Nucleolar Changes in Lemon Fruit Explants (*Citrus limon* L.) Incubated in a Non-Aqueous Medium

Variations in nucleolar morphology, especially the formation of highly refractile nucleolar inclusions, have been considered as being early cytological indications of growth of lemon fruit explants inoculated onto a mineral-sucrose nutrient medium[1,2]. Refractile inclusions were evident in very few nucleoli (1–2%) in explants incubated on distilled water[1]. The suggestion was made that the qualitative and/or quantitative availability of nutrient materials may have been responsible for these differences in nucleolar morphology between the explants incubated on distilled water and on a mineral-sucrose medium[1]. The formation of highly refractile nucleolar inclusions in lemon fruit explants incubated in a non-aqueous medium in the complete absence of any external source of nutrient material is reported here.

Materials and methods. Excised vesicle stalks from mature lemon fruits (*Citrus limon* L.) were: 1. inoculated onto a mineral-sucrose-agar medium[3]; and 2. completely immersed in liquid paraffin (light grade) 3–4 mm deep in 'Pyrex' Petri dishes. The Petri dishes of both treatments were sealed with 'Parafilm' and placed in the dark for 48 h at 25–28°C. The nutrient medium and the liquid paraffin were sterilized by autoclaving for 15 min at 121°C. At the end of the 48-hour incubation period, the stalks from both treatments were placed in Lillie's AAF solution[4] for 24 h in the cold. In the case of treatment 2, the stalks were first blotted thoroughly with filter paper to remove the excess liquid paraffin adhering to the tissue surface before placing the stalks in the fixative. The explants of treatment 2 were swirled vigorously in the fixative every 15 min during the first 2 h of fixation as a means of minimizing the amount of liquid paraffin adhering to the tissue surface. BDH Laboratory Reagent Light Grade Liquid Paraffin was used in this investigation as it does not contain any kind of added stabilizers (personal communication with BDH Chemicals Ltd., Poole, England).

Longitudinal paraffin sections of the fixed explants were prepared as described previously[2] except that the dewaxed sections were hydrated and dehydrated in a graded *n*-propanol series instead of a graded isopropanol series before mounting them in the unstained condition. Mounting was done with 'Sira' mountant after clearing with xylene or in Euparal after dehydration with *n*-propanol. Unstained longitudinal paraffin sections of freshly excised stalks fixed in AAF solution served as physiological and cytological controls.

Results and discussion. The homogenous-appearing nucleoli in spindle-shaped nuclei were evident in the control tissue (Figure 1) as was observed in previous investigations[1,2,5]. Many of the nucleoli in the explants from treatment 1 contained the highly refractile inclusions (Figure 2) which have been considered as being indicative of growth of the cells in which such nucleolar inclusions are found[2]. Large numbers of nucleoli in the explants from treatment 2 likewise possessed these highly refractile inclusions (Figure 3). These refractile nucleolar inclusions

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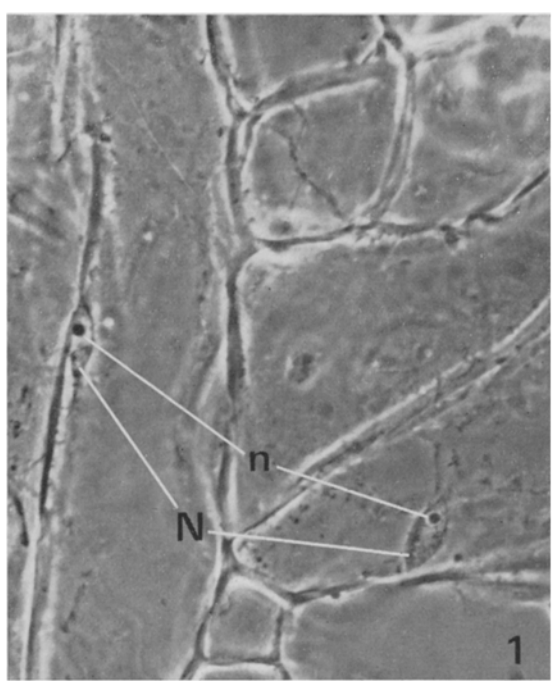

Fig. 1. Control tissue showing the spindle-shaped nuclei and the homogeneous-appearing nucleoli. Phase-contrast microscopy, unstained preparation. ×800.

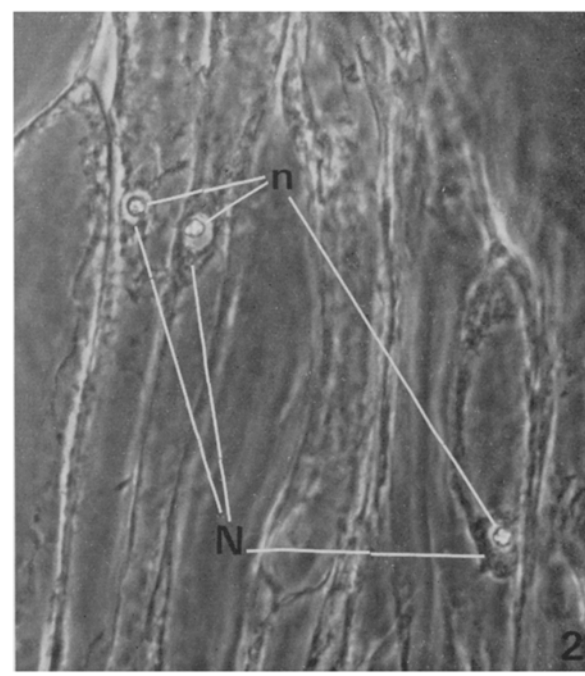

Fig. 2. Tissue incubated on the nutrient medium for 48 h showing nuclei with the highly refractile material in the nucleoli (see reference[2]). Phase-contrast microscopy, unstained preparation. ×800.

in tissue from treatments 1 and 2 showed the same optical properties under phase-contrast and polarized light microscopy as was observed in squash preparations and sectioned material of lemon fruit cultures and germinating seedlings[2]. In addition to the formation of the refractile nucleolar inclusions, all other aspects of change in nucleolar morphology observed in growing vesicle stalks[2] were also evident in the nucleoli of vesicle stalks from treatment 2 in this investigation.

Injured cells at both ends of each vesicle stalk are invariably introduced at the time the stalks are excised from the fruit. It is possible that the injured cells leak some of their contents to the external aqueous medium when the stalks are placed on distilled water which may have an adverse effect with respect to the formation of the refractile nucleolar material. Although leakage from the injured cells may also occur when the explants are placed on the nutrient medium, the nutrient materials evidently provide a physiological situation which is conductive to the development of this refractile nucleolar material.

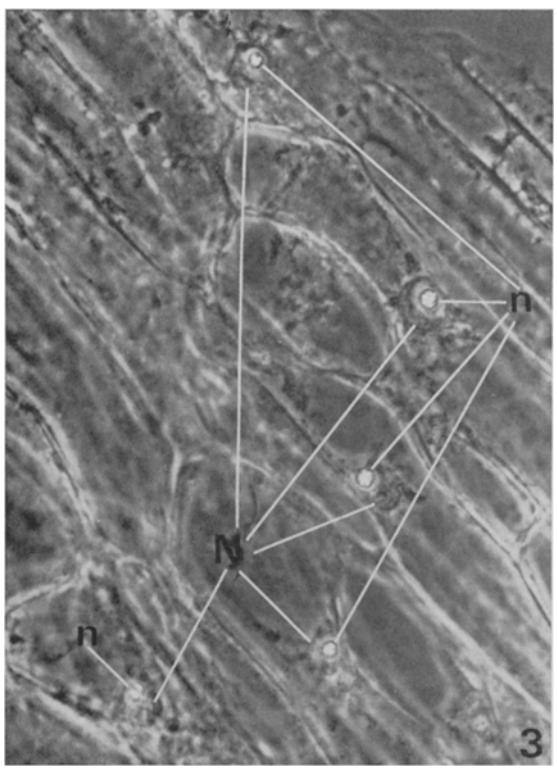

Fig. 3. Tissue immersed in liquid paraffin for 48 h showing nuclei with the highly refractile material in the nucleoli as in Figure 2 above (see reference[2]). Phase-contrast microscopy, unstained preparation. ×800. N = nucleus; n = nucleolus.

Liquid paraffin was used here as a means of sealing the excised tissue against water loss while at the same time permitting gas exchange by virtue of its permeability to gases such as oxygen and carbon dioxide. The physical conditions imposed by treatment 2 also enable the cells to remain isotonic with themselves since there is no possibility of water exchange occurring between the cells and their external environment. In addition, the liquid paraffin also probably prevents or minimizes any leakage of the cell contents from the injured cells since it is unlikely that the aqueous contents of the injured cells would be able to migrate into this non-aqueous oily external medium.

The results of this investigation show that liquid paraffin and the mineral-sucrose nutrient medium provide physiological conditions which yield like transformations in nucleolar morphology in explanted lemon fruit tissue. Also evident here is that all materials required for such transformations in nucleolar morphology under the conditions imposed by treatment 2 must come solely from endogenous sources within the explants themselves since there are no external supplies of nutrients or growth-regulating substances in the liquid paraffin medium.

By employing distilled water and liquid paraffin as incubating media, it becomes possible to separate out different stages of nucleolar morphology in lemon fruit explants in the complete absence of exogenous sources of nutrient materials or growth-regulating substances and in the absence of meristematic activity. Only the physical act of removing the tissue from its natural environment within the fruit is needed to bring about the physiological changes which lead to these transformations in nucleolar morphology.

Two recent publications describing optical anisotropy in the Nucleoli of onion root tips[6] and in animal cells[7] show that this optical phenomenon is not restricted to the nucleoli of the plant material used in previous investigations[8].

Zusammenfassung. Zitronenfruchtgewebe, welche in eine nichtwässrige Flüssigkeit (Paraffinöl) inkubiert wurden, zeigen Veränderungen der Nuklearmorphologie, analog zu denjenigen Geweben, die auf einem Mineralsalze und Saccharose enthaltenden Nährmedium kultiviert wurden. Daraus ergibt sich, dass alle Stoffe, die für eine unklearmorphologische Umwandlung notwendig sind, in den Geweben selbst erzeugt werden, wobei keine Nährstoffe oder wachstumsregulierenden Substanzen zugeführt werden und auch keine meristematische Aktivität festzustellen ist.

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Discovery of Toxicogenic *Stachybotrys chartarum* Strains in Finland

Stachybotryotoxicosis is a mycotoxicosis caused by feeding animals with fodder or grain contaminated with the fungus *Stachybotrys chartarum*. Stachybotryotoxicoses have been investigated in the Soviet Union[1,2] and in other East-European countries[3,4]. Toxicogenic *S. chartarum* strains have not previously been reported in Scandinavia.

According to the literature, *Stachybotrys chartarum* (Ehrenb. ex Link) Hughes (Syn. *S. atra* Corda, *S. alternans* Bon.)[5] is a saphrophyte commonly occurring on cellulosic