photographs, estimations were made by 2 persons with a measuring magnifier. Data was processed in a Digital PD P8/e (Digital Equipment Co., Maynard, Mass.).

*Results.* In the control rats, the whole thickness of the urothelial membranes was about 110 A at the level of the plaque areas. The unit membrane was asymmetric, since there was a significant difference in width between outer and inner leaflets (Figure 1 and Table).

In the EFAD rats, the overall thickness of the membrane was reduced, measuring about 100 A, whereas the asymmetry was notably diminished due to a marked decrease in the width of the thick, luminal leaflet (Figure 2). Changes in the thickness of inner and middle leaflets were statistically not significant. Similar changes in width were observed in the interplaque areas of membrane. No significant difference was noted between luminal plasmalemma and vesicle membrane in control and experimental animals.

Preliminary observations indicated that the ultrastructural changes were reversed by adding to the diet a source of EFA, such as corn oil.

*Discussion.* Our observations are in keeping with data showing that polyunsaturated fatty acids made up on molar basis 52.3% of the total fatty acid content of the bladder luminal membrane[12]. A perusal of the Table indicated that the 3 EFA gave the high value of 46.6%.

We should like to propose that the unusual asymmetry of the urothelial membrane is primarily due to its content in EFA rather than to the membrane protein or mucoprotein[13]. Furthermore, it may be that EFA containing lipids may have an asymmetric arrangement in the membrane, as has been shown for phospholipid and glycolipid components in red blood cells[14].

The ultrastructural changes of urothelial membrane in the EFAD would support the view that the composition of the dietary fatty acids may affect the pattern of these lipids in subcellular fractions[15]. Furthermore, it would seem that the higher the content of EFA in a membrane, the greater the likelihood that changes will be induced by a deficient diet in these nutrients. Thus, liver mitochondria and plasmalemma[16–18], as well as red cell membrane[19], which are rich in EFA, showed chemical, ultrastructural and physical changes in the EFAD[20–22]. Yet, no variations in the thickness of the membrane of these organelles have been reported in the EFAD.

Since it has been indicated that the presence of lipids containing polyunsaturated fatty acids favours a loose by expanded arrangement of membrane constituents and controls, certain physical properties such as fluidity, as well as functional characteristics like permeability[23], it remains to establish the actual role of EFA in the structural and functional properties of the urothelial membrane which has been considered unusually rigid and impermeable[12–13].


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## Characteristics of the Nucleolini Observed under the Electron Microscope


A. Bolognari, A. Licata and M. B. Ricca

*Centro Universitario di Ricerca sui Tumori e Istituto di Zoologia dell' Università, Messina (Italy), 26 November 1975.*



*Summary.* Observations with the electron microscope permitted us to ascertain that in molluscs and echinoderms oocytes and in malignat tumour cells, the nucleolini, already seen with the phonton microscope, correspond to the 'clear fibrillar zones'. These present fibrils 40–60 Å thick, spread throughout a very clear matrix. All around these zones there are other closely thickened and interlacing fibrils.


In a previous article which was published in this journal[1], the characteristics of the nucleolini observed under the photon microscope were explained. These nucleolini could also be extracted by micromanipulator needles[2].

In a first series of investigations done by one of us[3], it appeared that under the electron microscope, corresponding to the nucleolini, there were found dense masses of granulations with a diameter not greater than 50 Å. However, the researches begun by Fabbri[4], and followed by other authors[5], have shown how 'clear fibrillar zones (or centres)' or simply 'clear zones (or centres)' correspond to the nucleolini.

Thus it seemed to us necessary to resume our investigations to see whether the previous results obtained by one of us could be confirmed or whether they should be suit-


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ably modified. We wished to reconsider the material fixed and included according to the former technique and to carry out new fixative and inclusion methods according to the present-day criteria.

In fact, what was affirmed in the past, especially about the nucleolini of the oocytes of the Molluscs *Patella coerulea* and *Aplysia depilans* and of the Echinoderm *Paracentrotus lividus*, must have been a consequence of the technique employed. This caused, at the level of the nucleolini, a precipitation of osmium in such a way as to give the appearance of accumulations (Figure 1). It must, however, be remembered that the so-called 'spotted nucleoli' have been described many times[6]. In these, in point of fact, there are more or less notable accumulations of granulations. No one has yet succeeded in giving an adequate explanation of these nucleoli, even if sometimes virus action has been admitted[6,7].

The observations now carried out under the electron microscope (pre-fixation in glutaric aldheyde at 4%, fixation in $OsO_4$ at 1% on a Millonig buffer; inclusion in araldite-epon, contrast with uranyle acetate at 5% and with lead citrate according to Reynold) on the oocytes of the Molluscs *Patella coerulea, Haliotis lamellosa, Aplysia depilans* and of the Echinoderms *Echinus melo* and *Paracentrotus lividus*, have permitted us to affirm that, corresponding to the nucleolini, there are clear fibrillar zones. (Figures 3 and 6) These, in a very clear matrix with respect to the electrons, present, in a spaced-out form, fibrils of a thickness of 40–60 Å. These zones, of pre-

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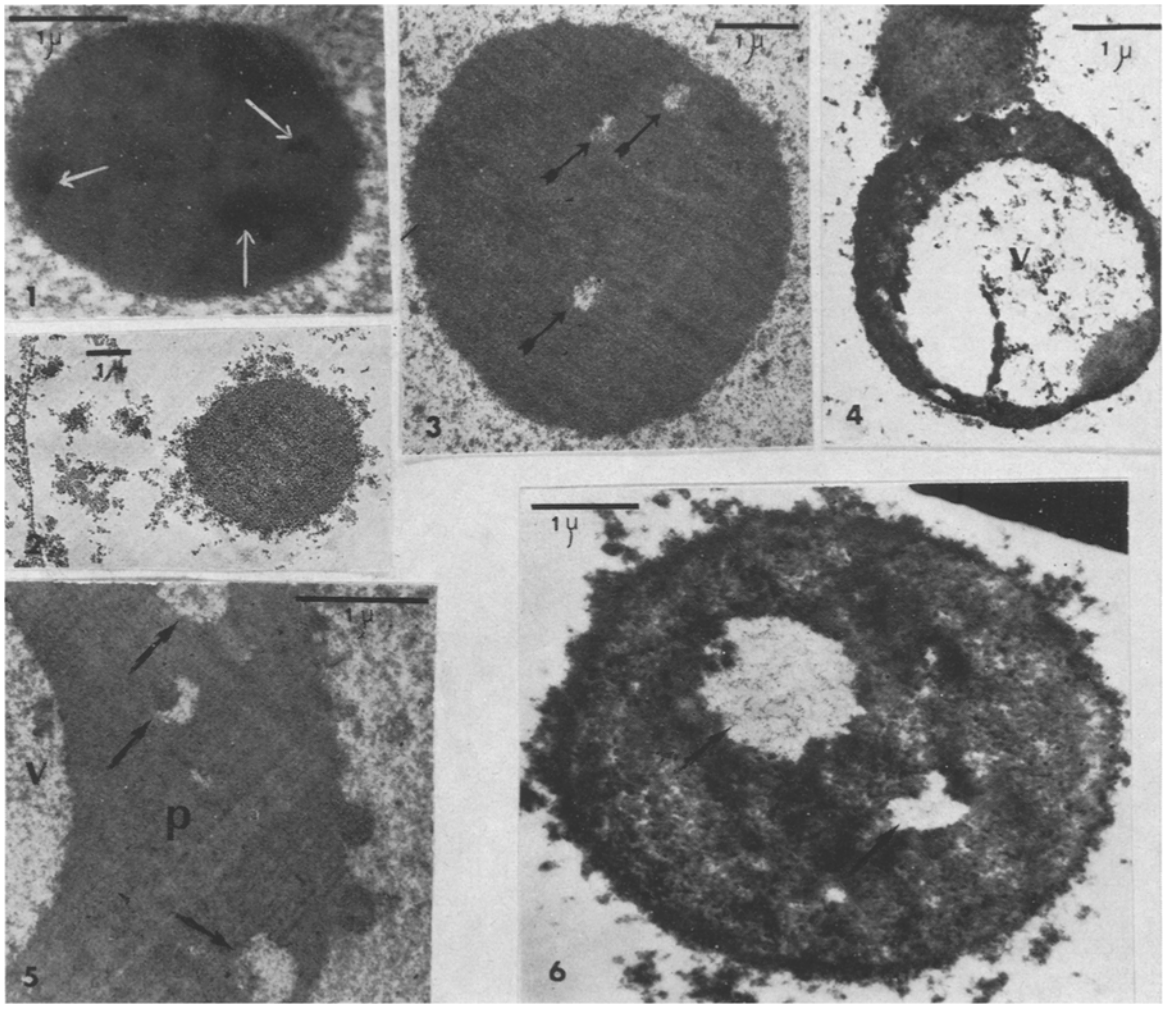


Fig. 1. Nucleolus of the oocyte of *Paracentrotus lividus;* the arrows indicate the areas erroneously held to correspond to the nucleolini. Fixation in $OsO_4$ at 1% buffered at pH 7.4; inclusion in a mixture of *n*-metacrylate. × 7,500.

Fig. 2. Nucleolus of a young oocyte of *Haliotis lamellosa;* this appears without clear fibrillar zones or nucleolini. Technique is outlined in the text. × 3,500.

Fig. 3. Nucleolus of a vitellogenetic oocyte of *Aplysia depilans;* thr arrows indicate the clear fibrillar zones. × 8,000.

Fig. 4. Nucleoli of vitellogenetic oocyte of *Patella coerulea;* in the amphinucleolus can be found a large vacuole (V) whose contents resemble the rest of the karyoplasm. × 8,000.

Fig. 5. A portion of the nucleolus of a vitellogenetic oocyte of *Haliotis lamellosa;* note that the internal vacuole (V) resembles the content of the rest of the nucleus and that in the peripheral part (P) clear fibrillar zones are found. × 8,500.

Fig. 6. Nucleolus of a vitellogenetic oocyte of *Halitios lamellosa;* the arrows indicate the clear fibrillar zones whose limits are marked by a kind of a wall. × 10,000.

dominantly circular outlines, have, all around, other fibrils of 40–60 Å closely crowded together among themselves so as to give the appearance of a surrounding wall (Figure 6).

Other observations carried out on the cells of the Walker tumour, on those of the Yoshida tumour and on those of human breast adenocarcinoma, have permitted us to establish that the nucleolini, present also in great numbers in the nucleoli, there corresponds to 'clear fibrillar zones' (Figure 7) with ultrastructural characteristics similar to those seen in the nucleoli of the oocytes.

Like the nucleolini, the clear zones are absent (Figure 2) in the smallest nucleoli and therefore they are more numerous in relation to the dimensions of the nucleoli. And this is the case as much in the oocytes as in the tumour cells. In the oocytes of *Patella coerulea* the same zones, like the nucleolini, are constantly absent in the so-called 'primary nucleolus' and present in the so-called 'amphinucleoli'. While the first always remain at a distance from the nuclear membrane, the second come alongside it an adherre closely to it. In the oocytes of *Haliotis lamellosa* the clear fibrillar zones, like the nucleolini, are only found in the peripheral part (Figure 5) of the more developed nucleoli. In fact, in the central part one finds a very large vacuole (Figure 5), which, in the electron micrographs, appears to contain material similar to that of the rest of the karyoplasm.

Moreover, in the amphinucleoli of the oocytes of *Patella coerulea*, it is possible to find rather large vacuoles (Figure 4) which have ultra-structural characteristics similar to those of *Haliotis lamellosa*.

What has been said will help to establish that it is not opportune, as some authors[8,9] have done, to identify the clear fibrillar zones with the endonucleolar vacuoles. In fact, already under the photon microscope, it was possible to single out the differences between the nucleolini and the vacuoles[3]. Now the electron microscope has enabled us to confirm these differences. These can be assigned, besides to the ultra-structural characteristics, to the dimensions, as well. These dimensions remain rather limited for the clear fibrillar zones, but for the vacuoles are always quite notable.

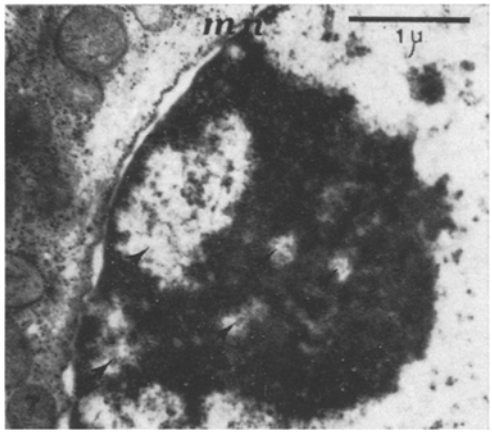


Fig. 7. Nucleolus of a cell of the Yoshida tumour adhering to the nuclear membrane (mn); various fibrillar zones are found (arrows). Technique is outlines in the text. × 16,000.

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## The Influence of Maltose and Other Carbohydrates on the Feeding Behaviour of *Heteronychus arator* (Scarabaeidae: Coleoptera)

O. R. W. Sutherland and J. R. Hillier

*Entomology Division, D.S.I.R., 120 Mount Albert Road, Auckland (New Zealand), 9 December 1975.*

*Summary.* Several of 17 carbohydrates stimulated ingestion by black beetle larvae (*Heteronychus arator*) and of these maltose induced an exceptionally strong response. Only maltose, glucose, fructose and sucrose stimulated feeding by adult beetles.

Investigations into chemical factors positively influencing ingestion by phytophagous insects have usually revealed a major role for sugars. Fructose, glucose, sucrose and maltose have been implicated in the feeding behaviour of a number of species. Sucrose has been particularly frequently cited as a strong phagostimulant, in comparison both with other sugars and with other plant constituents generally. In the case of those scarab larvae whose feeding behaviour has been studied[1,2], sucrose again emerged as the most effective carbohydrate; maltose, glucose and fructose were somewhat less so.

As part of a programme to identify the chemical basis for plant resistance to the black beetle, *Heteronychus arator*, we investigated the influence of 17 carbohydrates on ingestion by larvae and adults. Efforts by colleagues to rear this insect in the laboratory had been frustated by a failure of larvae and adults to feed vigorously on a standard artificial diet[3] containing sucrose and glucose (at concentrations of 3% and 0.5% respectively) as the only sugars. We hoped to resolve this problem.

Field-collected 3rd instar larvae and young adult beetles were starved for 24 h, then enclosed separately in 5.5 cm petri dishes, each with a single test disc of an artificial medium (4% agar, 4% cellulose powder) prepared with either distilled water or solutions of single sugars at concentrations of 0.1 *M*. Ingestion was estimated by counting the faecal pellets produced by each insect in 24 h[1].

Most of the carbohydrates induced some feeding by 3rd instar larvae; and sucrose, fructose, glucose and 1% starch can be considered effective feeding stimulants (Table I). But the response to 0.1 *M* maltose was exceptional. It far exceeded that to the other sugars and established that maltose is a major stimulant for feeding by these larvae.

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