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INTRANUCLEAR MEMBRANOUS INCLUSIONS IN OOCYTES OF A VIVIPAROUS TELEOST (*XIPHOPHORUS HELLERI*)

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SUMMARY

Intranuclear inclusions were observed in oocytes of *Xiphophorus helleri* during prophase I. In osmium-fixed leptotene nuclei, the inclusions were made up of groups of membrane-limited vesicles or tubules with pale contents, situated near the inner nuclear membrane with which some of them exhibited apparent continuities. In zygotene nuclei, larger vesicles also appeared bounded by two or three membranes and containing tubules apparently invaginated from their walls. In pachytene-dictyate nuclei most vesicular bodies had a wall formed by stratified membranes, or were entirely made up of membranous whorls. In glutaraldehyde-osmium fixed material some of these myelin-like bodies showed a peculiar arrangement, consisting of concentric bands each containing thick inner dense lamellae 2·0–3·0 nm thick and a 5·0-nm outer lamella.

It is suggested that these inclusion bodies arise from the inner nuclear membrane of oocytes when cells start to grow intensely during prophase I. The bodies seem to become more complex at late prophase, probably by association of individual vesicles and the occurrence of multiple membrane invaginations, which may be related to active metabolic phenomena taking place at this stage in oocytes.

INTRODUCTION

During a study of oogenesis in *Xiphophorus helleri*, a viviparous Teleost (Azevedo, 1974), membranous inclusions were observed in the nuclei of oocytes undergoing meiotic prophase I. The inclusions were made up of simple vesicles or tubulo-vesicular composite structures at leptotene-zygotene stages. In pachytene, diplotene and dictyate cells, most vesicular bodies were bound by concentric membranes or entirely made up of membrane whorls. The evolution of these bodies through meiosis and the fact that the more complex ones occurred in late prophase when oocytes grow intensely and are the sites of active metabolic phenomena suggested that the bodies might have a functional significance.

MATERIALS AND METHODS

We studied the ovaries of 18 normal females of *Xiphophorus helleri* Heckel (*Poeciliidae* family) from birth to 7 months of age. When animals are about 15 days old, oocytes start entering prophase I to reach sexual maturation at 7 months of age. During this time interval

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it is possible to find cells at the various stages of oogenesis in the ovary. Animals have been sacrificed at different times throughout the last 2 years without preference for any particular season or month.

In 6 animals, the ovaries were fixed by immersion in 2 % osmium tetroxide in 0·1 M phosphate buffer containing 0·05 % calcium chloride, pH 7·2, for 2 h at 4 °C. In the remaining animals, the ovaries were fixed in 2 % glutaraldehyde in the same buffer for 2 h at 4 °C, washed overnight and postfixed in 2 % osmium tetroxide for 2 h at 4 °C. The pieces were embedded in Araldite (Glauert & Glauert, 1958) or in Epon 812 (Luft, 1961). Semithin serial sections were cut and each one was immediately stained with methylene blue-azur II (Richardson, Jarett & Finke, 1960). When intranuclear bodies appeared in a semithin section, ultrathin sections of the same block were then cut. These sections were double stained with aqueous 2–5 % uranyl acetate for 20 min plus aqueous lead hydroxide (Karnovsky, 1961) for 10 min or lead citrate (Reynolds, 1963) for 15 min, and examined in an electron microscope Jeol 100 B operated at 80 kV. The specimen was tilted up to 30° from the horizontal by means of the manual goniometer stage IB-1004.

RESULTS

Osmium-fixed material

Intranuclear inclusions were seen in the light microscope in semi-thin sections as small dots near the nuclear contour (Fig. 1). Electron micrographs showed those dots to correspond to clusters of membranous bodies. In nuclei at the leptotene stage of prophase I, such bodies consisted of membrane-limited vesicles and tubules, close to the inner nuclear membrane (Figs. 2, 3). The vesicles were round or oval with pale contents and the limiting membrane measured ~ 9·0 nm in thickness (Fig. 2). Apparent continuities of some tubules with the inner nuclear membrane were often observed (Fig. 3). The inner nuclear membrane measured ~ 9·0 nm in thickness. The same intranuclear bodies appeared in zygotene cells, together with larger oval-shaped bodies bound by two or three concentric membranes and containing individual tubules and small vesicles (Fig. 4). Some of those tubules sometimes appeared to represent invaginations of the limiting membrane (Fig 4). The membranes of these bodies measured ~ 9·0 nm.

In pachytene nuclei, the intranuclear bodies observed in previous stages were again encountered (Fig. 5). In addition, bodies with pale contents but limited by concentric membranes occurred in these cells (Fig. 6). Sometimes the membranes were sinuous and seemed to fill the whole body, which took the shape of a myelin-figure. The membranes of these bodies measured ~ 8·5 nm in thickness. During the next

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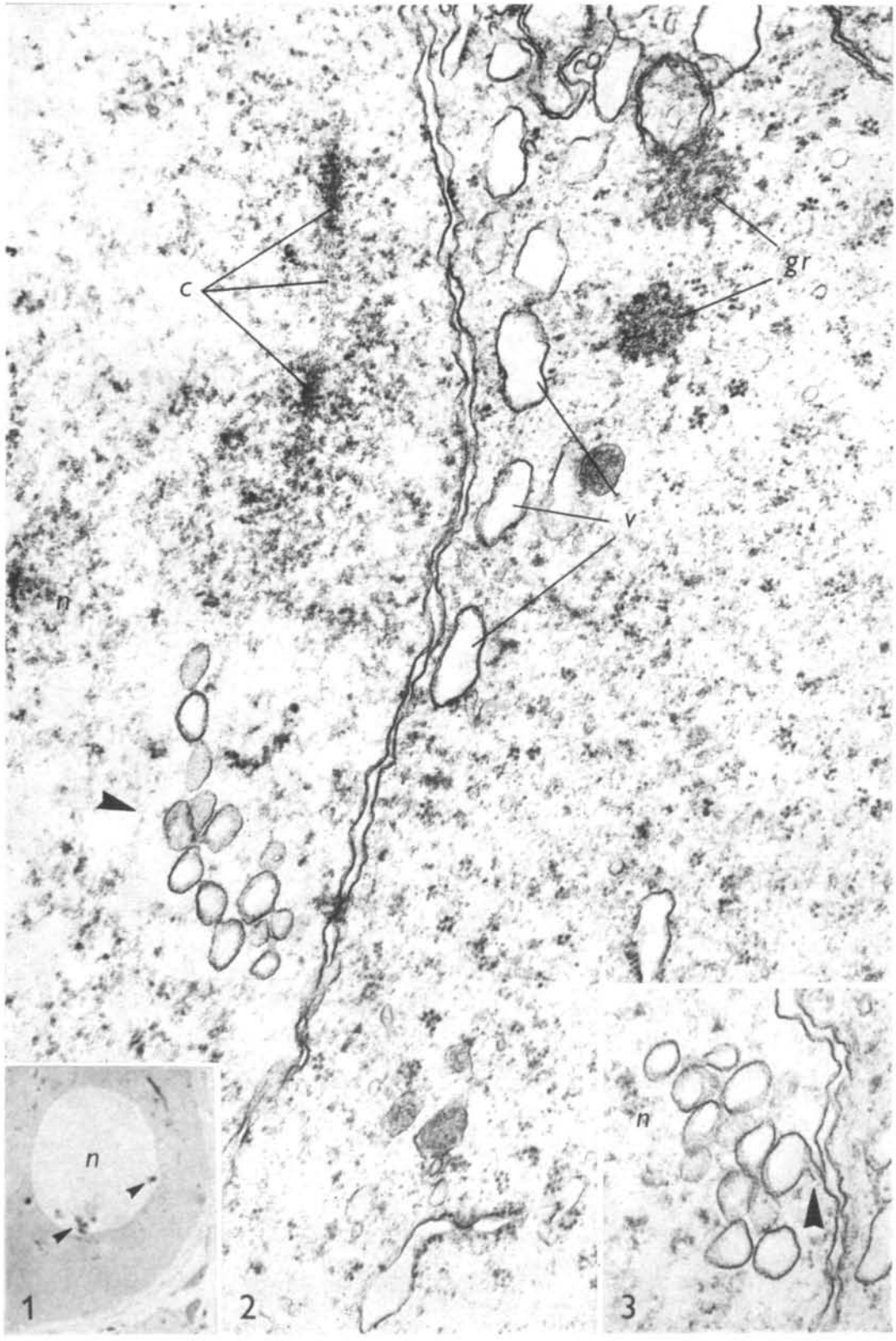
Fig. 1. Semithin section of a diplotene oocyte with intranuclear inclusions (arrows). *n*, nucleus. Osmium fixation. × 2400.

Fig. 2. In an oocyte at leptotene, a cluster of membranous vesicles (arrowhead) in the nucleus (*n*). Note one leptotene chromosome (*c*), and in the cytoplasm abundant polysomes, some RER cisternae, smooth-walled vesicles (*v*), and 2 perinuclear granular bodies (*gr*) with fine structure similar to that of the *pars granulosa* of the nucleolus (Ulrich, 1969). Osmium fixation. × 54000.

Fig. 3. A similar group of intranuclear vesicles, one of them apparently relayed to the inner nuclear membrane by a short tubule (arrowhead). *n*, nucleus. Osmium fixation. × 45000.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stages of diplotene and dictyate these myelin-like forms were often observed (Fig. 8) while simple vesicles, connected or not connected with the inner nuclear membrane, became rare. Intranuclear bodies were found in meiotic oocytes from all fish studied; they were absent from cells which were not undergoing meiosis.

Glutaraldehyde-osmium fixation

During leptotene-zygotene the images were the same as those observed in osmium-fixed material. In pachytene-dictyate cells some of the myelin-like bodies had a more complex arrangement of the membranes (Figs. 8, 9). These bodies were formed by 4–6 curved concentric wide bands, 40·0–50·0 nm thick, separated by 4·0–8·0 nm spaces. At high magnification the bands were seen to consist of 8–14 dense lines alternating with light spaces. The 2 outer lines of each band were 5·0 nm thick, the inner ones 2·0–3·0 nm and the intervening spaces 1·0–2·0 nm thick (Fig. 9). The lines were stacked within each band with a periodicity of 4·0 nm (Fig. 9).

Cells with nuclear inclusions, whatever the meiotic stage in which they were found, in addition contained mitochondria with membranous whorls in the matrix or at the periphery; the membranes of these were continuous with the limiting membranes of the mitochondria. Similar whorls appeared in connexion with Golgi and RER cisternae, though these were much rarer. Such whorls were not found in osmium-fixed material. Intranuclear bodies were found in double-fixed oocytes of 8 animals out of 12.

DISCUSSION

A number of intranuclear organelles, such as the ribonucleoproteic interchromatin and perichromatin granules or the proteinaceous simple nuclear bodies, appear to be of general occurrence in normal eukaryotic cells (Bouteille, Laval & Dupuy-Coin, 1974; Monneron & Bernhard, 1969). Other nuclear structures have been shown only in certain cells and their presence seems to be highly dependent on the physiological state of the cell. Among the latter are the intranuclear rodlets, frequent in neurons (Feldman & Peters, 1972) at certain development stages (Masurovsky, Benitez, Kim & Murray, 1970); the intranuclear annulate lamellae observed mainly in differentiating germ cells of invertebrates (Folliot, 1968; Hsu, 1967; Kessel, 1968); and inclusions formed by concentric membranes recently described in young oocytes of *Triturus helveticus* (Humeau, 1968), in tumoral glial cells (Tripier, Bérard & Toca, 1974) and in renal tubular cells of lead-poisoned rats (Franke & Scheer, 1974).

The bodies described here partly belong in this last category since they are confined

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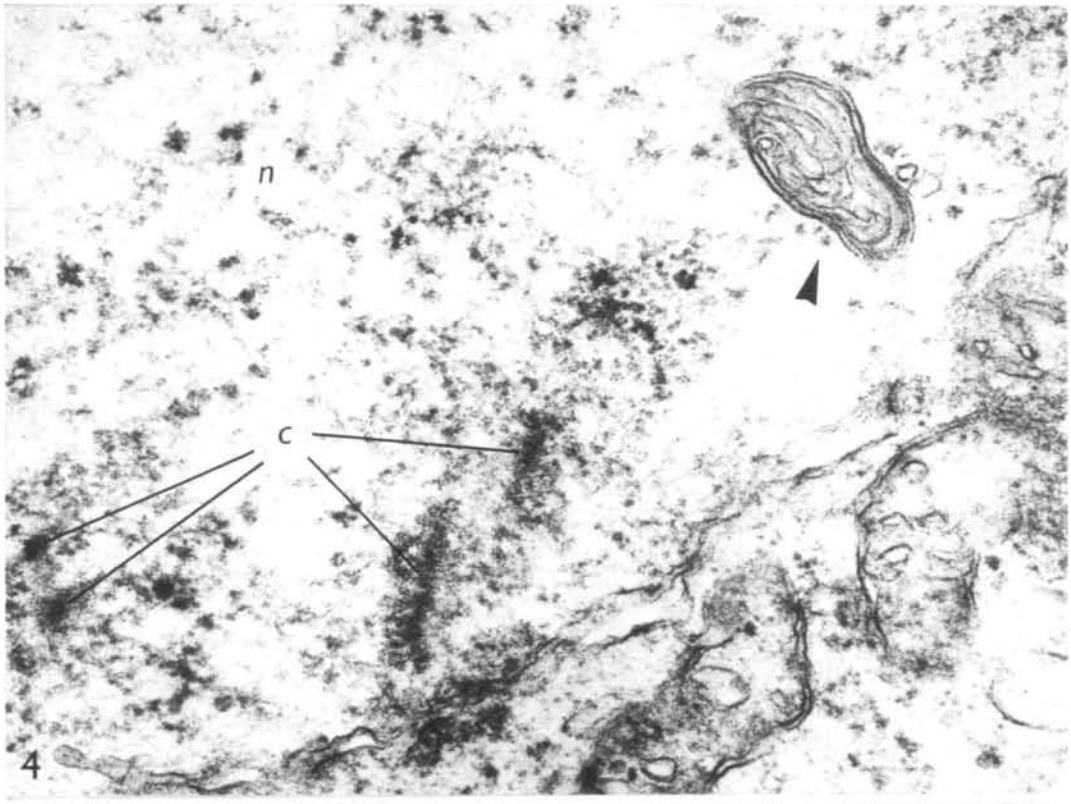
Fig. 4. A zygotene nucleus (*n*) with 2 chromosome pairs (*c*) and a membranous body containing tubules (arrowhead). Osmium fixation. × 54000.

Fig. 5. At pachytene, numerous simple vesicles and tubules close to the inner nuclear membrane (*) and 3 vesicles (arrowhead) with 2 or 3 membranes at the wall and a tubule in the interior of the larger vesicle. Of the 3 pairs of chromosomes (*c*) the synaptonemic complex is evident in the one situated on the right. Osmium fixation. × 37800.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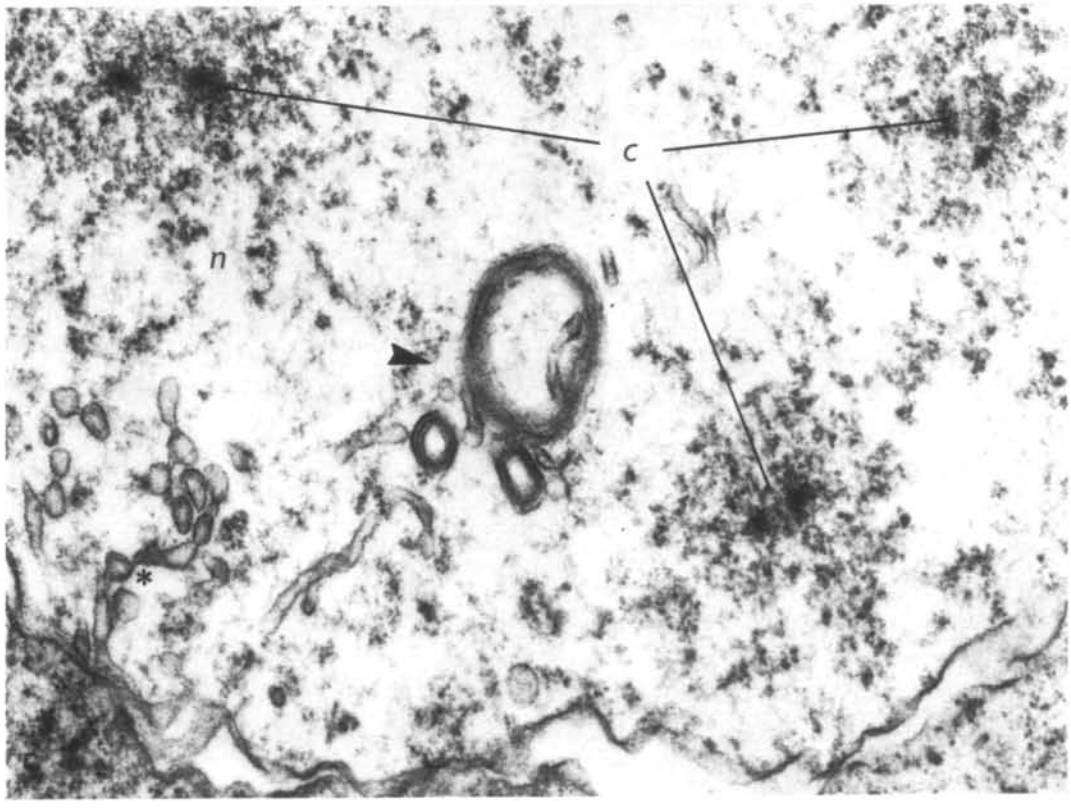
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to a certain stage of oocyte development and because some of them have a stratified wall. However, they exhibited a gamut of different forms not found in any of the examples listed above. The exclusive presence of simple vesicles at leptotene and the gradual occurrence of more complex forms thereafter were indeed strongly suggestive of a progressive elaboration of the initial vesicles, which might associate together forming complex arrangements finally leading to the myelin-like variety.

On the other hand, in osmium-fixed material the membranes of all these bodies including the most complex ones, exhibited unit membrane structure with a thickness very similar to that of the inner nuclear membrane. This fact, as well as the continuities sometimes observed between the latter and the simpler vesicular or tubular forms (see Figs. 3, 5) seemed to indicate a mode of formation based on blebs of the nuclear envelope, as suggested by Kessel (1968) with regard to the genesis of certain nuclear annulate lamellae.

It should be noted that in the whole variety of oocytes present in the ovaries of the animals here studied, no fine alterations in the cytoplasm or nuclei were detected at the ultrastructural level. Furthermore, oocytes containing inclusions showed no signs of atresia, such as RER and Golgi dilations, swollen mitochondria, or the presence of leukocytes between oocytes and follicular cells (Bouteille *et al.* 1974). These data do not support a pathological cause for these bodies, nor any association with atresia as proposed by Humeau (1968) in the case of *Triturus* oocytes. On the contrary, since oocytes of *X. helleri* undergo a striking size increase during late prophase it seems more likely that our nuclear bodies may result from an exaggerated production of perinuclear membrane.

Curgy (1968) observed membrane whorls in embryonic liver cells double fixed in glutaraldehyde-osmium, which were attributed to the artifactual reorganization of the cell phospholipids by that type of fixation. We do not think that the present membranous bodies may have had such an origin, because: (*a*) they were present in material fixed in osmium tetroxide alone; (*b*) the intranuclear bodies were of different conformation according to meiotic stage of the oocytes; and (*c*) all other oocytes in the same ovaries which were not in prophase I of meiosis did not show them. However, the presence of many invaginated membrane whorls in cytoplasmic organelles in glutaraldehyde-fixed material, as well as the complexity of some intranuclear bodies, may have been due to such fixative effects.

On this point, it is curious that the bodies depicted in Figs. 8 and 9 show a lamellar arrangement nearly reproducing the pattern of the lipid-protein-model preparation Stoeckenius (1962) obtained after surrounding phospholipid in a globin solution. The periodicity of the inner lines (4·0 nm) and their width (2·0–3·0 nm) were identical to those of the lipid layers in the model, whereas the outer lines of each stack had a width

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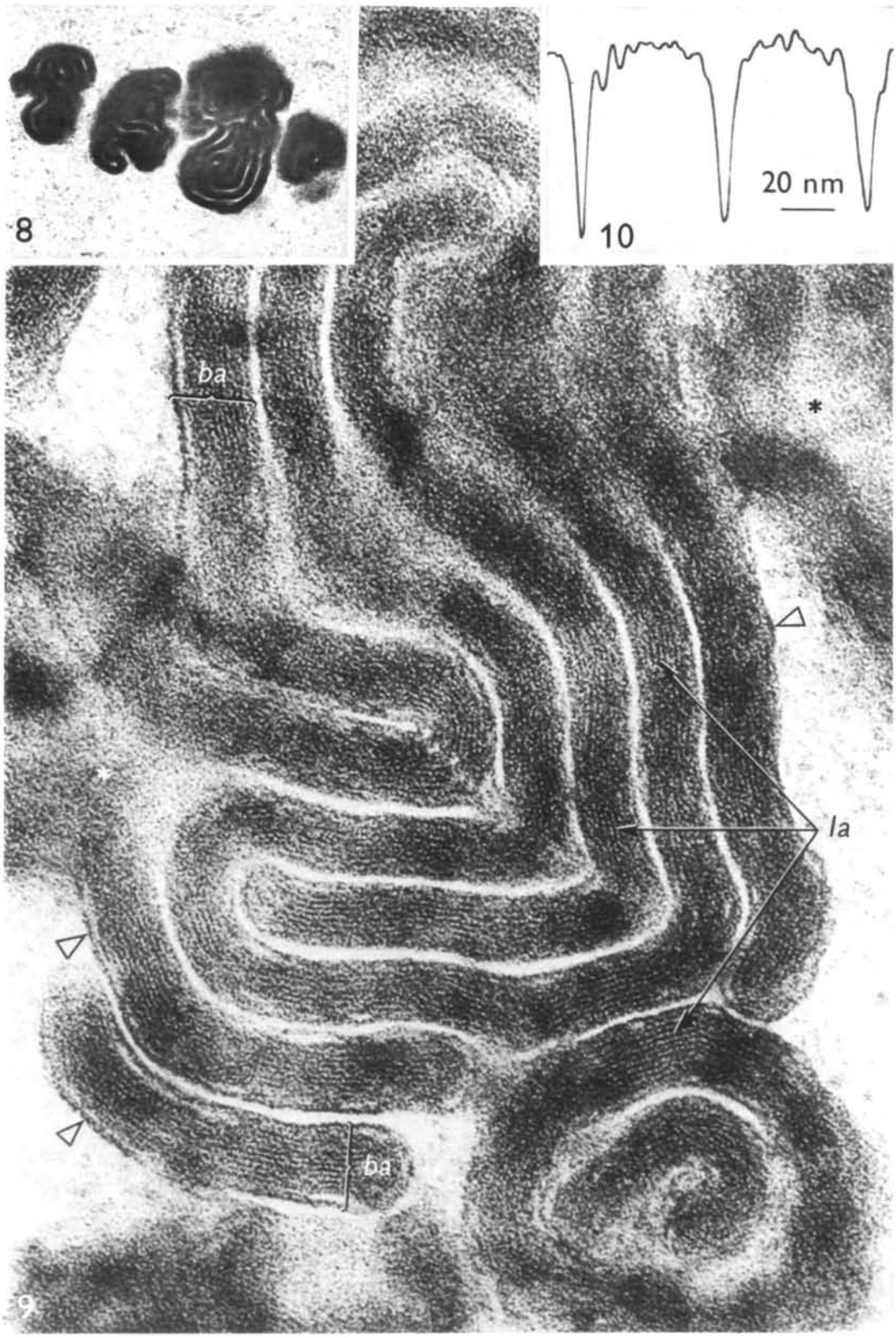
Fig. 6. In the (upper) oocyte nucleus (*n*) at pachytene, a body with stratified membranous wall can be seen (arrowhead). The nucleus (*n*) at the lower left belongs to a follicular cell. Osmium fixation. × 36000.

Fig. 7. Two intranuclear bodies (arrowheads) made up of membranous whorls. Note the unit membrane structure of the latter. Osmium fixation. × 120000.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(~ 5·0 nm) comparable to that of the protein film absorbed on to the outermost phospholipid layers in the Stoeckenius (1962) preparation. Although this elaborate arrangement may be a consequence of a glutaraldehyde artifact, it may also be argued that only glutaraldehyde would be capable of adequately preserving the protein components of the outer layer of such bands.

Finally, it should be emphasized that the larger and more elaborate bodies occurred at late prophase when cells not only grow rapidly, but also start storing lipid and protein yolk, while intense cytoplasmic exchanges of nucleolar material are indicated by the occurrence of perinuclear extrusion bodies (Fig. 6). The intranuclear bodies described here may thus result not only from exaggerated membrane growth, but may also represent special sites of metabolic phenomena taking place at this particular time in the life of the cell.

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REFERENCES

AZEVEDO, C. (1974). Évolution des enveloppes ovocytaires, au cours de l'ovogenèse, chez un Téléostéen vivipare, *Xiphophorus helleri*. *J. Microscopie* **21**, 43–54.

BOUTEILLE, M., LAVAL, M. & DUPUY-COIN, A. M. (1974). Nuclear structure. Localization of nuclear functions as revealed by ultrastructural autoradiography and cytochemistry. In *The Cell Nucleus*, vol. 1 (ed. H. Busch), pp. 5–71. New York and London: Academic Press.

CURGY, J.-J. (1968). Influence du mode de fixation sur la possibilité d'observer des estructures myéliniques dans les hépatocytes d'embryons de poulet. *J. Microscopie* **7**, 63–80.

FELDMAN, M. L. & PETERS, A. (1972). Intranuclear rods and sheets in rat cochlear nucleus. *J. Neurocytology* **1**, 109–127.

FOLLIOT, R. (1968). Les lamelles annelées intranucléaires des cellules du tissu germinal mâle avant la méiose chez *Philaenus spumarius* L. (Insecte Homoptère). *Z. Zellforsch. mikrosk. Anat.* **92**, 115–129.

FRANKE, W. W. & SCHEER, M. (1974). Nuclear structure. Structures and functions of the nuclear envelope. In *The Cell Nucleus*, vol. 1 (ed. H. Busch), pp. 219–347. New York and London: Academic Press.

GLAUERT, A. M. & GLAUERT, R. H. (1958). Araldite as an embedding medium for electron microscopy. *J. biophys. biochem. Cytol.* **4**, 191–194.

HSU, W. S. (1967). The origin of annulate lamellae in the oocyte of the ascidian, *Boltenia villosa* Stimpson. *Z. Zellforsch. mikrosk. Anat.* **82**, 376–390.

Fig. 8. Three inclusion bodies in a dictyate nucleus formed by concentric dense bands. Glutaraldehyde-osmium fixation. × 33 000.

Fig. 9. High magnification of the same type of bodies depicted in the anterior figure showing the wide bands (*ba*) composed of tightly dense lines (*la*) the outer ones of which are thicker (hollow arrowheads). A grazing section shows blurred membrane profiles (*). Glutaraldehyde-osmium fixation. × 225 000.

Fig. 10. Densitometric reading of the bands, each small peak corresponding to a dense line.

HUMEAU, C. (1968). Formations membranaires dans le noyau et le cytoplasme périnucléaire due jeune ovocyte de *Triturus helveticus* Raz. *C.r. Séanc. Soc. Biol.* **162**, 183–186.

KARNOVSKY, M. J. (1961). Simple methods for 'staining' with lead at high pH in electron microscopy. *J. biophys. biochem. Cytol.* **11**, 729–732.

KESSEL, R. G. (1968). Annulate lamellae. *J. Ultrastruct. Res.*, Suppl. **10**, 1–82.

LUFT, J. H. (1961). Improvements in epoxy embedding methods. *J. biophys. biochem. Cytol.* **9**, 409–414.

MASUROVSKY, E. B., BENITEZ, H. H., KIM, S. U. & MURRAY, M. R. (1970). Origin, development, and nature of intranuclear rodlets and associated bodies in chicken sympathetic neurons. *J. Cell Biol.* **44**, 172–191.

MONNERON, A. & BERHNARD, W. (1969). Fine structual organization of the interphase nucleus in some mammalian cells. *J. Ultrastruct. Res.* **27**, 266–288.

REYNOLDS, E. S. (1963). The use of lead citrate at high pH as an electron-opaque stain in electron microscopy. *J. Cell Biol.* **17**, 208–212.

RICHARDSON, K. C., JARETT, T. & FINKE, E. H. (1960). Embedding in epoxy resins for ultrathin sectioning in electron microscopy. *Stain Technol.* **35**, 313–325.

STOECKENIUS, W. (1962). The molecular structure of lipid-water systems and cell membrane models studied with the electron microscope. In *The Interpretation of Ultrastructure*, vol. 1 (ed. R. J. C. Harris), pp. 349–367. New York and London: Academic Press.

TRIPIER, M.-F., BERARD, M. & TOCA, M. (1974). 'Membranous nuclear bodies' as possible intermediate structure between nuclear bodies and invaginations of the nuclear envelope. *J. Microscopie* **21**, 21–30.

ULRICH, E. (1969). Étude des ultrastructures au cours de l'ovogenèse d'un poisson téléostéen, le Danio, *Brachydanio rerio*. *J. Microscopie* **8**, 447–478.

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