Atti Accademia Nazionale dei Lincei

Classe Scienze Fisiche Matematiche Naturali

Rendiconti

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On the "test cells" of Molgula impura

Atti della Accademia Nazionale dei Lincei. Classe di Scienze Fisiche, Matematiche e Naturali. Rendiconti, Serie 8, Vol. **59** (1975), n.1-2, p. 147–151.

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Atti della Accademia Nazionale dei Lincei. Classe di Scienze Fisiche, Matematiche e Naturali. Rendiconti, Accademia Nazionale dei Lincei, 1975.

Embriologia. — *On the "test cells" of* Molgula impura (*). Nota (**) del Corrisp. GIUSEPPE REVERBERI.

RIASSUNTO. — Nella cavità dell'intestino anteriore delle larve natanti di *Molgula impura* viene rilevata la presenza di cellule che, per molti caratteri, sembrano doversi considerare come « cellule testali » dislocate.

Le masse presenti in queste cellule, come pure quelle che si riscontrano nelle cellule testali degli oociti giovani, vengono interpretate come aspetti volutivi di microrganismi simbiotici.

Le « cellule testali » di *Molgula impura*, secondo questa interpretazione, sarebbero da considerare come agenti che trasmettono la condizione simbiotica, da una generazione all'altra.

1. In the course of an investigation carried out with the electron microscope on the tadpoles of *Molgula impura* we met some strange " formations " which are illustrated in Plates I, II, III. As shown by the figures, these formations are situated within the lumen of the gut; they can clearly be distinguished from the surrounding entodermic cells for these are characterized by the presence in their cytoplasm of many yolk-granules.

Within the " formations ", which result to be cells, one can discover the presence of mitochondria, large vesicles of the endoplasmic reticulum, and a nucleus; but besides that they possess three large, oval (or spherical) masses, which occupy nearly all the cytoplasm. The three great oval masses differ from each other in their structure. One of them, indicated in the figures with *c* is limited by a very thin membrane, and contains a great quantity of small vermiculated rods; another, indicated with *b* is limited by a thick membrane (a true capsule) and its content is made up of electron dense aggregates; the third, finally, indicated with *a*, is also limited by a thick capsule, and its content is composed of a great quantity of small granules. The three masses are certainly successive stages of a unique developmental process.

Of great interest is the structure of the vermicular rods, which at high magnification result to be made of small spheres, connected to each other in form of a chain (Plates I B; II B; III B).

The granules of the mass (*a*), at higher magnification, appear also as small spheres (Plate I D).

Within the intestinal lumen two or more of these peculiar cells were sometimes found (Plate III A).

(*) Lavoro eseguito presso l'Istituto di Zoologia dell'Università di Palermo.

(**) Presentata nella seduta dell'11 giugno 1975.

2. The characteristics of these cells are very similar to those shown by the " test cells " embedded in the cytoplasm of the young oocyte. Plate IV A represents a young oocyte (at the beginning of the vitellogenesis), surrounded by a voluminous sheet of follicular cells; its cytoplasm is filled with numerous " test cells ". These, at higher magnification (Plates IV B; V A, B; VI A, B) show many features, the most interesting being that constituted by three great bodies, each limited by a distinct membrane.

The body which is indicated in the Plates with x shows a homogeneous structure; on it, however, one can distinguish, here and there, a few spots which sometimes appear as small vesicles (Plates IV B; V A; VI A; VI B). The body indicated with y has a very fine granular structure: it sometimes (Plate VI B) is delimited by a thick membrane. The body indicated with z, on the other hand, is constituted by coarse granules, or small spheres (Plates IV B; VI B). Between the three bodies which occupy nearly all the cytoplasm, one can observe vesicles of the endoplasmic reticulum, some mitochondria (Plates V A; VI A) and a degenerating nucleus (Plate V A).

3. Before we attempt to give an interpretation of the above described features we want to refer the observations which have been made by some other researchers on the same, or similar, material.

First the observations of Kessel [5] on the egg of *Styela* must be mentioned. According to the Author the " test cells " of the egg (late oocytes) of this species are replenished by masses of rods, which ultimately, if observed at high magnification, appear as chains of small spheres. The rods would be elaborated within the cells as shown by the fact that the test cells of the young oocytes show vacuoles filled with fine, beaded filaments, those of the medium oocytes with bigger filaments, and finally those of the oocytes at the end of their growth with chains of small spheres. The Author holds that these features should be interpreted as succession phases of the synthesis of the yellow-orange pigment, a pigment which the test cells pour into the cytoplasm of the oocyte. The role of the " test cells " at least in Styela would thus be explained: they see to the synthesis of the pigments of the egg. This hypothesis is moreover supported by an interesting fact, namely that these " test cells " possess an exceptional Golgi body [6]; the granules of pigment should be synthesized or elaborated in its vesicles. The elaborated pigment is afterwards poured into the growing oocytes: the test cells, having so ended their function are new reabsorbed by the oocyte.

An analogous investigation has been carried out by Kessel and Kemp [7] on the " test cells " of *Molgula manhattensis*, a species which is strictly related to *Molgula impura*. The Authors note with particular emphasis a singular feature of the nucleus which would " differentiate " into two zones: one, the inner, which at the electron microscope is dense; the other, the outer, which is light: this, moreover, shows scattered spots which look like pores; these, however, do not seem to correspond to the pores of the nuclear membrane.

The low electron dense zone, comes to form part of the peripheral zone of the nucleus; as this zone is PAS positive probably it contains polysaccharides.

As regards the "test cells" the two researchers hold that they exert the function of synthesizing the pigments: a histochemical or biochemical proof, however, is not alleged.

4. Having reported these bibliographic indications I will now pass on to examine the possible significance of the cells found in the lumen of the fore gut of *Molgula impura*, and of their strange features.

As above remarked these cells do not differ much in their aspect and in their features from the "test cells" of the young oocytes. These "test cells", too, contain three spherical or ovoidal bodies in their cytoplasm, and each body is surrounded by a membrane: this sometimes is very thick and looks like a true capsule. The three bodies probably represent different phases of a unique process: the phases would precede the phases noticed in the cells enclosed in the foregut of the tadpoles. From the similarity of the aspects of both sorts of cells we deduce that also the cells which are in the lumen of the larval intestine should be considered "test cells". The question now arises: what function do these "test cells" dislocated in such an unusual site exert? The solution of the question is suggested by the accurate examination of the vermicular rods contained within them. As remarked, these rods are not continuous but are constituted by a linear series of small vesicles. The sequence seems to be produced by the repetitive reproduction of isolated vesicles as in microrganisms. The "rods" in other words should be in our hypothesis linear colonies of symbiotic microorganisms. If this hypothesis is accepted the "test cells" should be considered as the elements deputed to the transmission of the symbiotic microorganisms from one generation to the other.

5. This hypothesis is not new. Numerous observations on many animals, mostly insects [3] show that nurse and follicular cells are used very frequently to transmit from one generation to another the symbiotic organisms contained in the cells of their mycetomes (cfr. [4]): but what is the matter in the Tunicates? Fortunately we are in a very good situation, as shown by the observations made many years ago on the pyrosomes. These possess in the peripharingeal sinus, two peculiar organs whose cells emit an intense light [10].

Julin [9] studying their origin through the embryonic development showed that these organs derive from the "test cells" which are also luminescent. A feature of both kinds of cells is that their cytoplasm is filled with a peculiar formation which is described by him as a "boyau décrivant des méandres nombreux et serrés" and whose significance was not understood by him. This was a merit of Pierantoni [11, 12], who showed that the "boyau" of Julin, in fact, is an assemblage of vermicular rods, and that the luminescence of the cells is due to them.

Besides that, from the observation that the rods take the colorations which are specific for the bacteria and possess "granules" like these, he came to the conclusion that the vermicular rods are symbiotic bacteria. Their transmission from one generation to another is operated in the following way: the "granules" situated in their cytoplasm assemble together and form the "spores"; these leave the cells of the peripharingeal bodies, enter the blood circulation, reach the ovary and penetrate into the folicular and "test cells" of the egg. Within these cells the spores evolve, assume the form of vermicular rods, reproduce. The "test cells" filled up with the microorganisms come afterwards to form the peripharingeal bodies of the primary ascidiozoids and finally with the concourse of the mesenchymatic cells of the "dorsal organs" ("dorsal glands" of Panceri), give rise to the typical luminous organs which one observes in the adult animals.

6. The occurrences now described in the pyrosomes support the hypothesis here advanced that the "features" noted in the "test cells" of *Molgula impura*, and in the cells found in the foregut of the tadpoles, are symbiotic microrganisms. Of course the hypothesis requires proof; and that is precisely the aim of future investigation.

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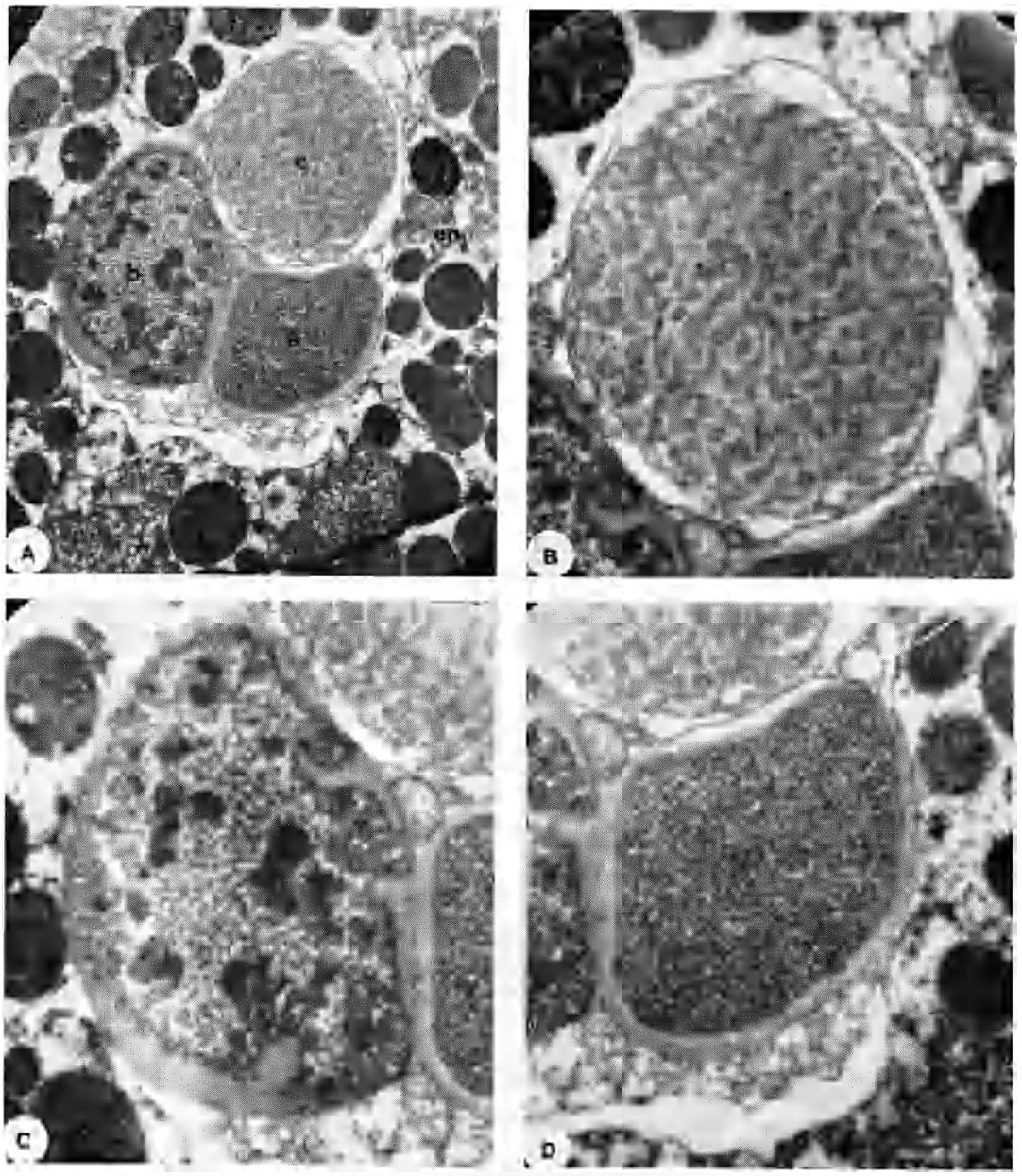
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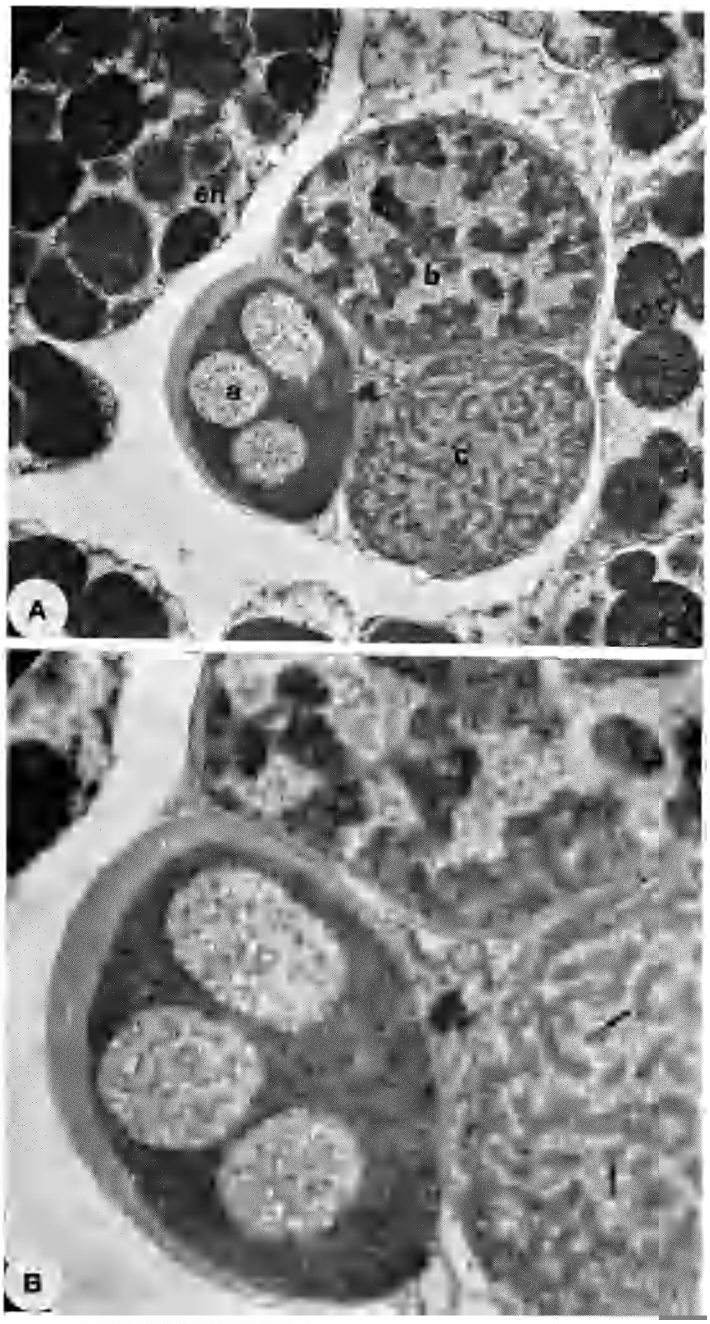
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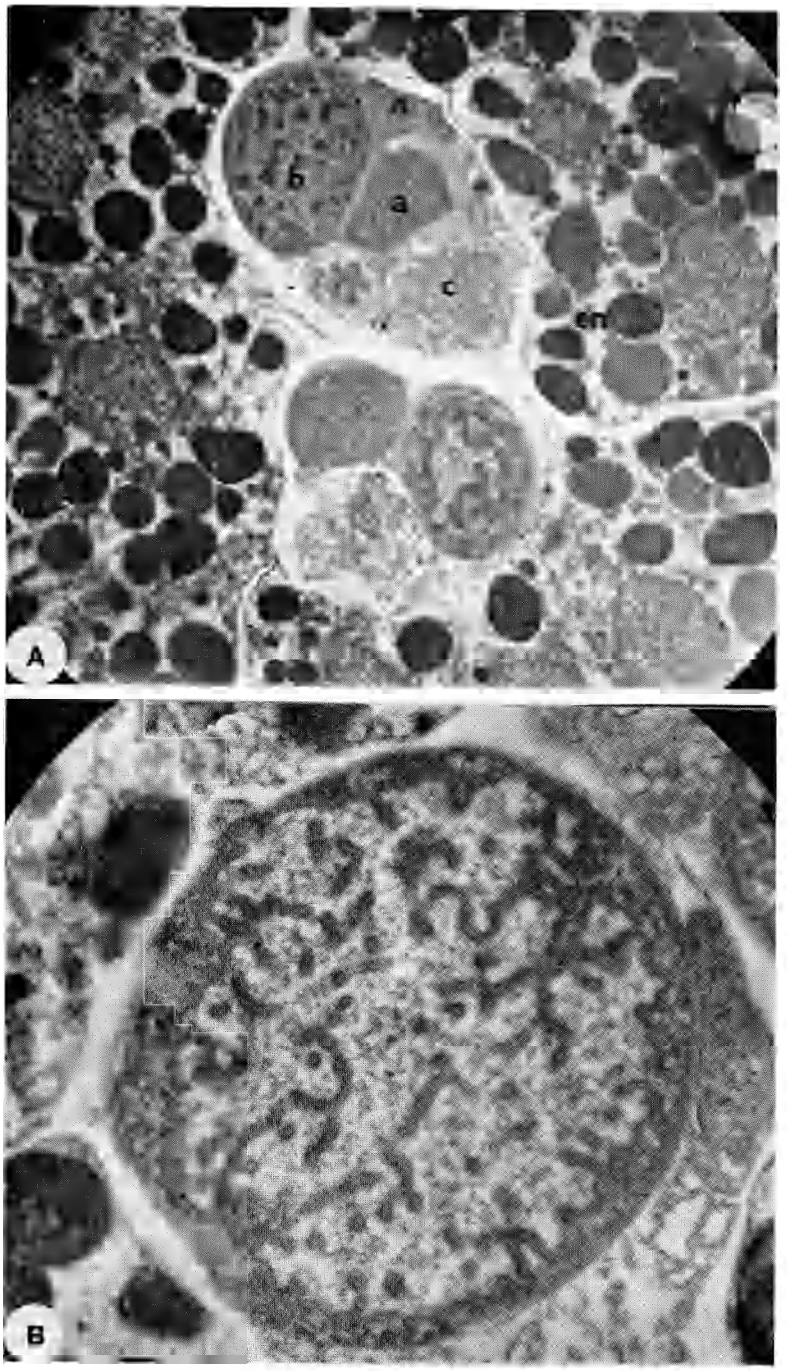
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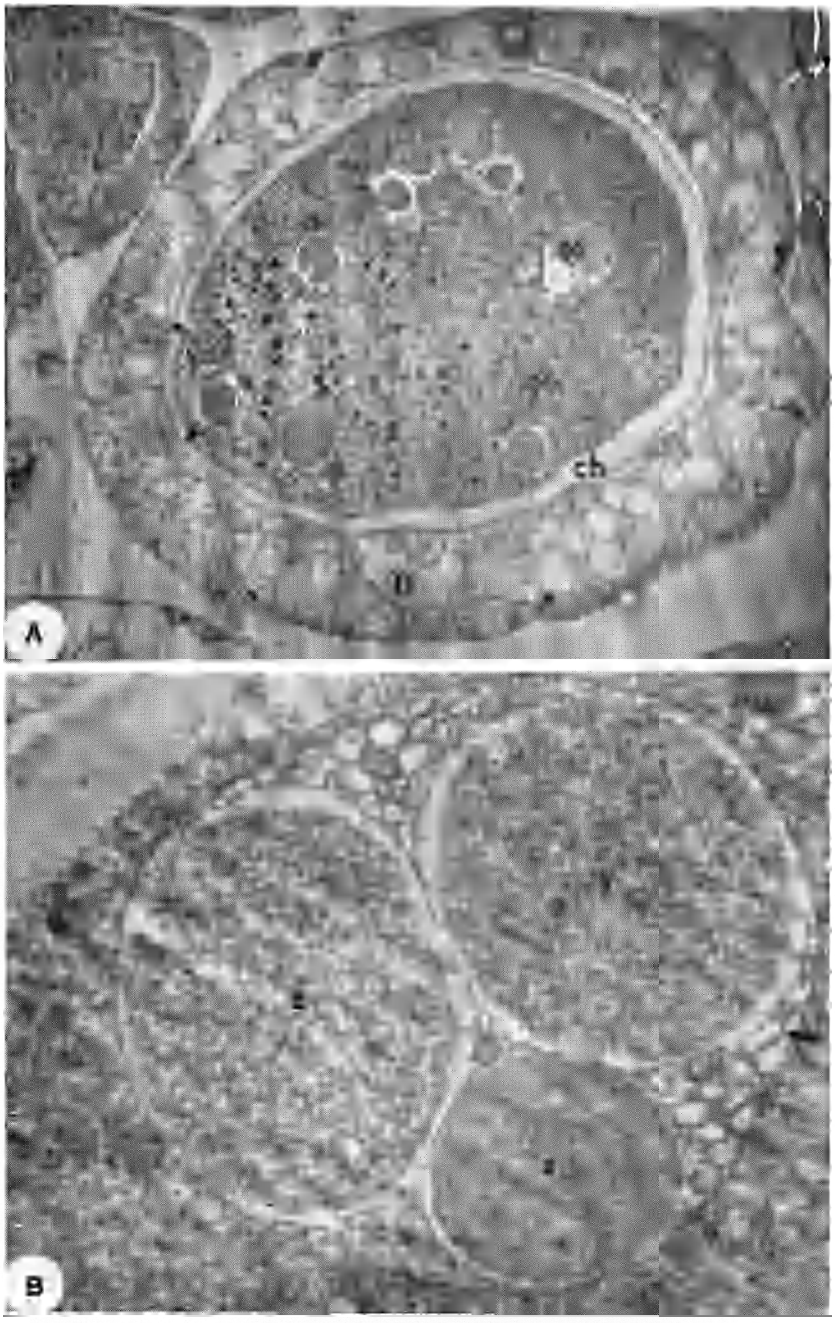
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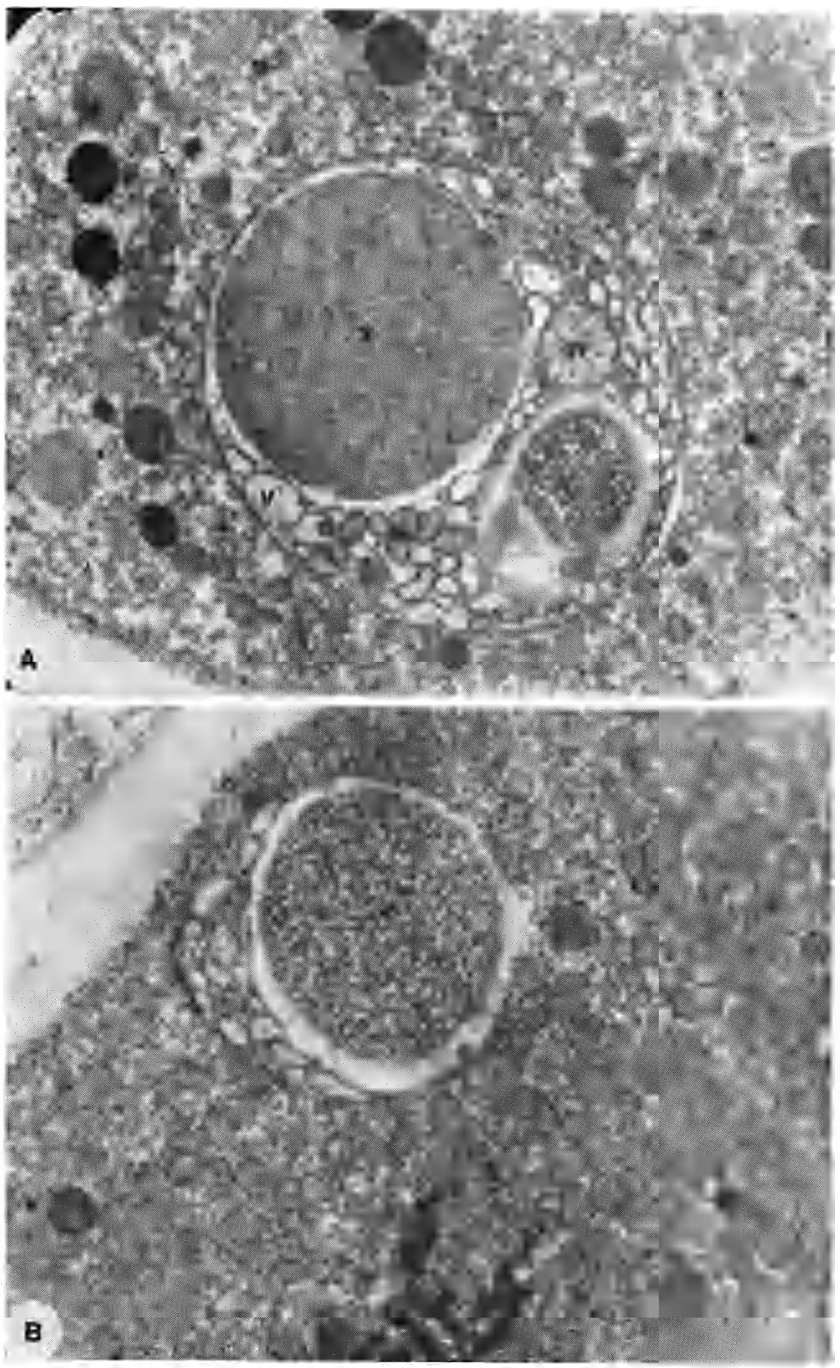
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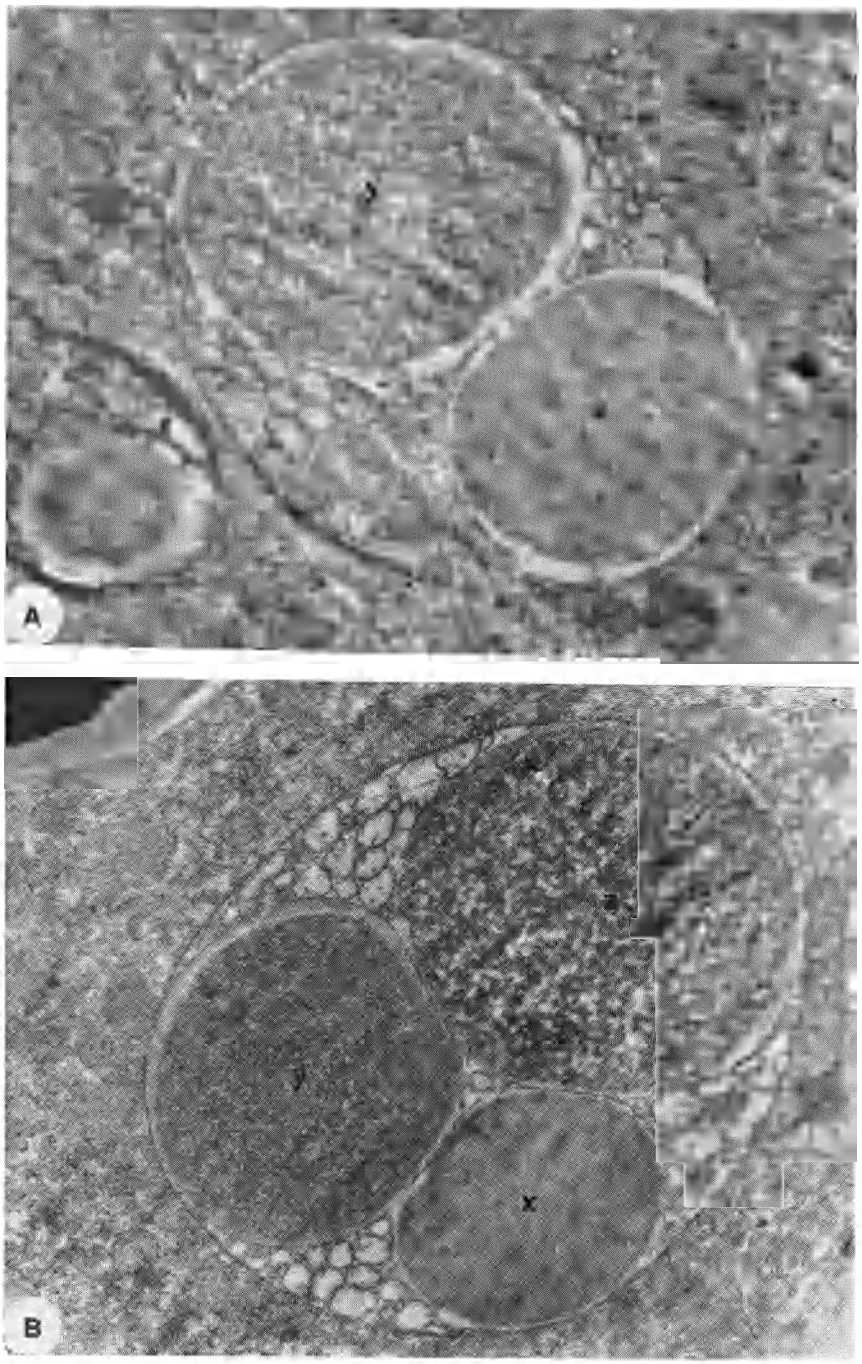
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EXPLANATION OF PLATES I-VI

PLATE I

A) Within the lumen of the foregut of a larva of ***Molgula impura*** one can observe a large cell, with the cytoplasm entirely occupied by three large oval bodies. One of them (*a*) has a granular structure; the other (*b*) contains massive elements; the third (*c*) shows many vermicular rods: (*a*) and (*b*) are limited by a thick membrane; (*c*) on the contrary, by a thin membrane. The entodermic cells which constitute the larval gut are characterized by the presence of big granules (8.000×). B, C, D) the three oval bodies at higher magnification (16,000×): note the peculiar chain-like constitution of the "rods" in B.

PLATE II

A) A cell within the intestine of a tadpole of ***Molgula***: note the presence of the three bodies described in Plate I: (*c*) is filled up with rod-like elements which are interpreted as symbiotic microrganisms in multiplicative phase (8,000). B) the three bodies at higher magnification: the vermicular elements appear constituted by a linear series of small vesicles (arrows 15,000×).

PLATE III

A) Within the foregut of another tadpole of ***Molgula*** one can observe two cells: each contains two or three bodies: (*a*) granular; (*b*) with small masses; (*c*) with vermicular elements: *n* nucleus (8,000×). B) the globular body (*c*) with its vermicular elements at higher magnification (16,000×).

PLATE IV

A) Young oocyte of ***Molgula impura***. The oocyte is surrounded by the chorial membrane and a very thick follicular sheet: eight "test cells" can be counted within the cytoplasm. B) "a test cell" whose cytoplasm is filled up with three big bodies (*x*, *y*, *z*); (*y*) shows a granular structure; (*z*) shows a rather vesicular aspect; (*x*) has a homogeneous aspect, however with some electron dense spots which confer on it a porous feature. The free cytoplasmic areas are occupied by vesicles of the endoplasmic reticulum and mitochondria (A = 1,600×; B = 10,000×).

PLATE V

A) A "test cell" within a young oocyte: (*x*) shows electron dense spots; *n* = nucleus; *m* = mitochondria; *v* = vesicles (8,000×). B) a "test cell" within the cytoplasm of a young oocyte: note the vesicular aspect of (*z*) (8,000×).

PLATE VI

A) A "test cell" within the cytoplasm of a young oocyte: two bodies *x* and *y* occupy nearly all its cytoplasm; notice the homogeneous structure of *x* with a pattern of electron dense spots; *y* shows a granular or vesicular structure; mitochondria and some large vesicles are also observed (10,000×). B) a "test cell" within the cytoplasm of a young oocyte; note the three large bodies *x*, *y*, *z*: *x* = with the typical electron dense spots; *y*, with fine granules and a thick capsule; *z* = with vesicular elements (10,000×).