Nucleolar Changes in Non-Growing and Growing Lemon Fruit Explants (*Citrus limon* L.)

Variations in nucleolar morphology, especially the formation of highly refractile nucleolar inclusions, are early cytological indications of growth of explants from mature lemon fruits on a mineral-sucrose medium[1,2]. This report describes changes in nucleolar morphology associated with non-growing and growing lemon fruit explants.

Vesicle stalks excised aseptically from mature lemon fruits (*Citrus limon* (L.)) were inoculated onto distilled water and basal nutrient medium[3]. Paraffin sections of 24- to 72-hour-old explants fixed in Randolph's CRAF solution[4] were mounted unstained after dewaxing. Freshly excised tissue prepared in the same manner served as controls. Photomicrographs were made with positive phase-contrast microscopy.

Control tissue nucleoli were predominantly spherical uniform-appearing organelles approximately 1 μ in diameter (Figure 1). Within 24 h enlarged uniform-appearing nucleoli (1.5–2 μ diameter) became evident in explants on distilled water and their number increased markedly after 48–72 h (Figure 2). Some enlarged nucleoli after 48–72 h showed regions with differing light transmission properties similar to the Figures 3, 4a and 4e. There was no evidence of refractile nucleolar inclusions in the distilled water explants within the 72 h period.

Nucleolar behaviour in tissue on basal medium was similar to distilled water explants in that there were enlarged uniform-looking nucleoli as well as nucleoli with regions differing in light transmission properties with diameters 2–2.5 μ and at times 3–4 μ (Figures 3, 4a, 4e). In addition, many nucleoli contained highly refractile inclusions which varied in size and number (Figures 4b–e) and which showed optical properties described previously[5,6]. In tissue which did not show signs of growth (e.g., starch formation[2,3]) (Figure 4d) nucleolar behaviour was like that in distilled water explants, namely enlargement without formation of refractile inclusions. The magnitude of nucleolar enlargement in growing and non-growing explants on basal medium was somewhat greater than in non-growing explants on distilled water within the same given period. Possibly qualitative and/or quantitative availability of nutrient materials required for this phenomenon to occur may have been responsible for this difference for in explants on distilled water material requirements were solely endogenous in origin.

As spheroids there was a pronounced increase in nucleolar volume in enlarged nucleoli of explants on distilled water and basal medium when compared with the nucleolar volume of the control tissue. The absence of a nucleolar membrane[7] points away from nucleolar enlargement being an osmotic phenomenon and evidence has shown a relationship between increase in nucleolar

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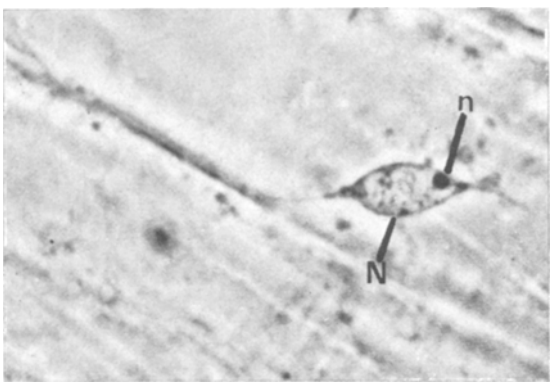

Fig. 1. Nucleus (N) and nucleolus (n) of control tissue. × 1250.

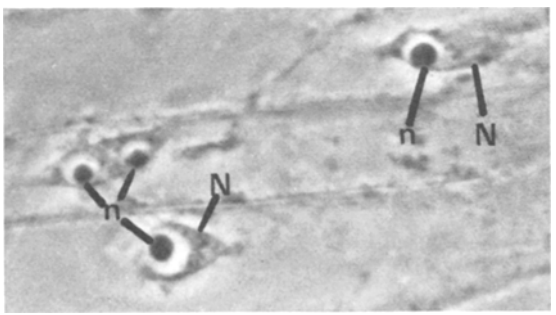

Fig. 2. Nuclei and enlarged nucleoli of explant on distilled water for 48 h. One binucleolate nucleus is visible. × 1250.

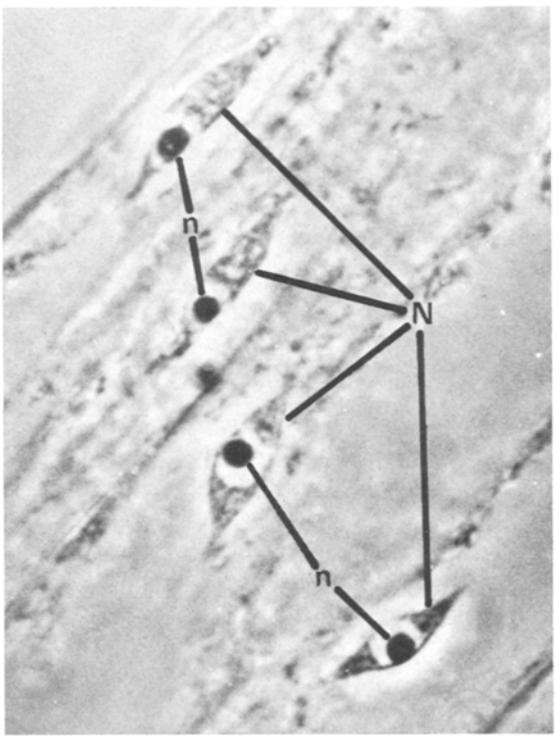

Fig. 3. Nuclei and enlarged nucleoli of explant on basal medium for 48 h. The topmost nucleolus shows regions with differing light transmission properties. × 1250.

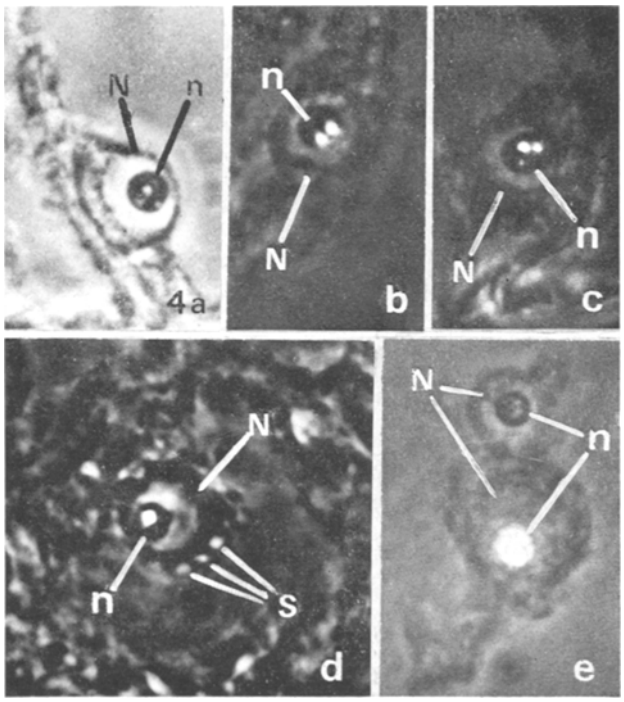

Fig. 4. Nuclei and enlarged nucleoli of 60-hour-old explants on basal medium. × 1250. (a) Nucleolus with regions of differing light transmission properties. (b, c) Nucleoli each with highly refractile inclusions. (d) Nucleolus with a single large refractile inclusion occupying the majority of the nucleolus (Compare with Figure 15 of reference[11]). Note the starch granules (S) adjacent to the nucleus. (e) 2 nucleoli with the upper one showing regions with differing light transmission properties; lower nucleolus contains numerous highly refractile inclusions. Overlapping and juxtaposition of one inclusion with another obscures their individuality (see reference [5])[12].

volume and synthetic activities in cells[8–10]. By withholding nutrients it has been possible to separate nucleolar enlargement, an activity associated with non-growing and growing lemon fruit explants, from the formation of refractile nucleolar inclusions, an activity associated with growing tissue. Cytochemical and cytomorphological investigations of these 2 nucleolar phenomena are under way[12,13].

Résumé. L'expansion des nucléoles et la formation de corps réfractaires nucléolaires sont deux étapes dans l'activité cytologique observée dans la croissance de tissus de citron in vitro. En privant les tissus de substances nutritives, on a réussi à séparer ces deux étapes.

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[12] It should be pointed out that in the unstained material the contrast between the refractile nucleolar inclusions and the nucleus is so high that the background invariably becomes dark as the refractile inclusions are brought out during printing.
[13] Addendum. (a) Dehydration and paraffin infiltration here and in previous investigations were done with isopropanol. (b) Occasionally stalks from some lemons showed 1–2% nucleoli with refractile inclusions after 48–96 h on distilled water. Physiological age differences between the fruits used, especially with respect to available endogenous nutrients, could account for the infrequent occurrence of this nucleolar morphology on distilled water. Another possibility is contamination of the stalks with nutrients from the sac juice during excision from the fruit.

Chemical Sympathectomy: Histochemical and Submicroscopical Consequences of 6-Hydroxy-Dopamine Treatment in the Rat Iris

Tranzer and Thoenen[1] recently reported that systemic administration of 6-Hydroxydopamine (6-HODA) results in a degeneration of postganglionic autonomic nerve fibers in the cat. In this present study, the effects of this drug upon catecholamine fluorescence and electron microscopic structure of postganglionic adrenergic fibers in the rat iris will be reported, as compared with the alterations seen after a surgical removal of the superior cervical sympathetic ganglion.

Investigations were carried out on male albino rats. The animals were injected 4 times with 20 mg/kg 6-HODA with 12 h intervals and sacrificed 24, 48 and 72 h after the first injection. Both irides were dissected either in physiological saline (for fluorescence microscopy) or in Karnovsky's aldehyde fixative (for electron microscopy). Pineal gland and vas deferens was excised for electron microscopy only. The standard formaldehyde-condensation technique of Falck was used to locate catecholamines[2,3]; the usual electron microscopic embedding and sectioning procedures were employed for ultrastructural studies, using a Reichert Ultratome with glass knifes, Reynold's lead citrate staining and a Tesla 242 D table electron microscope.

Twenty-four hours after the first injection, no changes in the peripheral autonomic innervation apparatus could be observed, either by means of fluorescence microscopy or with electron microscopy. 48 h after the first injection, the terminal branches of the adrenergic plexus exhibited signs of depletion, yet no characteristics of degeneration could be seen. 72 h after the first injection, however, fluorescence of the terminal arborization of the adrenergic neurons completely ceased, and only large (pre-terminal) branches of the adrenergic axons exerted fluorescence, that, however, appeared to be completely normal. At the same time, well-defined degenerative signs became apparent in the terminal adrenergic axons, characterized by the appearance of large vacuoles and in a complete disappearance of axoplasmic material from the axolemmal sheath. This kind of 'osmiophobic' degeneration pattern is characteristic for the postganglionic adrenergic fibers

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