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Submicroscopic morphology of hyperplasic ovaries of ex-fissiparous individuals in Dugesia gonocephala s.l.

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SEZIONE III

(Botanica, zoologia, fisiologia e patologia)

Citologia. — *Submicroscopic morphology of hyperplasic ovaries of ex-fissiparous individuals in* Dugesia gonocephala s.l. (*). Nota di VITTORIO GREMIGNI e ROSALBA BANCHETTI, presentata (**) dal Socio M. BENAZZI.

RIASSUNTO. — Sono stati studiati gli ovari iperplasici di individui sterili ex-scissipari di *Dugesia gonocephala s.l.* I piccoli ovociti previtellogenetici hanno una struttura regolare simile a quella degli ovociti di planarie normalmente sessuate dello stesso gruppo di specie. Gli ovociti che hanno iniziato la vitellogenesi mostrano invece vari sintomi di patologia cellulare: condensazione della cromatina, vacuolizzazione del nucleo, progressivo incremento del sistema lisosomale. Gli Autori prospettano che un blocco della maturazione determini durante il diplotene varie alterazioni del metabolismo cellulare che portano alla degenerazione degli ovociti. Questa sarebbe una delle fondamentali cause della sterilità di questi individui.

INTRODUCTION

Some specimens from races of *Dugesia gonocephala s.l.* which usually multiply by fission and are therefore devoid of the reproductive apparatus (ovaries, testes, copulatory organ) may spontaneously reach sexual maturity [1].

These individuals named by Benazzi " ex-fissiparous " are fertile and have normal ovaries in some populations, while in others they are completely or almost completely sterile and have abnormal ovaries. In the latter instance single specimens are unable to produce fertile cocoons and their ovaries are enlarged and occupy a wide area of the anterior part of the body.

The abnormal constitution of the ovaries has been attributed to a hyperplasia of female germ-cells, probably due to a more intense transformation of neoblasts into oogonia which subsequently produce a very high number of oocytes; these, however, only rarely leave the ovary and become enclosed in the cocoons [2, 3].

An analogous phenomenon has been recently observed in a Canadian population of *Fonticola morgani* [4].

This paper explores the ultrastructure of hyperplasic ovaries to evidentiate eventual pathological aspects that may cause the sterility of ex-fissiparous specimens.

(*) This research was carried on in the department of Zoology, University of Pisa, and it was supported by grants from the Italian National Research Council (CNR).

(**) Nella seduta dell'8 aprile 1972.

Materials and methods

Planarians of two populations collected, respectively, in Israel (River Jordan) and Algery (Ruisseau des singes) were used. All the specimens, when brought to our Institute, were fissiparous; later on, some reached sexual maturity and showed hyperplasic ovaries.

Specimens which had become sexual a long time before were studied from each population; some had already laid at least one cocoon which was, as usual, sterile.

Light microscopy study was carried out on paraffin sections 8–10 μ thick, stained with haematoxylin-eosin, and on araldite-epon sections 1–2 μ thick, stained with toluidine blue and methylene blue.

Controls for caryological aspects of meiotic prophase were made on *in toto* ovaries stained with acetic carmine.

Preparations for electron microscopy were carried out as follows: small body pieces containing the ovaries were fixed in 3 % glutaraldehyde or in Carnovsky's solution [5] in 0.05 M phosphate buffer (pH 7.3) and postfixed in identically buffered 1 % osmium tetroxide.

For demonstration of acid phosphatase activity we used a modification of Gomori's method [6]. Specimens were fixed in 3 % glutaraldehyde in 0.05 M cacodylate buffer (pH 7.3) with 0.5 $CaCl_2$, washed in the same buffer with 7 % sucrose overnight. Incubations in Gomori's medium for 15′, 30′ and 60′ at 37° C were made. Controls in the same conditions were carried out on specimens kept in the medium without substrate. Specimens were postfixed in osmium tetroxide or directly embedded.

All the samples were embedded in Araldite–Epon [7]. Ultrathin sections were stained with uranyl acetate and lead citrate [8], micrographs were taken in a Siemens Elmiskop 101.

Results

A) *Light microscopy.*

No substantial differences appear in the nuclear and cytoplasmic structure of oogonia and young oocytes (less than 15–20 μ in diameter) of ex-fissiparous when compared with those of normally sexual planarians. During vitellogenesis oocytes (more than 20 μ in diameter), on the contrary, show progressive vacuolation of the nucleus and atypical staining of cytoplasm.

Some degenerating oocytes are clearly recognizable in each ovary. Studies on *in toto* ovaries show that diplotenic oocytes are more numerous than the younger ones, and confirm the nuclear vacuolation in vitellogenic oocytes.

B) *Electron microscopy.*

Ultrathin sections support that oocyte maturation is quite regular until the vitellogenesis stage. Structure of young oocytes is similar to that of

normally sexual planarians. Nucleus is regularly shaped, nuclear envelope has few pore-complexes, nucleolus is compact and contains granules 150–200 Å in diameter mixed with fibrils 50 Å thick. In the cytoplasm many free ribosomes are uniformly distributed, mitochondria show a regular structure, endoplasmic reticulum is scant and Golgi complexes are few (Plate I, fig. 1).

Afterwards endogenous vitellogenesis begins in the way described by one of us [9] in normally sexual individuals of *D. benazzii* (1), endoplasmic reticulum develops, Golgi complexes increase in number and small yolk globules accumulate in the cytoplasm. Mitochondria, as well, increase in number and form clusters close to finely granular, electron dense bodies probably coming from the nucleus [10, 11, 12] (Plate I, fig. 2). Near the bodies stacks of annulate lamellae are often found. In tangential sections nuclear envelope shows closely spaced annuli. In the caryoplasm synaptinemal complexes are visible; each complex is no longer made of a unique band of filaments as it is in the first meiotic stages, but it already shows a tripartite structure typical of pachytene and diplotene [13] (Plate II, fig. 3).

Structural damages noticed in 20–30 μ in diameter oocytes are: chromatin condensation especially near the nuclear envelope, progressive vacuolation of the caryoplasm (Plate II, fig. 4). Nucleolus is still compact and often shows a clear " segregation " between granular and fibrillar components [14, 15, 16] (Plate II, fig. 5), but it is never ring-shaped as happens in the stages just before the metaphase.

In the cytoplasm mitochondria decrease in number and show localized structural alterations (Plate II, fig. 6), endoplasmic reticulum becomes swollen; dense bodies surrounded by a single membrane are first seen: they show an acid phosphatase activity and are interpreted as lysosomes (Plate III, figs. 7 and 9). These autophagosomes increase in number and in size and occupy almost the whole cell. They have a very heterogeneous morphology and contain granular and amorphous material, myelin-like figures and lipofuscin-like granules. (Plate III, figs. 8, 10 and 11).

DISCUSSION

The specimens from *D. gonocephala* characterized by hyperplasic ovaries, object of our research, were completely sterile even if they had reached sexual maturity many months before. The very few cocoons they laid actually lacked oocytes. The morphology of the ovaries is very atypical both regarding the whole gonad and each oocyte maturation.

Previtellogenic oocytes are regular as to the morphology and the fine structure; they are less numerous than the vitellogenic ones. This observation suggests that oocyte maturation occurs with regular mechanism up to the initial diplotene.

(1) This species belongs to « *D. gonocephala* group ».

On the contrary oocytes that for their large size, the presence in their nucleus of tripartite synaptinemal complexes, and the presence in their cytoplasm of yolk globules, can be considered as diplotenic, show clear symptoms of cellular pathology, i.e. progressive nuclear vacuolation, increasing of the lysosomal system followed by demolition of cellular structures.

The above described phenomena suggest that a maturation blockage occurring during the diplotene stage in hyperplasic ovaries determines metabolic alterations and causes the degeneration of almost all oocytes. This maturation blockage must be very slow, because many diplotenic oocytes, in progressive stages of degeneration, are recognizable in the same ovary, while the necrotic ones are rare.

It is to be noted, in this regard, that some oocytes can degenerate also in normal individuals of *D. gonocephala s.l.*; but this phenomenon generally occurs only in ripe oocytes which do not enter the oviduct, but become overripe in the ovary simulating aberrant meta-anaphase.

Recently sexual individuals with hyperplasic ovaries have been obtained from a population of *D. gonocephala s.l.*, giving the agamous animals a " pap " made of fragments of *Polycelis nigra* specimens [17]. This treatment, however, has not modified till now the morphology of the ovaries and the sterility of the ex fissiparous planarians we studied.

Further investigations are planned to establish, also with autoradiographic methods, possible metabolic alterations occurring in degenerating oocytes.

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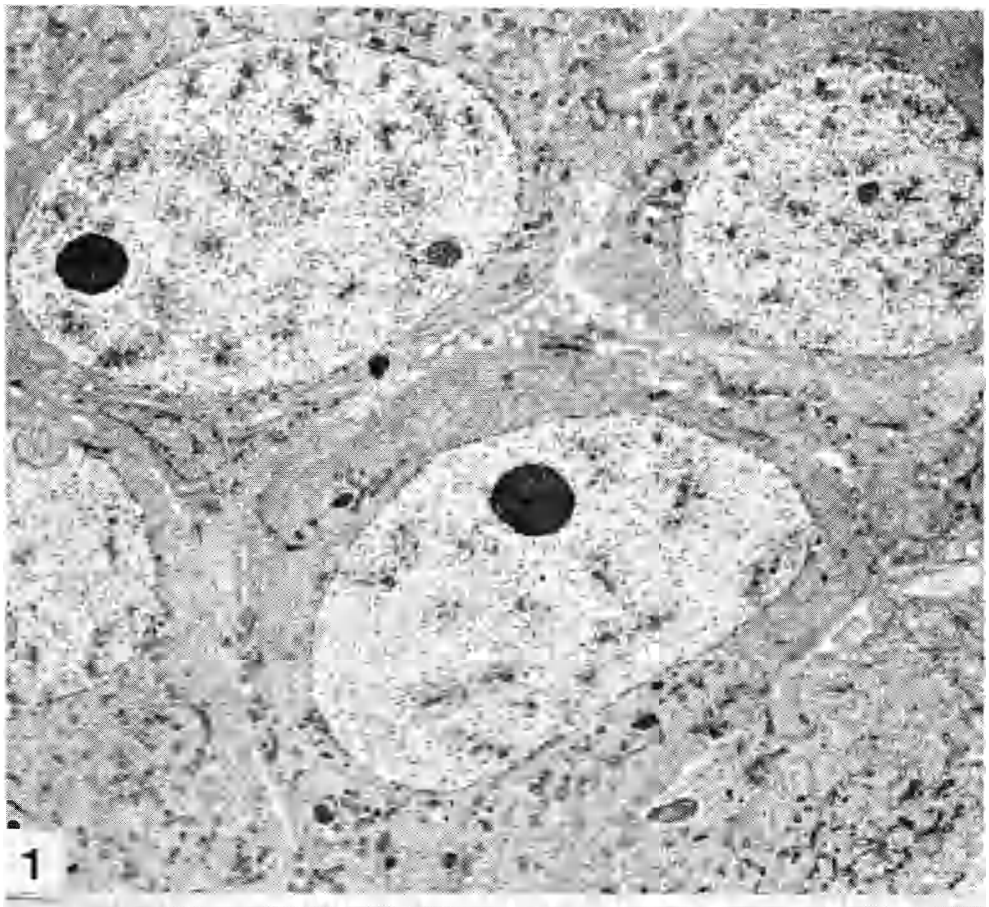

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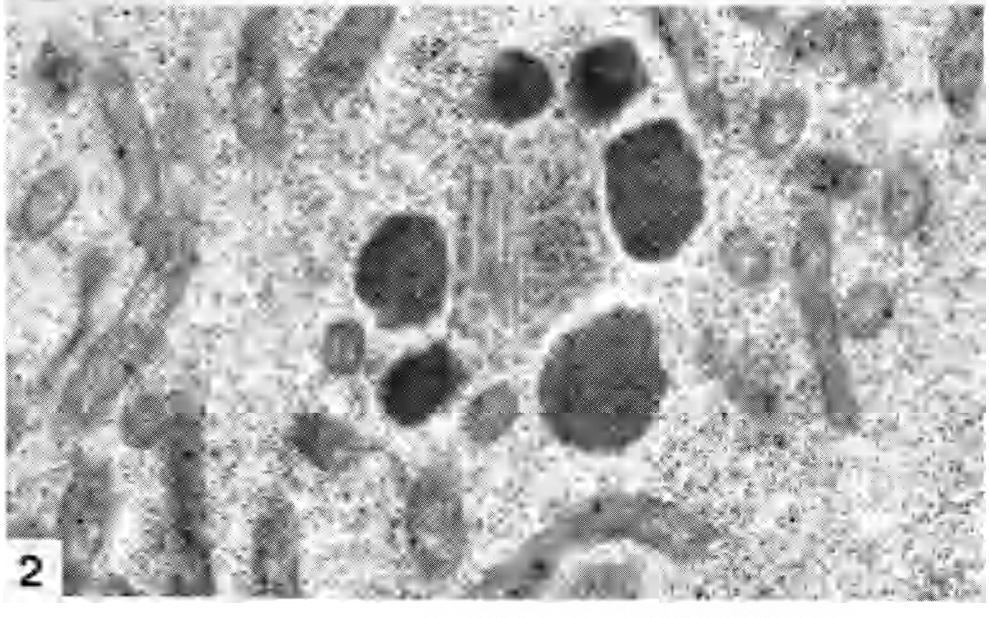

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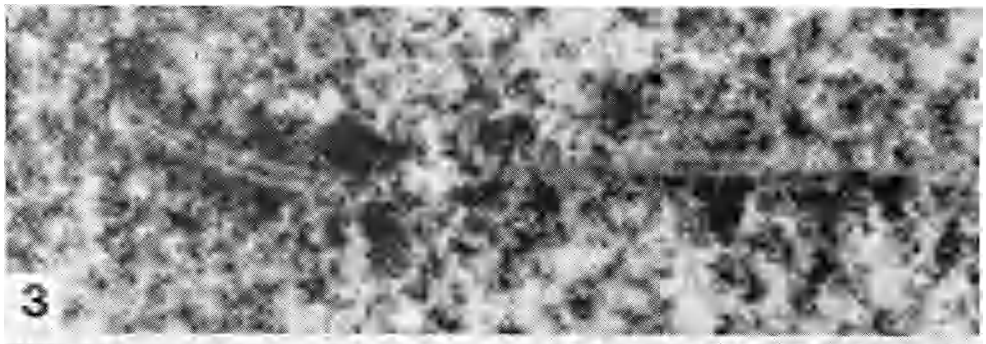
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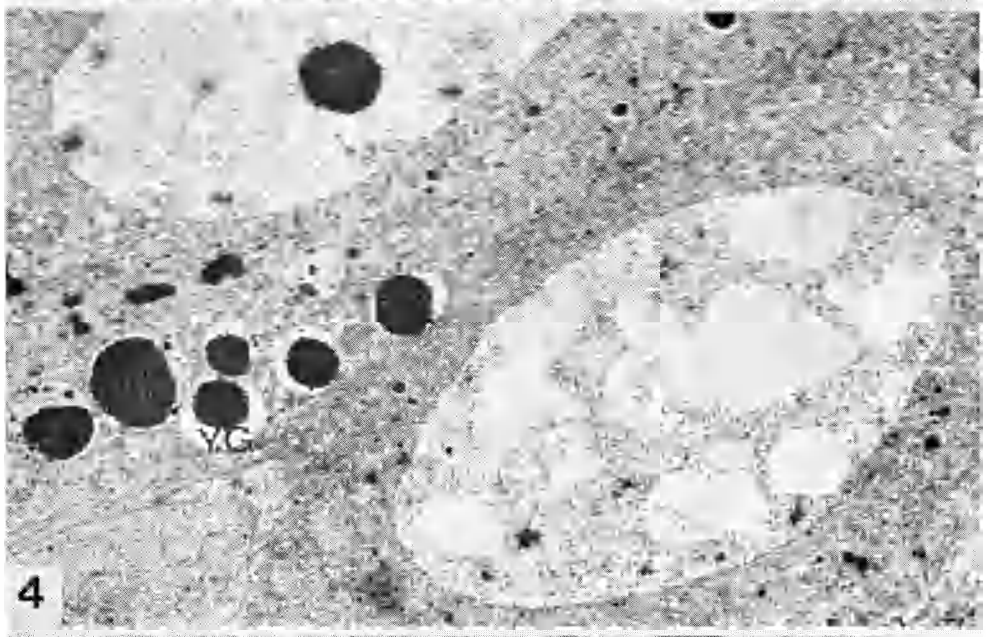
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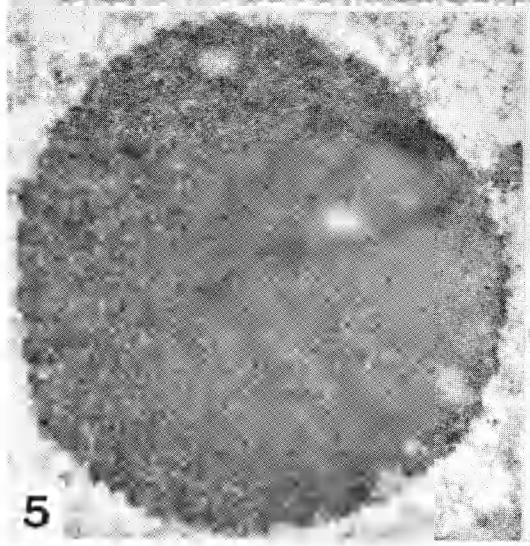
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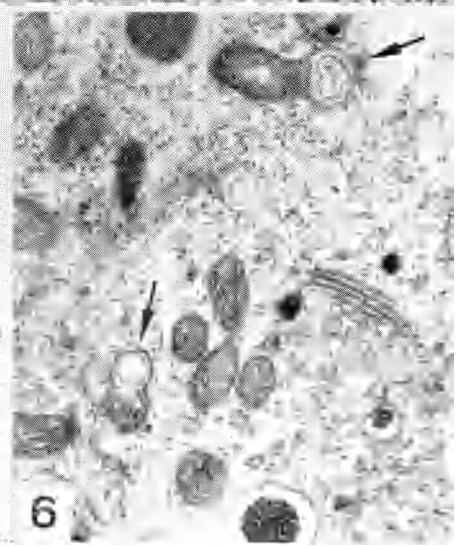
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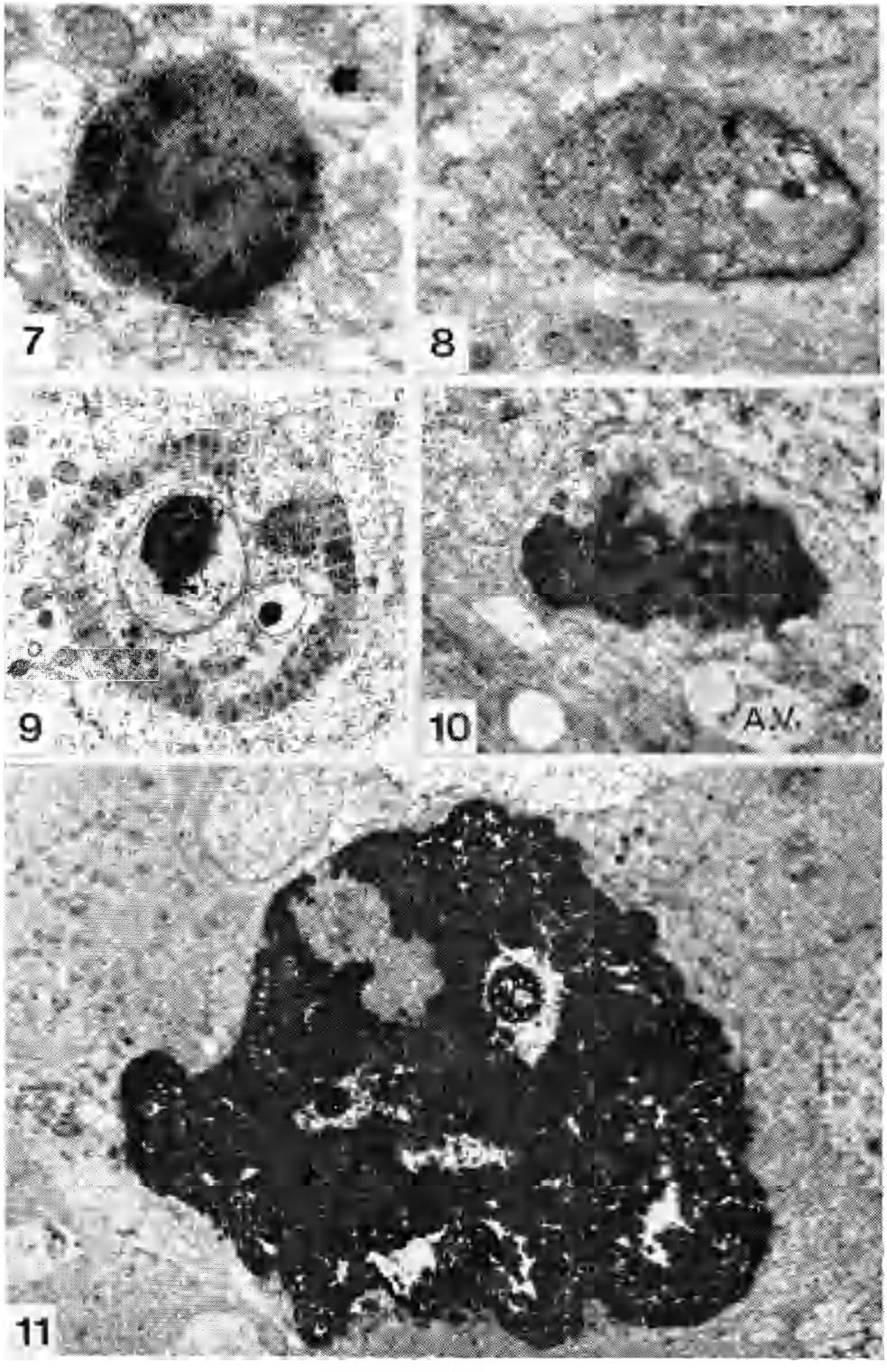
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EXPLANATION PLATES I–III

PLATE I

Fig. 1. – Low magnification electrograph showing a group of young oocytes with typical morphology (×4.800).
Carnovsky–OsO_4.

Fig. 2. – Dense bodies of possible nuclear origin closely associated with mitochondria (×37.000).
Carnovsky–OsO_4.

PLATE II

Fig. 3. – Frontal–radial section of a synaptinemal complex in a diplotenic chromosome. Note the typical tripartite structure (×32.000).
Carnovsky–OsO_4.

Fig. 4. – Degenerating oocytes. Note the chromatin condensation and the vacuolation of the nuclei. Y.G. = yolk globules (×4.000).
3% Glut.–OsO_4.

Fig. 5. – Nucleolus from a degenerating oocyte showing segregation between granular and fibrillar components (×23.000).
Carnovsky–OsO_4.

Fig. 6. – Anomalous mitochondria from a degenerating oocyte (→). Note granular yolk bodies near a Golgi complex (×34.000).
Carnovsky–OsO_4.

PLATE III

Fig. 7. – A dense body containing acid phosphatase reaction products (×42.000).
3% Glut.–OsO_4. Incubation in Gomori medium 30′.

Fig. 8. – A lysosome, containing heterogeneous material, located near the cell membrane (×22.000).
Carnovsky–OsO_4.

Fig. 9. – A myelinated body, containing enzyme reaction deposits, surrounded by annulate lamellae (×20.000).
3% Glut.–OsO_4. Incubation in Gomori medium 30′.

Fig. 10. – A lipofuscin–like lysosome near an autophagic vacuole (A.V.) (×13.000).
Carnovsky–OsO_4.

Fig. 11. – A very large lysosome which occupies almost the whole cell (×4.000).
Carnovsky–OsO_4.