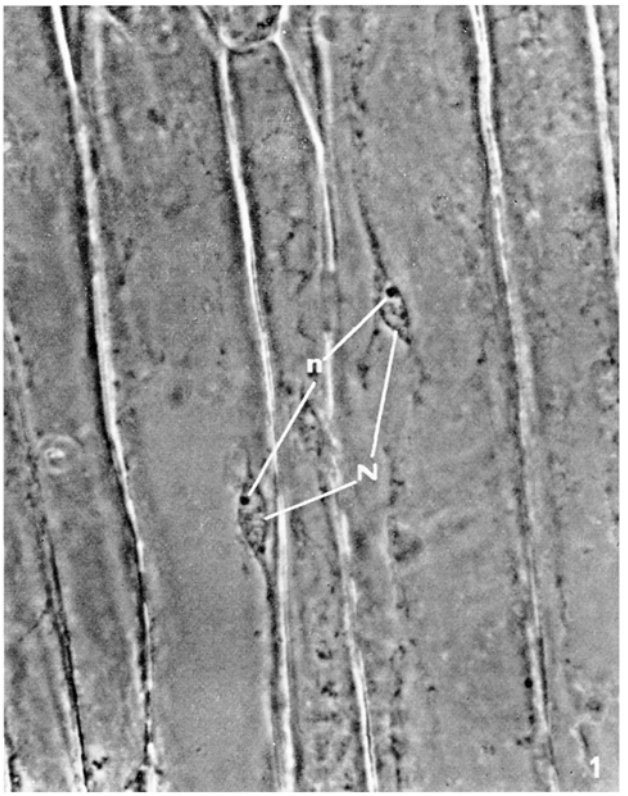
Nucleolar Enlargement in Lemon Fruit Explants (*Citrus limon* L.)

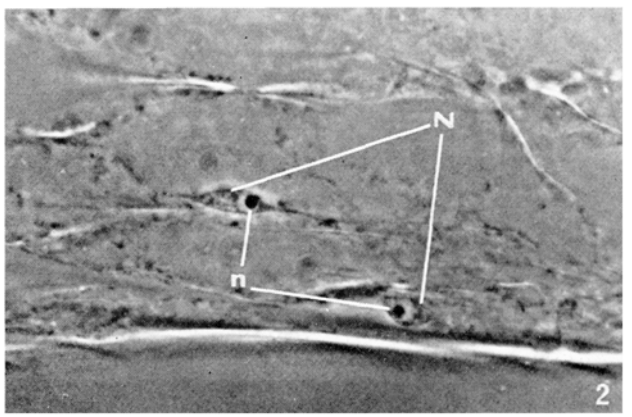
An enlarged nucleolus is usually considered as being a sign of increased protein synthesis[1–6]. STICH[7] has indicated that a hypertrophic nucleolus may not be an indication of high protein synthesis in cells under abnormal conditions. This communication presents observations on nucleolar enlargement in quiescent non-dividing cells under conditions which would not be regarded as favourable for increased synthetic activity.

Vesicle stalks were removed aseptically from mature lemon fruits (*Citrus limon* L.) and inoculated onto distilled water and 1 *M* mannitol in 'Pyrex' Petri dishes and placed in the dark at 25 °C after sealing the dishes with 'Parafilm'. Explants were harvested after varying periods in vitro and unstained paraffin sections of CRAF and FPI-fixed tissue were made as described previously[8]. Unstained paraffin sections of freshly excised stalks served as physiological and cytological controls and all specimens were examined and photographed with positive phase contrast microscopy.

Nucleoli of the control tissue were predominantly spherical-shaped uniform-appearing structures (ca. 1 µm in diameter) in spindle-shaped nuclei as described previously[9] (Figure 1). Spindle-shaped nuclei have also been observed in living lemon fruit tissue[10] and are not, therefore, fixation artefacts. Nucleolar enlargement was apparent after 6 h in vitro and maximum size (ca. 3 µm in diameter) was attained after 24 h on distilled water. Many cells with enlarged nucleoli were apparent after 48 h (Figure 2). The nucleoli were progressively smaller after 72–96 h which indicated that the enlargement process had ceased and that the nucleoli were possibly returning to their normal size. The nuclei were predominantly spindle-shaped throughout the entire period of treatment.

Nucleolar enlargement occurred more rapidly on mannitol than on distilled water, maximally enlarged nucleoli (ca. 3 µm in diameter) being evident after 12 h. Many of the cells contained enlarged nucleoli by 48 h (Figure 3).





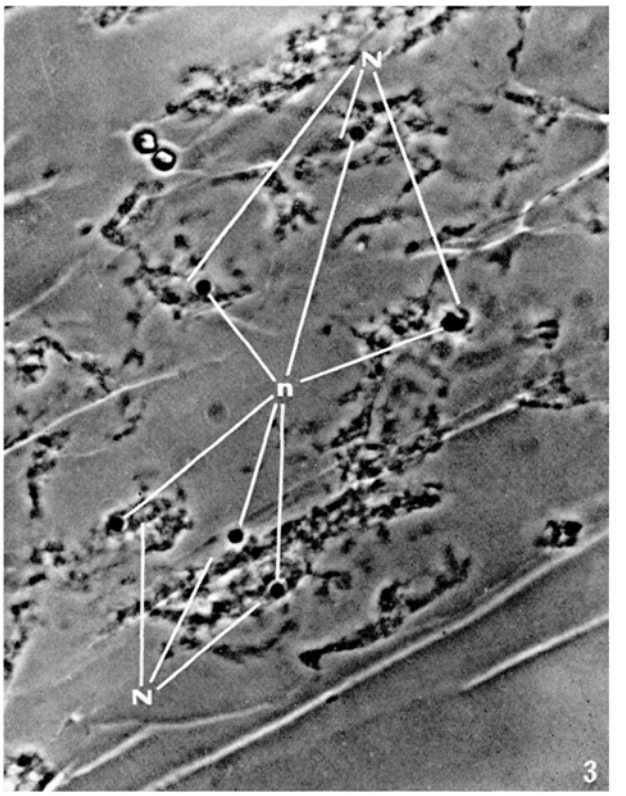


Fig. 1. Control tissue showing the nucleoli in the spindle-shaped nuclei. Note the state of the cytoplasm. ×800.

Fig. 2. Spindle-shaped nuclei with enlarged nucleoli in stalk incubated on distilled water for 48 h. Note the state of the cytoplasm. ×800.

Fig. 3. Spindle-shaped nuclei with enlarged nucleoli of a stalk incubated on 1 *M* mannitol for 48 h. Note the complete plasmolysis of the cytoplasm. ×800. N, nucleus; n, nucleolus.

The cytoplasm of the plasmolyzed cells was reduced to approximately $^2/_3$ its original volume by 12 h and to approximately $^1/_3$ its original volume by 24 h. At 48 h, plasmolysis was complete, the cytoplasm of the cells having been reduced to relatively small masses (Figure 3). Though the shape of the nuclei in the plasmolyzed cells was distorted at times, their spindle shape was still discernible.

Nucleolar enlargement under the abnormal conditions of cell plasmolysis described here lends support to the suggestions of STICH[7,11], that nucleolar growth may be dependent upon the metabolic activity of the cytoplasm, and that under abnormal conditions an enlarged nucleolus is not necessarily a sign of increased protein synthesis. Nucleolar enlargement in lemon fruit cells incubated on distilled water and on a hypertonic mannitol solution indicate that this phenomenon is not a result of changes in the water content of the nucleoli. This is in agreement with the finding[7] that the increase and decrease of nucleolar volume in *Acetabularia mediterranea* was not due to changes in water content.

Note added in proof. The following observation was made after this article was sent to press. Marked nucleolar enlargement was evident in many cells of lemon explants that were placed on a dry glass surface immediately upon removal from the fruit and immediately covered with liquid paraffin (Light Grade) and placed in the dark for 48 h at 25°C.

Riassunto. L'ingrossamento nucleare è stato osservato in cellule plasmolizzate e non plasmolizzate di tessuto di frutto di limone. Queste osservazioni confermano le precedenti investigazioni secondo le quali l'ingrossamento nucleare può dipendere da una attività citoplasmatica e che sotto condizioni non normali un nucleo ingrossato non rappresenta necessariamente un incremento di sintesi proteica.

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Rotation and First Reversion in the *Octopus* Embryo - A Case of Gradual Reversal of Ciliary Beat

Coleoid Cephalopoda show two types of egg deposit. Decapoda enclose their eggs in jelly cases. The egg chorion (a product of the ovary) strongly increases in size during embryonic development, giving the embryo ample space in the perivitellin fluid which it circulates by means of ciliary motion[1]. The Octopoda, on the other hand, lay eggs that are surrounded but by an elongate, stalked chorion, which swells only slightly during development; the embryo remains tightly enclosed.

In 1875, LANKESTER[2] noted that the *Octopus* embryo at an early stage reverses its position in the chorion, turning around from the micropyle to the stalk side. This important phenomenon sunk into oblivion, however, for more than half a century[3]. PORTMANN[4] rediscovered it in 1933, and – referring to it as the first reversion – he described a second reversion performed by animals at a late embryonic stage, compensating for the first reversion in order to bring about a position better suited – but not necessary[5] – for hatching. A double reversion is also known in *Eledone* and *Tremoctopus*[6,7]; most observations were made on *Octopus*, however[8,9]. A time-lapse film study by PAINLEVÉ and ORELLI[10] illustrates all movements of the *Octopus* embryo. Lately, SACARRÃO[11] critically revised the entire problem of the first reversion or 'blastokinesis'[6].

Inspite of this large body of information, the mechanism achieving the first reversion remained unknown, mainly because Octopod embryos were assumed to be entirely devoid of cilia[12]. This is true for the actual embryo, but I observed that the ectodermic surface of the outer yolk sac is covered with ciliary cells. The cilia are very delicate and cannot be seen through the chorion which is transparent, but on bare embryos, they can easily be observed in vivo under the microscope; examination of histological sections also reveals their presence.

Earlier observations. In order to understand the function of the cilia during the first reversion, we have to recall the observations described by the afore-mentioned authors.

The yolk mass is penetrated with a network of plasmic processes of the yolk syncytium that are likely to bring about the contraction along the longitudinal axis of the egg preceding the first reversion[7,11]. The yolk mass forms together with the overlaying embryo and yolk envelope a unity; a gliding of the blastodisc on the yolk[9] is inconceivable[11].

From stage VI (staging according to NAEF[3]) on, the embryo slowly rotates around its longitudinal axis[9]. This rotation is directed clockwise when seen from the micropyle end of the chorion and remains so before, during and after the first reversion[10].

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