Discussion. The above results give direct experimental support to the hypothesis that calcium plays a key role in the control of cytoplasmic streaming in plant cells. Our finding that ionophore A 23187 stops cytoplasmic streaming even after free or loosely-bound calcium has been eliminated by thoroughly washing out cell walls with 10 mM EDTA suggests that a release of membrane-bound Ca^{2+} into the cytosol is required for cessation of streaming rather than influx of Ca^{2+} from outside into the cell. This does not conflict with Barry's finding that the action potential does not suppress *Nitella* cyclosis in the absence of external Ca^{2+} since release of Ca^{2+} from many excitable membranes does not occur in the absence of external free calcium[17,18].

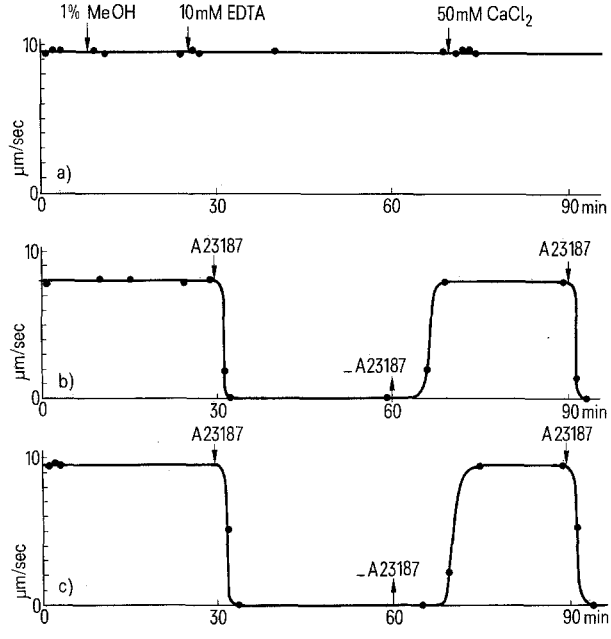

Fig. 2. Rates of streaming of staminal hair cells. *a* Staminal hair in aerated pure water. Arrow points to successive substitution of aerated 1% methanol, 10 mM EDTA and 50 mM $CaCl_2$ for pure water. *b* and *c* Arrows point to addition (+) or elimination (−) of ionophore A 23187. 1 µl of 200 µM methanolic solution of ionophore was added to 100 µl of 1 mM $CaCl_2$ (B) or 100 µl of 10 mM EDTA (C). In experiment C staminal hair was first washed rapidly several times with 10 mM EDTA.

Our results contrast, however, with Herth's findings that ionophore A 23187 stops tip growth, but not cytoplasmic streaming in pollen tubes of *Lilium longiflora*[19], which led him to conclude that A 23187 does not interfere directly with the contractile elements involved in cytoplasmic streaming. Growing pollen tubes, however, drive considerable currents which maintain a Ca^{2+} gradient along them[20,21]. Such currents, which antagonize ionophore action, would be expected to decrease sensibility to ionophore.

How Ca^{2+} redistribution within the cell can induce changes in cytoskeleton organisation remains largely speculative at present[22]. Cytoplasmic streaming, which allows rapid and quantitative studies of such changes, might provide a tool for elucidating the regulatory mechanisms which control the assembly of contractile proteins into supramolecular forms.

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Characterization of polyphenol-containing dense cells[1]

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Summary. Dense cells of *Rubus chamaemorus* L. (cloudberry) root meristems have been characterized by histochemical staining reactions at light and electron microscope levels. Presence of polyphenolic compounds in the cellular matrix contribute to the density of the dense cells.

In recent years dense cells have been reported from growing and differentiating plant tissues. Some of these cells have denser cytoplasm due to increased concentration of ribosomes in the matrix[2], thereby showing more basophilia and appearing more electron dense when viewed with the electron microscope. A different kind of dense cell has been also observed at the junction of the shoot apical meristem and leaf primordia in maize[3] and in the root meristems of cloudberry[4]. The density of these cells is uniformly distributed over the cytoplasm as well as the nucleus and it has been postulated[4] that polyphenolic compounds present in the cell matrix are responsible for the intense staining reaction. This communication presents evidence to support the above hypothesis.

Materials and methods. Healthy root tips of *Rubus chamaemorus* L. (cloudberry) were fixed in a mixture of paraformaldehyde and glutaraldehyde[5] in phosphate buffer pH 7.2 and in 2.5% glutaraldehyde in the same buffer at 25 °C for 1–2 h. After thorough washing in buffer at 4 °C for 18 h, the samples were treated with 1% OsO_4 in phosphate buffer. Dehydration was done through ethanol series followed by embedding in Epon. Thick sections (0.5–1.0 µm) were stained in toluidine blue pH 11 and pH 4[6] for light microscopic observations. Ultrathin sections were stained with uranyl acetate and lead citrate preceeding observations with a Zeiss 9S electron microscope.

For ultrastructural localization of polyphenols, root tips were fixed in a mixture of 3% $FeCl_3$[7,8], paraformaldehyde and glutaraldehyde in phosphate buffer (see above) for 1 h. The samples were then washed in buffer and processed for electron microscopy without OsO_4 treatment.

Histochemical tests were made on free hand sections of fresh root tips. The sections were treated with a) DMB reagent (1% 2,4-dimethoxy-benzaldehyde in 95% ethanol and equal volumes of 18% HCl) according to Mace and Howell[9] and b) nitroso reagent[10] for localization of catechins. Gossypol and related terpenoids were tested for by using DNP reagent (2,4-dinitrophenylhydrazine in 2N HCl) and $SbCl_3$ in 60% $HClO_4$[11].

Results and discussion. The root apex of *Rubus chamaemorus* L. revealed dense cells when stained with toluidine blue (figure 1). These cells occur randomly within the meristematic tissue. Except for their staining intensity there seems to be no morphological difference from the adjacent cells. A gradation of staining intensities can also be observed in certain areas of the tissue. At lower pH values toluidine blue produces a faint metachromatic green colouration in the dense cells, which is indicative of phenols[6].

Figure 2 represents a dense cell as seen with the electron microscope after aldehyde-OsO_4 fixation and uranyl acetate/lead citrate staining. The density is uniformly distributed over the cytoplasm as well as the nucleus as seen in the light micrograph (figure 1).

Positive results for polyphenolics were obtained in histochemical tests on fresh root tip sections. Although good sections were generally difficult to obtain, colour reaction could be clearly detected within cells of the tissues. Both DMB and nitroso reaction produced orange-red colour in scattered cells throughout the epidermis, outer cortex and endodermis. Some of the cells adjacent ot xylem vessels also showed positive reaction. DNP and $SbCl_3$ reaction was localized mostly near the cell walls. The degree of staining increased noticeably towards the root apex.

For precise localization of polyphenols, $FeCl_3$ used in conjunction with aldehyde fixatives yielded most revealing reaction products within the cells. Fe is known to form a complex with polyphenols which is electron dense and therefore visible with the electron microscope. Such a preparation is shown in figure 3, where Fe-polyphenolic complexes appear as dense reaction products within the cytoplasm, nucleus, and the interface between the cell wall and the plasma-membrane. The adjacent cells show considerably less amount of the reaction product. The electron micrograph demonstrates the distribution of polyphenols in the cell matrix (arrows). Therefore it is possible to differentiate dense cells with a specific polyphenol reaction at the electron microscope level. During post fixation treatment osmium also possibly forms a complex with polyphenols to increase the electron density of the dense cells as shown in figure 2.

There are other reports of dense cells in embryonic tissues[12,13]. It is necessary to characterize these cells as to their polyphenol content by using similar techniques. The density of the cells may not be solely due to increased ribosomal

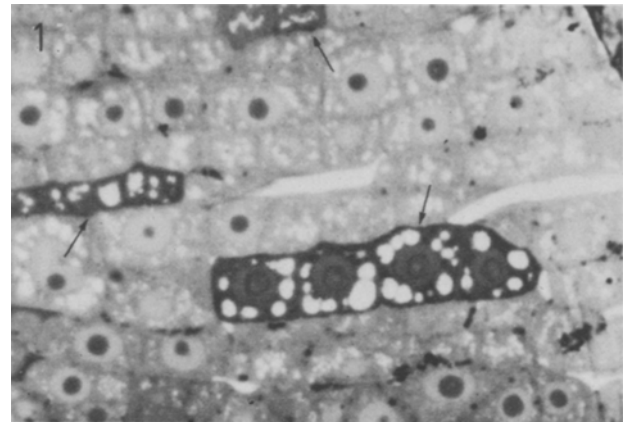

Fig. 1. Photomicrograph of a 0.5-µm epon section of *R. chamaemorus* root meristem stained with toluidine blue showing dense cells (arrows). Note the density is more or less uniformly distributed all over the cytoplasm and the nuclear matrix, but not in the vacuoles.

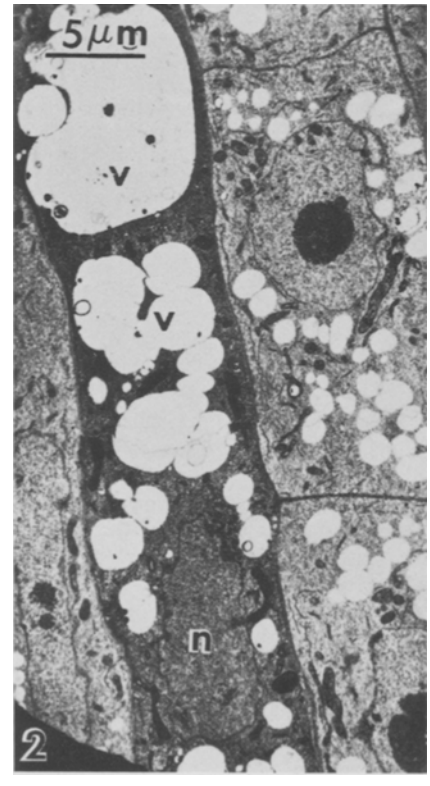

Fig. 2. Electron micrograph of a dense cell fixed in a mixture of paraformaldehyde and glutaraldehyde followed by OsO_4 treatment and stained with uranyl acetate/lead citrate. Note the density in the nucleus (n) and in the cytoplasmic matrix, but not in vacuoles (v).

population. Since polyphenols have been implicated in the protective mechanism against pathogens[14] the polyphenol-containing dense cells may have a protective role in the embryonic tissue. Mueller and Beckman[15] have referred to cells that accumulate polyphenols in vacuoles as polyphenol-storing cells in banana roots, whereas Ledbetter and Porter[16] have used the term tannin cells in *Pinus*. In the dense cells described here the polyphenols do not appear to

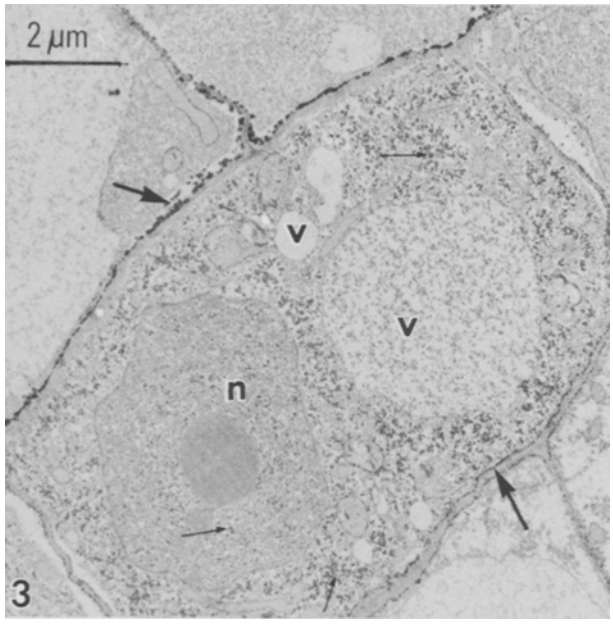

Fig. 3. Electron micrograph showing localization of polyphenols by $FeCl_3$. Note the Fe-polyphenolic complexes in the cytoplasmic and nuclear matrix (small arrows). The preparation was stained with uranyl acetate and lead citrate. The vacuoles (v) do not show reaction product; the fine granulation in the vacuole is not due to Fe-polyphenol complex and does not appear in unstained sections. Reaction product can also be seen at the interface of the cell wall and the plasma membrane (large arrows).

be localized in the vacuole and therefore may not be in the storage form. Their localization within the cell matrix suggests an active physiological participation within the cytosol.

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In situ aging of auricular chondrocytes is not due to the exhaustion of their replicative potential

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Summary. Multiplication of chondrocytes during growth of rabbit auricular cartilage was estimated on the basis of total DNA determination and compared with the population doubling level reached by these chondrocytes in vitro. The results indicate that the in situ aging of auricular chondrocytes is caused by factors other than the intrinsic depletion of their growth potential.

It has been shown that diploid cells have a finite capacity for replication in vitro[1]. Growth potential of cells seems to be related to aging since it is decreased in older donors[2,3]. Nevertheless, recent studies indicate that the cessation of growth in cultures of diploid cells may be due to terminal differentiation of cells rather than to aging[4,5]. It also appears that the finite limit of replication occurring in vitro is rarely, if ever, reached by cells in vivo and therefore is not responsible for the aging of the organism[6,7].

While the aging of auricular cartilage is hardly critical for the individual, this organ offers a good opportunity for studying the latter point. Auricular chondrocytes taken from 7-day-old rabbits pass in culture 10–14 population doublings (PD) before the onset of phase III[8] and may be easily matched with chondrocytes aging in situ, since auricular cartilage forms a closed system in which migration of cells is prevented by an intercellular matrix. It is also noteworthy that nearly all chondrocytes in auricular cartilage from 7-day-old rabbits are mononuclear, while in 4-week and older rabbits centrally located chondrocytes contain 2 nuclei and are much larger[9]. These chondrocytes, in adult rabbits, display pycnotic condensation of the chromatin and accumulate a large amount of fat[10]. Binuclear chondrocytes seem to represent terminally differentiated cell types; mononuclear chondrocytes from older rabbits are able to grow in culture for a limited period[11]. It appears, therefore, that in mature auricular cartilage there exist both cells which are capable of replication and those that are not, they show a characteristic spatial distribution, and in the latter cells changes which may be interpreted as aging occur relatively early. In this work actual multiplication of auricular chondrocytes during growth of cartilage was estimated on the basis of total DNA determination in cartilage of varied ages to see how it corresponds with the replicative potential displayed by these cells in culture.

Materials and methods. Auricular cartilages were carefully cleared of surrounding tissues, including, as far as possible, perichondrium, then lyophilized and pulverized in an agate mortar. The pulverized material was dried to constant weight, weighed, and divided into suitable samples. The samples were digested overnight in preactivated 0.5% papain in 0.1 M phosphate buffer a t 37 °C[12]. The partially digested material was cooled and precipitated with trichloroacetic acid (TCA) added to the final concentration of 5%.