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.

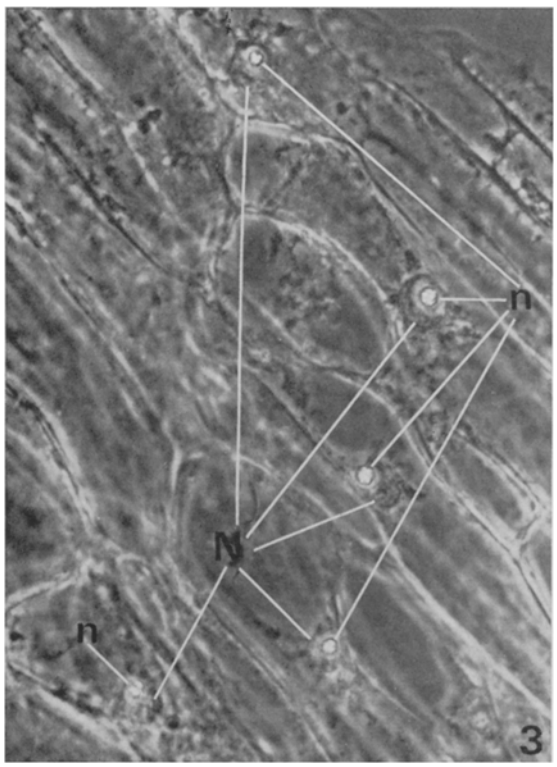

Fig. 3. Tissue immersed in liquid paraffin for 48 h showing nuclei with the highly refractile material in the nucleoli as in Figure 2 above (see reference[2]). Phase-contrast microscopy, unstained preparation. ×800. N = nucleus; n = nucleolus.

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

materials which it can decompose rapidly; it has sometimes been found on farm seeds and various types of fodder. Mycelium is creeping, branched, septate, at first hyalin becoming black with age. Conidiophores are erect, septate, simple or sympodially branched, hyalin at first, the upper portion becoming darker and roughened with age; each is terminated by a group of 6 to 10 dark coloured, ovoid phialides. Conidia are borne single at the ends of phialides, are elliptical to ovate, dark-coloured, roughened 8–11 × 4.5–5.5 µm.

Three S. *chartarum* strains were investigated. 2 of the strains were isolated during a mycological investigation in which all species of fungi found from the grain samples were registered. One of the 2 strains originated from a mouldy sample of barley and the other from a sample of wheat. The 3rd sample was isolated from a commercial pig feed, suspected of causing abortions in pigs and giving birth to mummified foetuses.

The fungi (Figure 1) were isolated on a wet filter paper in a large petri dish at room temperature. The strains were cultivated for 2 months in Roux flasks at room temperature on a grain mixture composed of $^1/_3$ wheat, $^1/_3$ barley and $^1/_3$ oats.

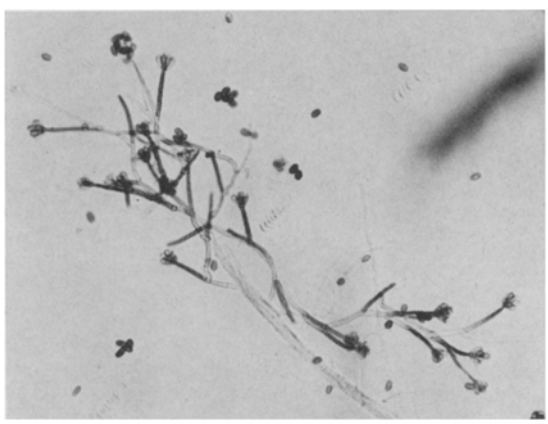

Fig. 1. *Stachybotrys chartarum*. Conidiophores showing sympodial branching. Lactophenol, unstained. ×200.

The infected grains were fed to mice. Every test group comprised 2 white mice, 1 of either sex, weighing 15–18 g, strain IRM, Orion, Finland. The mice were fed solely on the grain mixture. The same grain mixture, sterilized but not inoculated with the fungi, was given to the control group.

On the 3rd day some mice showed symptoms of disease and all the mice died in 5–10 days, except the control group which was killed on the 14th day. The mice became apathetic, moved slowly and showed symptoms of ataxia, shivering, circling movements and peritoneal pain. The ears, nose and paws became cyanotic. The nose became swollen and partly lost its hair.

The pathologic picture showed haemorrhages in the stomach, duodenum and jejunum as well as in the cerebral meninges. Microscopic examination revealed degenerations in the liver (Figure 2), kidney and heart. Necrotic processes were found along the digestive tract in the mucous membrane. Bacteriologic and mycologic investigations from the kidney, liver and lungs failed to show any infections by bacteria or fungi.

An attempt at extraction of the toxic substance from the grain was carried out in a Soxhlet apparatus according to PALYUSIK[6]. From the ether extract a substance, supposedly stachybotryotoxin A, was obtained by FIALKOV and SEREBRYANIU's procedure as modified by PALYUSIK. This substance was subjected to the resorcine test, which is considered a reliable and specific indicator of stachybotryotoxin (PALYUSIK). The resorcine test gave, as a sign of positivity, red colour for strain No. 1 and dark brown colours for strains Nos. 2 and 3.

Although only very few mice were used in this preliminary test, the clinical and pathologic-anatomical pictures and the positive resorcine test unequivocally support the suggestion that the 3 *Stachybotrys chartarum* strains were producers of stachybotryotoxin.

ВЫВОДЫ. Три штаммы *Stachybotrys chartarum*, которые были обнаружены в пробах зерна в Финляндий, вызывали смерть мыш, которым кормили. *S. chartarum* в образе зараженного зерна. *S. chartarum* был первый раз обнаружен в скандинавских странах.

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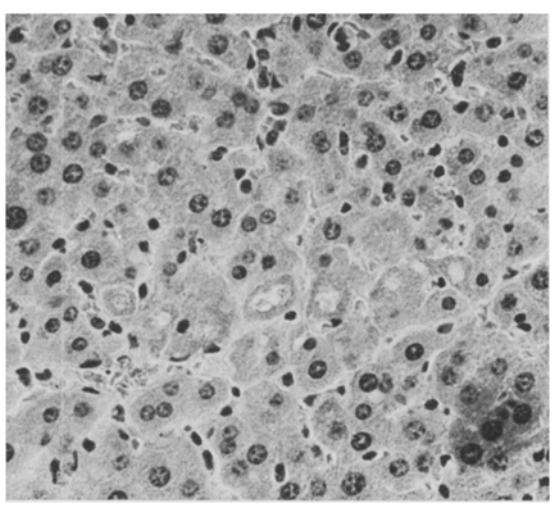

Fig. 2. Degenerated liver cells (centre) of a mouse fed with *S. chartarum* infected grain. Formaline fixation, H.E. ×1000.

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