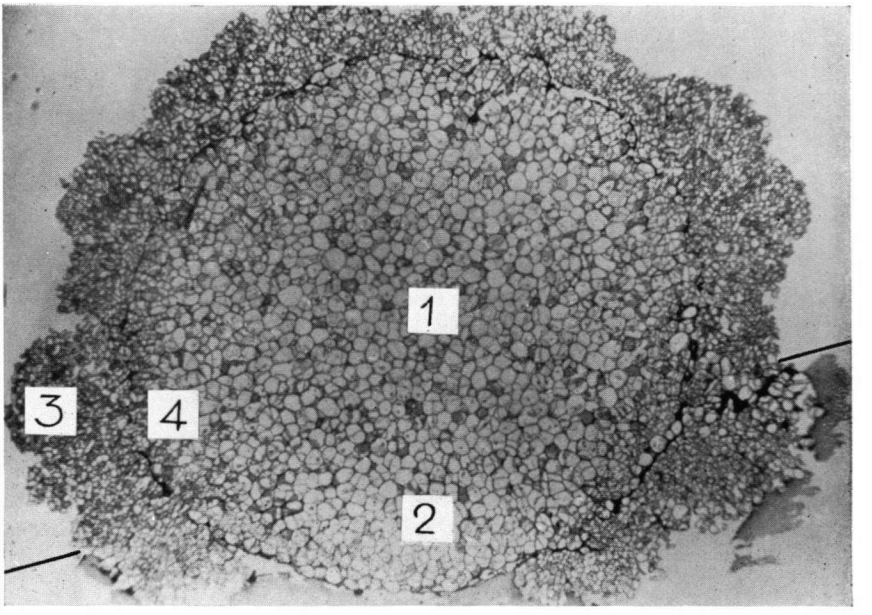

Plate 3a. *Sambucus nigra* var. *pendula*. Transverse section of a horizontally placed pith cylinder after 46 days of cultivation. Zones 1, 2, 3 and 4 are clearly visible. The heavy line indicates the border of the nutritive medium of which stained patches are discernable. 18 ×.

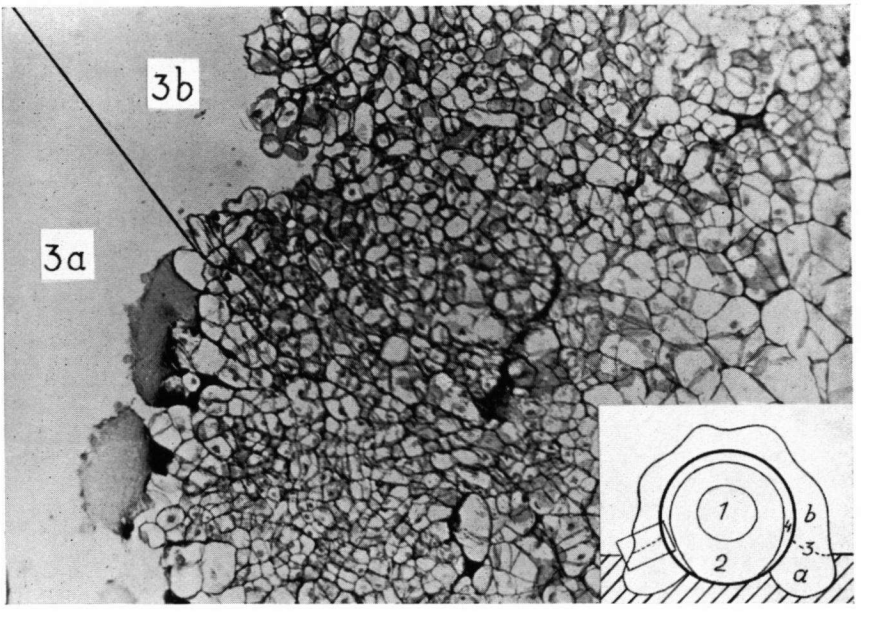

Plate 3b. *Sambucus nigra* var. *pendula*. Details of the proliferation zone (3); 3a pictures the proliferation in contact with the nutritive medium (stained patches visible), 3b shows the proliferations in the air. The heavy line indicates the partition between the two portions of (3). 50 ×.

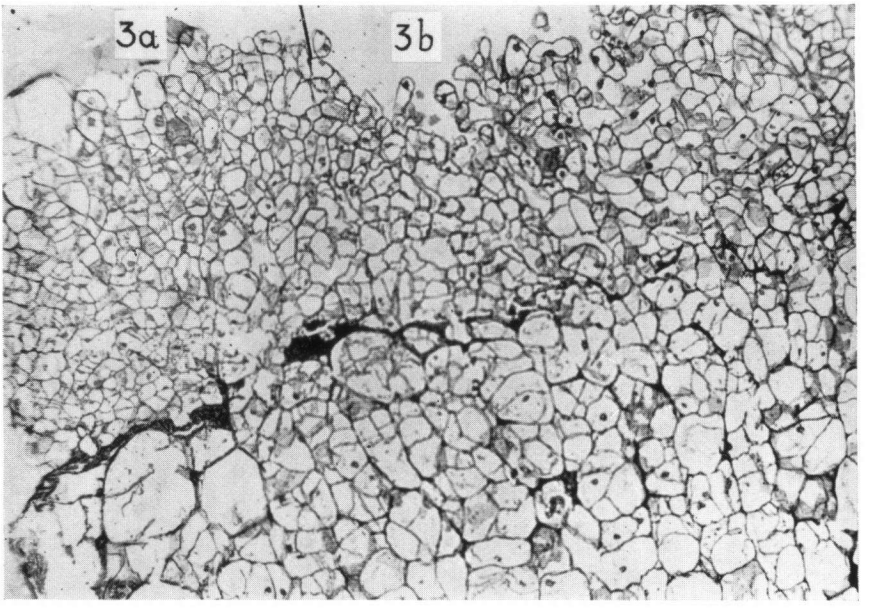

Plate 4a. *Sambucus nigra* var. *pendula*. Details of the proliferationzone (3); 3a shows a typical thin walled closely built fanlike proliferation in contact with the nutritive medium; 3b shows the thick walled loosely built outgrowth in contact with the air. 50 ×.

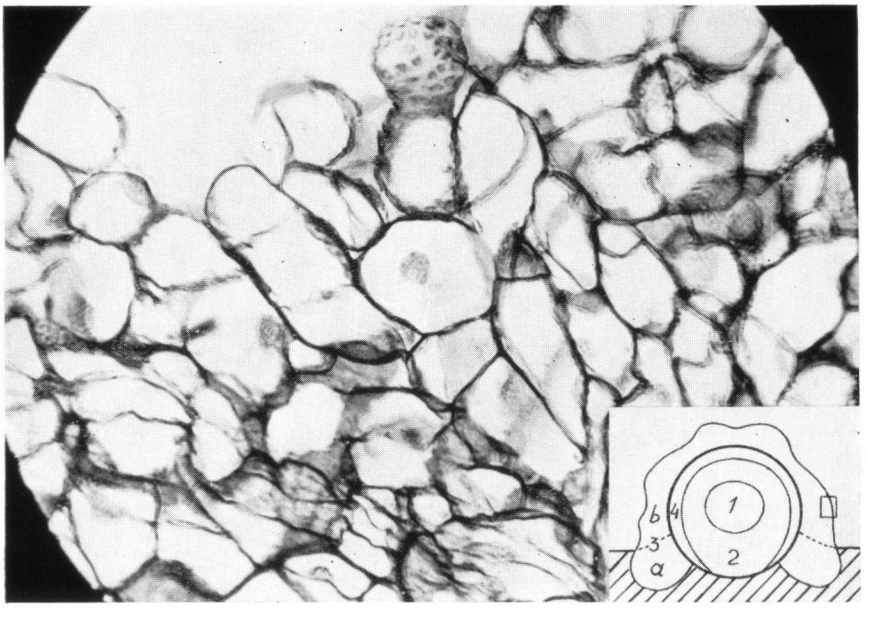

Plate 4b. *Sambucus nigra* var. *pendula*. Marginal cell (zone 3b) with pectic warts and reticulate lignification. 230 ×.

III and IV, finally, represent stages after 46 and 117 days of cultivation resp. Unchanged parenchymatous tissue is indicated with (1), while (2) represents a zone in which all or almost all cells have divided more or less, however, without loss of the original cell shape. In older explantates zone 2 exhibits the differentiation of a lateral meristem-like border, marked as (4), by the formation of a series of tangential cell walls. The areas 1, 2, and 4 are situated within the circumference of the original explantate, the borderline of which is indicated by a heavy line. Zone 3, finally, is produced by fusion of proliferations breaking through the original border. A distinction between 3a and 3b has to be made, indicating new growth in contact with the atmosphere and with the nutritive medium resp.

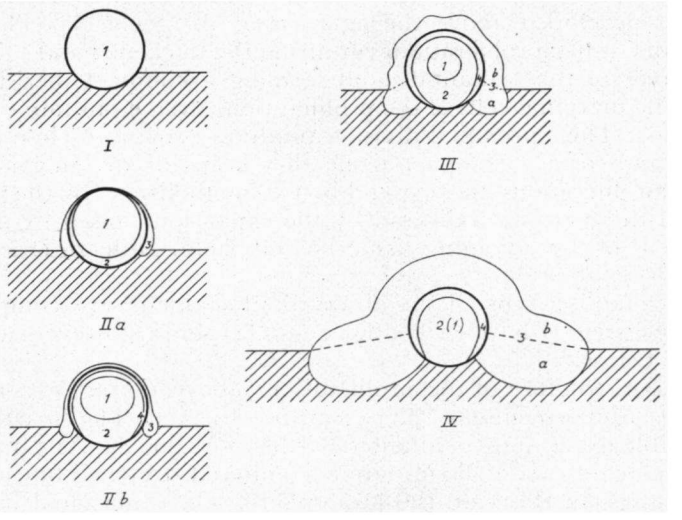

Fig. 1. Development of excised cylinders horizontally placed upon culture medium. Schematical representation of transverse sections. Culture medium hatched, border of the original explantate drawn with a heavy line. For further explanation, see text.

After having given this rough draft of the facts observed some details may follow here. Plates 1b, 2a, and 2b represent different parts of explantates which have been cultivated during 25 days. In Plate 1b part of the original explantate and a portion of the marginal proliferations can be recongized. The major part of the picture shows the split up cells of zone 2 (Fig. 1, IIa). To the left the still limited outgrowth of zone 3 on the border of the nutritive medium and atmosphere is apparent. In this part of the section the thick suberized outer layer of the original explantate has been ruptured by outgrowing cell groups (See also Plate 2a) Several cells of zone 2 show lignification in a scalariform pattern (Plate 2b). This same pattern is found in cells of the upper part of zone 3.

Fig. 1, III and Plate 3a show the gross structure of an explantate after 46 days of cultivation. Only a small portion of unchanged cells (zone 1) is left, the zones 2, 3, and 4, however, are much more developed than in the explantate cultivated for 25 days. The outline of the original cylinder remains visible, the diameter, however, has grown somewhat through the activity of zone 4. The greater part of the expansion of the whole system is due to the activity of zone 3. Apart from the heavy ridge in contact with the culture medium a 1 mm thick, small-celled layer of new tissue covers the explantate. The part of the lower side of the explantate between the collateral ridges shows no proliferation at all.

Zone 1, 2, and 4 do not exhibit new features, zone 3, however, shows some details worth mentioning.

First of all there exists a striking difference between the structure of the new tissue grown into the culture medium (Fig. 1, III, zone 3a) and that developed above the agar (zone 3b). Zone 3a (Plate 2b) consists of fan-like proliferations rupturing the thickened and suberized borderlayer of the explantate and forming a compact meristematic tissue. The marginal cells of the proliferations stretch out into the agar and divide. Their proximal division products come into close mutual contact and form a compact tissue that keeps its dividing activity: thus the proliferations may expand by two methods, i.e. by the pioneer action of the marginal cells and by the expansion caused by division of the cells of the adjoining tissue behind them. Differentiation does not occur in zone 3a.

As to zone 3b, some of the observed phenomena are comparable with those previously described for zone 3a; some, however, we have not met before.

As to the structure of the proliferations above the agar (zone 3b), a primary and a secundary phase can be observed. The first phase is comparable to the state of affairs described for zone 3a. As a matter of fact, here again marginal cells are encountered stretched out into the surroundings, in this case the atmosphere. These marginal cells are often much longer than the corresponding elements of zone 3a. In addition the division products form a relatively loose and irregularly built tissue.

It is remarkable that contrast to our knowledge of the internal structure of plant tissue cultures, hardly anything is known about the methods by which a plant tissue culture expands on the outside. GAUTHERET (1942–43) describes the presence of longer or shorter, more or less branched chains of cells, arising from dividing cells scattered over the surface of many types of explantates cultivated in vitro to which he gave the name of "pseudo-thalles" because of the resemblance of the thallus of certain fungi. They have been described earlier by MAGNUS (1918) who gave these formations the name of "Wundhaare". GAUTHERET (1937) was at first of the opinion that these formations were comparable to the migrating cells of animal tissue cultures. The fact that the cell chains cannot be subcultured, which is in contrast with the recently described animal cells, led him to the conclusion that his former comparison was not longer allowable.

Gautheret's "pseudo-thalles" remain free; they never fuse. A substantial expansion of the explantate is, according to this author, only possible if the proliferation of cells "se généralise à toute la surface du tissu, dont toutes les cellules se multiplient: le fragment produit alors un parenchyme compact légèrement mamelonné que l'on pourrait interpréter comme étant formé d'une grande quantité de pseudo-thalles étroitement unis les uns aux autres."

CAPLIN (1947) investigated the way by which a cube cut out of a tobacco tissue culture grew in subculture. According to this author knobs of new tissue are formed in two ways. One way is by a process of cleavage caused by failure of a cell, or a row of cells, on the surface to continue dividing. Instead such cells enlarge greatly, while the surrounding cells continue to divide and give rise to new knobs. The other method is by the formation of groups of actively dividing cells in the subsurface of existing knobs. Tangential walls are formed and by increment or size of the newly formed cells the knob grows out with the cells in radially diverging rows, resulting in a fan-like structure when sectioned and fountain-like when seen in space. There was no mention of the activity of marginal cells although some of the figures of Caplin show their presence.

From the above it will be clear that our material behaved in a different way. Practically all cells just below the suberized skin at the margin of the explantate become active but only on a relatively small number of places the activity is such that the suberized skin becomes ruptured. From these points pseudo-thallus like and fan-like proliferations originate, together forming a sort of pseudo-parenchyma which gradually becomes more solid (Plate 4a).

In so far zone 3a and 3b present essentially the same picture, except that the cells of zone 3a are smaller (Plate 3b). However, in zone 3b changes occur at a certain moment. As a matter of fact, differentiation takes place in zone 3b, while zone 3a never shows any. As in zone 2 some elements exhibit lignified cell walls, some, as is the case in zone 2 (see Plate 2b,) of a scalariform pattern others of a reticulate lignification. Sometimes isolated marginal cells exhibit both pectic warts and a reticulate lignification (Plate 4b).

Another difference is the occurrence of cup-shaped or gutter-shaped cambial zones with matching internal xylem and external phloem elements (Plate 5a).

Finally, some remarks have to be made concerning a cylindrical pith explantate after having been cultivated for a period of 117 days.

Fig. 1, IV illustrates a continuation of the course of things observed in explantates of a younger age. This is specially true of the development of zone 3a and 3b.

In a certain sense the same is the case with the original explantate. The original medullary parenchyma e.g. has practically disappeared, here and there scattered remains are encountered.

The most spectacular difference, however, is a totally different orientation of the elements within the original and suberized borderline. In the explantates of 25 and 46 days cultivation more or less

concentric changes have taken place in the old tissue (zones 1, 2, and 4). This is in complete agreement with the original stem pattern. After 117 days of cultivation this situation is fundamentally changed. A very distinct orientation towards the culture medium has manifested itself, resulting, as seen in transverse section, in a kind of peacock's tail (Plate 5b). A radiating structure originating from the bottom of the explantate results, presumably by expansion of composing cells along new axes. In addition, cell complexes in zone 4 have expanded in such a way that the suberized borderline has become undulated. In the central part groups of cells of a bizarre shape and with heavily lignified cell walls have been formed.

The original expansion of the explantate (see page 196) has probably undergone a regression by necrosis of the tissue, followed by inward expansion of zone 3. A free contraction from the nutritive medium has taken place in the bottom part of the explantate (Plate 5b).

DISCUSSION AND SUMMARY

The results obtained by cultivating excised medullary parenchyma of *Sambucus nigra* L. var. *pendula* Dipp. clearly indicate that the properties differ greatly from the corresponding tissue of the Jerusalem Artichoke, *Helianthus tuberosus* L. While in the case of *Helianthus* virtually the whole explantate proliferates by cell division and differentiation this proves not to be the case with *Sambucus*-pith. Here, on the contrary, growth of the explantate takes place exclusively through the formation of a considerable layer of new tissue on the outside of the explantate.

External factors have a profound influence on the structure of the newly formed tissue. Contact with the atmosphere results in a parenchymateous tissue consisting of small cells, some of them exhibiting scalariform or reticulate lignification. Furthermore, more or less cup-shaped formations consisting of internal xylem and external phloem arise from isolated cambial zones.

A complete contrast is provided by the structure of the new tissue that develops in contact with the nutritive medium. A homogeneous tissue consisting of thin walled non-differentiating cells results.

As to the original explantate, this retains its individuality. Even after 117 days of cultivation the dimensions have not changed considerably and the original borderline is still visible. The pattern of the explantate, however, has undergone a very striking transformation. As might be expected alterations take place according to the original radial pattern of the pith resulting in a number of concentric zones. After prolonged cultivation, however, presumably by expansion of the composing cells along induced axes a different structure, as seen in transverse section not unlike a peacock's tail, originates.

Finally, the original explantate dies off.

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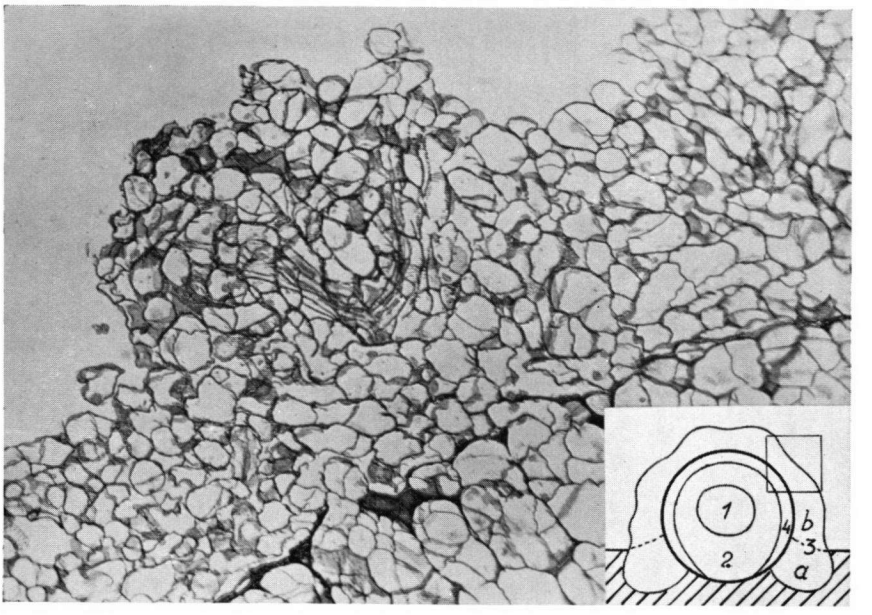

Plate 5a. *Sambucus nigra* var. *pendula*. Differentiation in (3b). A cambial zone with internal xylem and external phloem is present. 70 ×.

Plate 5b. *Sambucus nigra* var. *pendula*. New orientation in original explantate after 117 days cultivation. A group of lignified cells indicated by the arrow is reproduced much enlarged. 20 × resp. 120 ×.

Miss E. van Wijnen (now Mrs E. van Die née van Wijnen) has kindly made the photographs.

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