Table II. Developmental capacity of oocytes (matured in vitro with progesterone) after shedding by foster females and 'artificial' fertilization

	Totals	%
Oocytes shed	296	100
Activation reaction	158	53.3
Blastula stage	30	10.8
Hatching tadpoles	20	6.7
Feeding tadpoles	13	4.4
Metamorphosed frogs	8	2.7

eggs, spontaneous activation, i.e. cortex contraction and cleavage (in unfertilized eggs cleavage is abortive). The two types of *Xenopus laevis laevis* oocytes, those which had passed through an oviduct within 3 h and those which had been kept in maturation medium for 3 h, were so treated and their behaviour compared. Oocytes which had passed the oviduct were fertilized following WOLF et al.[5]. In addition to the parallel storage of oocytes in maturation medium (a), a second control was done as follows: 3 ovulating females were sacrificed, and their serum prepared. Oocytes matured with progesterone were transferred into this serum (medium b) after the maturation spot had appeared, and incubated for 3 h. All experiments were realized at 21–22 °C (temperature of the solutions used).

Results. Tables I and II.

Discussion. The process of maturation not only induces completion of meiosis and the transformation from the cytoplasmic state of an oocyte (RNA synthesis) to the cytoplasmic program of an egg (DNA synthesis), but also acts on the oocytes membrane (for review see[8]). The present results suggest that cortex maturation in vivo depends on contact of the oocyte with the oviduct, since the controls did not contain fully mature eggs. This observation is corroborated by the possibility of raising the low metabolic activity of a body cavity egg to the high level of a shed egg by treating the body cavity eggs with extracts from the uppermost part of the oviduct[9].

Summary. Contact of progesterone matured oocytes of *Xenopus laevis* with the oviduct reduces the time necessary to attain cleavage capacity from 24 h to 3 h. Full maturity has been demonstrated by normal development of the matured eggs after fertilization.

R. BRUN

Station de Zoologie Expérimentale, University of Geneva, 154, route de Malagnou, CH-1224 Chêne-Bougeries, Genève (Switzerland), 30 July 1975.

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Nuclear Envelope Changes Related to Cell Activation in *Helianthus tuberosus* L.

Nuclear pore complexes are sites of communication between nucleus and cytoplasm through which macromolecular exchange is effected[1]. Although the structural organization of the pore complex appears to be similar in most cell types, there is considerable variation in the number of pores in the nuclei of different species and tissues[1]. Furthermore pore frequency and pore number per nucleus can increase in a given cell type with an increase in its metabolic activity[2–5].

The activation of plant storage tissues is accompanied by many changes[6] including a rise in RNA synthesis, an increase in nucleolar size, polysome formation and protein synthesis. A thin-section study of *Daucus carota* root cells also revealed a doubling of nuclear pore frequency[7], suggesting that nuclear pore number might be an important controlling factor in the process of cell activation. In this preliminary report, we describe the results of an investigation into nuclear envelope ultrastructure of dormant and activated *Helianthus tuberosus* tuber cells, in which the freeze-fracture technique was employed to permit a more accurate analysis of nuclear pore frequencies.

Materials and method. Explants of *H. tuberosus* tuber tissue were prepared and incubated, as described previously[6]. Nuclei were extracted from dormant tissue, and tissue incubated for 24 h (termed activated tissue in this report), fixed in 0.1 *M* cacodylate buffered 2.5% glutaraldehyde, glycerinated, frozen in melting freon 12, and freeze-fractured using the BULLIVANT-AMES method[8]. Cleaned replicas were examined with an AEI EM6B

Frequency of nuclear pores in nuclei from *Helianthus tuberosus* tuber cells

	Nuclear pores ($\mu m^{-2} \pm SD$)	Sample size
Dormant cells (0 h incubation)	11.3 ± 1.8	363 μm^2 of nuclear envelope from 24 nuclei
Activated cells (24 h incubation)	11.9 ± 1.9	191 μm^2 of nuclear envelope from 34 nuclei [a]

[a] The smaller total area of nuclei sampled in activated cells results from the reduced frequency of large areas of face fractures of the convoluted nuclear envelopes.

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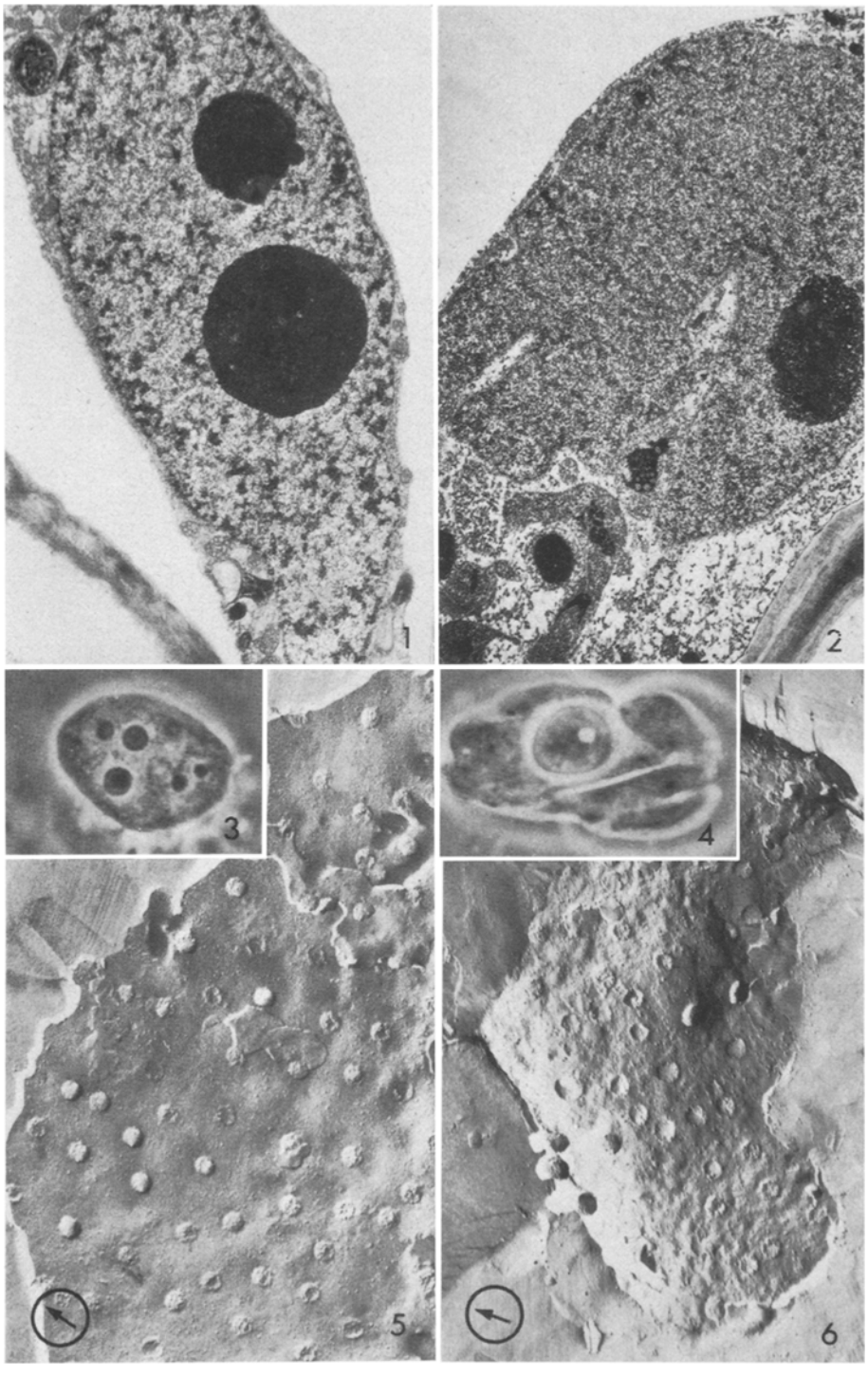

Fig. 1. Thin-sectioned 0 h (dormant) artichoke nucleus from tissue fixed sequentially in glutaraldehyde and osmium tetroxide, and embedded in araldite. The nuclear profile shows no indentations at this stage. ×7,000.

Fig. 2. 24 h (active) artichoke nucleus, prepared similarly to Figure 1. Note the convoluted outline of the nuclear envelope. ×7,000.

Fig. 3. Phase contrast photomicrograph of 0 h (dormant) nucleus isolated in cacodylate buffered glutaraldehyde, showing typical smooth outline and multiple small nucleoli. ×1,450.

Fig. 4. Phase contrast photomicrograph of typical 24 h (active) nucleus isolated in cacodylate buffered glutaraldehyde. The nucleus is larger than at 0 h, shows a convoluted outline, and has a single large nucleolus. ×1,450.

Fig. 5. Freeze-fracture replica of part of a 0 h (dormant) isolated nucleus, showing frequency and distribution of nuclear pores. ×30,000. Encircled arrow in this and the following figure indicate direction of shadow.

Fig. 6. Freeze-fracture replica of part of a 24 h (active) isolated nucleus. The frequency and distribution of nuclear pores appears similar to that observed at 0 h. ×30,000.

electron microscope. Material was processed for thin-section studies as described previously[6]. Changes in nuclear morphology were monitored using a Zeiss photomicroscope equipped with phase contrast optics.

Results. Light microscopy revealed a reduction in numbers of nucleoli, an increase in total nucleolar volume and development of nucleolar vacuoles on cell activation (compare Figures 3 and 4). Furthermore, the nucleus enlarged and developed an irregular outline with deep infoldings (Figures 1 and 2; 3 and 4). As a consequence of this, the nuclei isolated from activated cells had a much larger surface area. However, their pore frequency did not differ significantly ($p \gg 0.01$) from that of dormant cell nuclei (Table and Figures 5 and 6), despite the fact that at the relatively low pore densities observed, there appears to be sufficient space to accommodate many more pores. Both types of nuclei revealed a number of different appearances of their pores in freeze fracture replicas. These can be understood in terms of the position of the cleavage plane through the pore[9-11] and do not indicate any alteration in nuclear pore structure.

Discussion. The changes occurring in the nucleolus are in agreement with those previously reported[12]. The development of an irregular nuclear profile has been noted in activated *D. carota* root cells[7] and is not an uncommon feature of other active cells[13]. Although the nuclear changes in *H. tuberosus* follow those of *D. carota* it is interesting that they differ with regard to the density of pores in the nuclear envelope. In view of the fact that an increase in nuclear pore frequency commonly accompanies cell activation in a number of systems[2-5] and in particular, *D. carota* root cells activated in an identical manner to that used here for *H. tuberosus*[7], the lack of a corresponding change in *H. tuberosus* may seem surprizing.

If nuclear pore complexes are regarded as relatively stable entities having the capacity to vary the rate at which nuclear products are transported through them, then formation of new pores might not occur unless the amount of material to be exported to the cytoplasm is in excess of that which existing pores can process. Data on flow rates of material through nuclear pores shows variation between species and in the same system at different developmental stages[1,14,15]. Thus, the difference in response between *H. tuberosus* and *D. carota* may be accounted for in terms of differences in the capacity of the nuclear pores already present to meet the new transport demands.

Any explanation for the difference must also take into account the increase in nuclear surface area. Although a similar pore frequency is shown in dormant and active cells of *H. tuberosus*, the absolute number of nuclear pores per nucleus must rise with the development of undulations in the nuclear surface. It might be that although formation of new nuclear pores is required, a restriction of an unknown nature on pore frequency exists, making an increase in nuclear pores dependent on the formation of new nuclear envelope.

Whatever the explanation, it is clear that the relationship between nuclear pore frequency and cell metabolism is more complex than originally anticipated, and emphasizes that an increase in total pore number is not always accompanied by an increase in pore frequency. Further work is in progress to attempt to determine more precisely the factors which lead to nuclear pore formation in this and other cell systems[16].

Summary. The ultrastructure of nuclei from dormant and activated *Helianthus tuberosus* tuber cells has been investigated with particular reference to nuclear pore frequency and nuclear envelope invaginations, and the results discussed in relation to observations made on other cell types.

N. J. SEVERS and E. G. JORDAN

Biology Department, Queen Elizabeth College, University of London, Campden Hill Road, London W8 7AH (England), 12 June 1975.

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Occurrence of Extra-Ovarian Ovules in Sunflower Plants (*Helianthus annuus* L.) Treated with Chlorflurenol

Morphactins (derivatives of fluorene-9-carboxylic acid) have been reported to increase or decrease the number of flowers[1], favour femaleness[2-4], and cause suppression or fusion of flowers or of floral parts[2,5,6]. In this laboratory, the effects of chlorflurenol (2-chloro-9-hydroxy fluorene-(9)-methylate, EMD 7301 W) are being studied on the development of the inflorescence in some members of the Compositae. One of the interesting observations made with sunflower is reported here.

Plants of *Helianthus annuus* L. var. Armavirsakij, have a terminal inflorescence bearing a whorl of sterile ray florets and 800–2,000 bisexual disc florets which form fruits. The inferior ovary has a basally attached ovule (Figure 1).

A foliar spray of aqueous chlorflurenol solution was given to 6-week-old plants at the following concentrations: 3×10^{-3} *M*, 10^{-3} *M*, 3×10^{-4} *M*, 10^{-4} *M*, and 3×10^{-5} *M*, along with 0.01% Tween 80 as the surfactant. The controls received only the surfactant solution. 8 to 10 weeks later, certain inflorescences of treated plants were observed in which the initiation of the disc florets was haphazard instead of being in spirals as in controls. Some of the florets showed exposed ovules (Figure 2). 1 to 7 (rarely up to 14) ovules were found projecting through the narrow apex of the corolla tube, or lying in the middle of the split-open boat-like corolla, or emerging laterally through the ovary wall. Some florets showed 2 ovules each, one basally attached, normal ovule con-