

in fish⁵. Among the other remarkable features of the cells of these complexes are: 1. The vacuolated cytoplasm with rough endoplasmic reticulum restricted to the central and basal portions of the cell. 2. Micro-villi like surface projections intimately associated with the cuticular undersurface in the neck. 3. Substantial deposits of an electron dense material which accumulates preferentially in the cytoplasm immediately below the disc and upon vacuolar and other membranes. 4. Numerous interdigitating membrane folds, containing a single mitochondrion each, and restricted to the cup and neck region.

The distribution of the electron dense material is interesting: it is generally absent in the cytoplasm and within the vacuoles of the lower part of the cup, but invariably occurs as granules coating either the external or internal surfaces of the membrane delimiting the vacuoles of the upper regions of the cup. Conversely, the vacuoles in the lower region contain clusters of small uniform membrane-bounded vesicles similar in appearance to other vesicles in the surrounding cytoplasm. These vesicles are quite absent from the upper vacuoles. The relation between the vesicles and electron dense granules if any, is not known.

The interdigitating membrane folds represent deep invaginations of the plasma membranes of cells neighbouring the one which constitutes the bulk of the complex. The invaginations develop symmetrically near the top of and at the sides of the cup (Figure 5), and extend down to the neck region, where their ends are most clearly seen (Figure 4). The location of the cell bodies of the cells which contribute these folds is under investigation; it is most likely that they lie both within the complex as well as in the adjacent respiratory epithelium. The presence of desmosomes in and near the neck region (Figure 4)

supports this concept that the complex consists of two or more closely associated cells. Analogous cells, called 'chloride cells' and possibly involved with salt absorption, occur in the gill plates of ephemeropteran nymphs⁶⁻⁸.

Sereral of the features of the cells of the complex in *Paragnetina*, in particular the membrane folds associated with mitochondria, are shared by the so-called chloride cells of the Ephemeroptera. They suggest that these complexes are capable of providing metabolically active surfaces for the exchange of materials with the environment. However, until definitive physiological experiments have been concluded the exact function of these complexes is in doubt.

Résumé. Les coupes ultraminces d'un filament trachéobranchial d'une nymphe de plécoptère ont relevé dans l'épithélium branchial des complexes cellulaires spécialisés. Ces complexes sont présents tout le long du bord du filament, juste au-dessous de la cuticule et n'avaient pas encore été observés chez les plécoptères. Ils ressemblent aux «cellules de chlore» des poissons et à celles des nymphes des éphémères.

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Department of Biology, University of Waterloo, Waterloo (Ontario, Canada), 11 January 1973.

⁵ F. P. CONTE, *Fish Physiology* (Academic Press, New York 1968), vol. 1, p. 241.

⁶ H. KOMNIC and J. H. ABEL JR., *Cytobiologie* 4, 467 (1971).

⁷ H. KOMNIC, R. W. RHEES and J. H. ABEL JR., *Cytobiologie* 5, 65 (1972).

⁸ W. WICHARD and H. KOMNIC, *Cytobiologie* 3, 215 (1971).

Nuclear Multivesicular Bodies in Cultured Hamster Cells

The nuclear envelope is not a static structure separating the nuclear substance from the cytoplasm, but a dynamic membrane which is capable of participating in storage¹ and organelle formation processes²⁻⁴. Blebbing phenomena taking place from the outer nuclear membrane have been extensively reported in many different cells and species; almost all of these nuclear membrane extrusions have been related to the endoplasmic reticulum system^{5,6}. KILARSKI and JASINSKI⁷ reported on the formation of multivesicular bodies (MVB) from the nuclear envelope in cells of the gas-gland of the perch *Perca fluviatilis* L. under stimulated physiological activity.

These MVB have been observed in various types of cells; they are sacculs containing small vesicles or tubules which most probably represent a particular kind of secondary lysosome⁸⁻¹². In this paper we report on the formation of MVB from the inner nuclear membrane (INM) of BHK 21 cells and its presence in the nucleoplasm.

Material and methods. In the present study, we have used BHK 21 cells, a fibroblastic tissue culture-strain originated in the kidney of a baby hamster¹³. These cells were cultivated in Eagle's basal medium containing hydrolyzed lactalbumin and 20% calf serum. Cells were harvested during the logarithmic growth phase. Cell pellets were fixed in 2% osmium tetroxide in Palade's buffer solution for 1 h. After being dehydrated with gradually increasing concentration of ethanol, pellets

were embedded in Epon 812. Ultrathin sections were stained with uranyl acetate and lead citrate, and observed in a Philips EM 300 electron-microscope.

Results and discussion. The nuclear envelope of the BHK cells will normally show irregularities and invaginations similar to those described in the bibliography¹⁴. We have found a particular kind of structure in these cells, consisting of the invagination of the INM. The latter takes on the shape of a baglet which is filled with nume-

¹ G. STERBA, K. KABISCH, B. SCHNEIDER and G. HOHEISEL, *Experientia* 28, 934 (1972).

² P. R. BELL and K. MÜHLETHALER, *J. Cell Biol.* 20, 235 (1964).

³ R. G. KESSEL, *J. Cell Biol.* 19, 391 (1963).

⁴ B. SCHARER and S. WURZELMANN, *Z. Zellforsch. mikrosk. Anat.* 96, 325 (1969).

⁵ R. G. KESSEL, *Z. Zellforsch. mikrosk. Anat.* 98, 17 (1968).

⁶ M. L. WATSON, *J. biophys. biochem. Cytol.* 7, 257 (1955).

⁷ W. KILARSKI and A. JASINSKI, *J. Cell Biol.* 45, 205 (1970).

⁸ A. B. MOVIKOFF and W. Y. SHIN, *J. Microsc.* 3, 187 (1964).

⁹ B. GORDON, L. R. MILLER and K. BENSCH, *J. Cell Biol.* 25, 41 (1965).

¹⁰ J. L. E. ERICSSON and B. F. TRUMP, *Lab. Invest.* 15, 1610 (1966).

¹¹ D. S. FRIEND, *J. Cell Biol.* 47, 269 (1969).

¹² A. U. ARSTILA, H. O. JAUREGUI, J. CHANG and B. F. TRUMP, *Lab. Invest.* 24, 162 (1971).

¹³ I. MACPHERSON and M. G. STOKER, *Virology* 16, 147 (1962).

¹⁴ E. R. BURNS, B. L. SOLOFF, C. HANNA and D. F. BUXTON, *Cancer Res.* 31, 159 (1971).

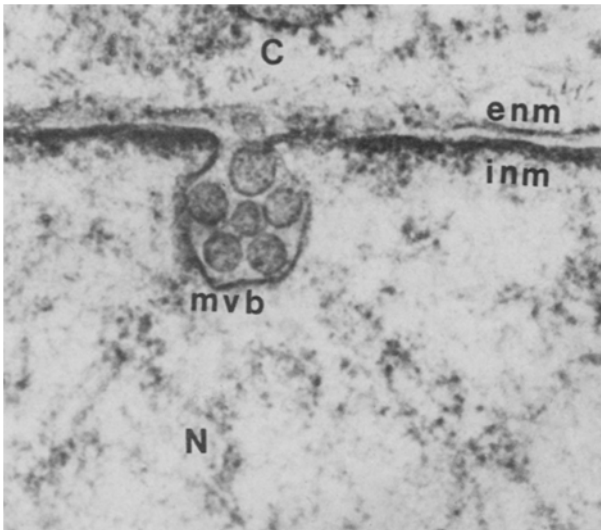


Fig. 1. A multivesicular body containing 6 vesicles is clearly seen as an infolding of the internal nuclear membrane. Note continuity of the MVB membrane with the INM. The external nuclear membrane is apparently not involved in the formation of the nuclear MVB; N, nucleus; C, cytoplasm; INM, internal nuclear membrane; ENM, external nuclear membrane. $\times 69,000$.

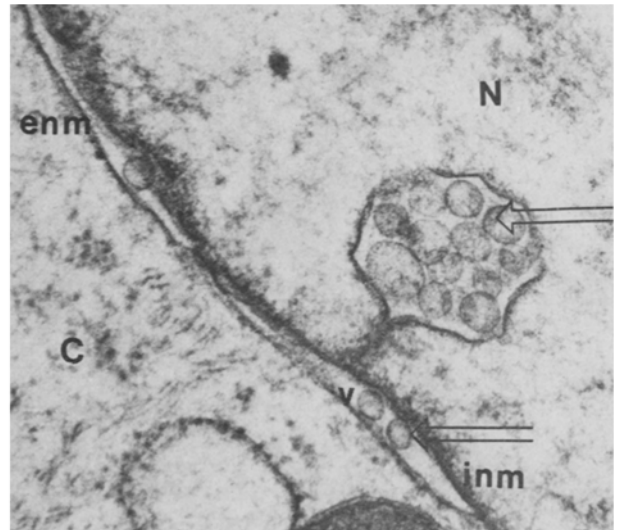


Fig. 2. Small vesicles (V) are found to lie free between the internal INM and external nuclear membranes (ENM). Note similarity between these vesicles and those filling the MVB (arrows). The vanishing and elongated aspect of the neck of the MVB suggests a possible separation of this structure from the nuclear membrane. $\times 66,000$.

rous vesicles or tubules (Figure 1). We have observed that, in some of these structures, the wide connection between the baglet and the intermembranous space narrows into a very thin neck (Figure 2).

The external nuclear membrane bridges over the neck of the INM, invagination without taking part in the process. The small vesicles inside these structures are approximately 90 nm in diameter and they are surrounded by a single membrane, probably originating in the INM. We refer to these vesicular invaginations as MVB due to the morphological similarities with the well described cytoplasmic organelles.

Furthermore we noted nuclear MVB apparently unconnected with the INM. In addition to and sometimes in association with MVB, several single-membraned small vesicles appeared between the internal and external nuclear membrane (Figure 2). These vesicles were found to lie free in the intermembranous space, and although no connection with the nuclear membranes was noted, their similarity with vesicles of multivesicular bodies suggests their probable nuclear membrane origin.

The formation mechanism of these nuclear MVB might be very similar to the one described by KILARSKI and JASINSKI⁷ (see figure legends). But, as only the internal nuclear membrane of BHK cells is involved, the development and possible separation of MVB from the INM could be more easily achieved.

As to the formation and nature of MVB, there are numerous reports relating these organelles to lysosomes, digestive vacuoles, Golgi system, and pinocytic vesicles⁸⁻¹².

In BHK cells, MVB are closely linked to the nuclear membrane and might be closely related to the intranuclear canaliculi described in many different tissues¹⁵⁻¹⁷. Furthermore their function could be similar to those ascribed to the canalicular system, i.e.: transport of nuclear and nucleolar products of the cytoplasm and vice versa.

Zusammenfassung. Mit elektronenmikroskopischer Untersuchung von BHK 21 cl 13 Zellen wurden multivesikuläre Körperchen im Karyoplasma festgestellt, Strukturen, die direkt der inneren Kernmembran entstammen. Die multivesikulären Körperchen wie auch die internen vesikulären Elemente (\varnothing 90 nm) waren von einer einzigen Membran umgeben.

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¹⁵ J. A. TERZAKIS, J. Cell Biol. 27, 293 (1965).

¹⁶ G. YASUZUMI and R. SUGIHARA, Expl. Cell Res. 37, 207 (1965).

¹⁷ S. KARASAKI, Cancer Res. 30, 1736 (1970).

¹⁸ Acknowledgment: we thank Miss. P. MARTINEZ VENTEO for her excellent technical assistance.

Effect of Light on the Synaptic Organization of the Inner Plexiform Layer of the Retina in Albino Rats

Visual receptors of albino rats are damaged by prolonged illumination¹⁻⁵. In rhesus monkeys degeneration of the receptors in the macular region is observed after the eye has been exposed to the light of an indirect

ophthalmoscope⁶. The extent of damage increases in severity with the length and intensity of illumination. When studying the effects of monocular deprivation (achieved by unilateral lid-suture) of pattern vision in