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The origin of hyperplasia in the ovaries of ex-fissi-parous specimens of Dugesia gonocephala s.l.

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Zoologia. — *The origin of hyperplasia in the ovaries of ex-fissiparous specimens of* Dugesia gonocephala *s.l.* (*). Nota di VITTORIO GREMIGNI e ROSALBA BANCHETTI, presentata (**) dal Socio M. BENAZZI.

RIASSUNTO. — In varie popolazioni di planarie ex-scissipare appartenenti a *Dugesia gonocephala s.l.* è stata studiata l'origine della iperplasia ovarica. Sono stati comparati i tempi di rigenerazione della testa e della gonade femminile in individui con ovari normali e con ovari iperplasici. In questi ultimi la notevole rapidità con cui si riorganizzano gli ovari e la presenza di numerosissimi neoblasti sparsi in tutto il corpo ancor prima del taglio fanno ritenere che la iperplasia sia determinata da un notevole differenziamento di neoblasti in ovogoni. Gli ovari infatti raggiungono dimensioni di 400-450 μ dopo soli 10-11 giorni dal primo tagliò e in questo momento nessun ovocita mostra segni di alterazione cellulare.

I risultati ottenuti su individui ex-scissipari divenuti sessuati spontaneamente o dopo nutrizione con frammenti di *Polycelis nigra* e tagliati ripetutamente a distanza di pochi giorni hanno confermato che il controllo della iperplasia è di natura genetica.

INTRODUCTION

In fissiparous strains of *Dugesia gonocephala s.l.*, some specimens reach sexual maturity and develop ovaries of remarkable size (900-950 μ in diameter). Benazzi calls these ovaries " hyperplasic ", because they contain an abnormally large number of oocytes, most of them blocked at the prophase of the first meiotic division. According to Benazzi, this hyperplasia probably depends not only on the fact that few or no oocytes leave the ovary, but mainly on an abnormally rapid transformation of neoblasts into oogonia [1].

Our recent histological and ultrastructural findings have shown that oocytes in hyperplasic ovaries develop quite regularly up to diplotene; a number of morphological changes then occur which lead to their very slow necrosis, and, by consequence, to the sterility of the ex-fissiparous specimens [6].

These findings might suggest that ovarian hyperplasia depends on the progressive accumulation of degenerating oocytes, and would do so even if the rate of transformation of neoblasts into oocytes remained almost regular. In this case the ovaries would be of almost normal size and appearance during the early stages of their development and only acquire hyperplasic features at a later stage, and, in any case, rather slowly. If, on the other hand, it is supposed that ovarian hyperplasia is due to the rapid transformation of neo-

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blasts into oogonia and/or to the higher mitotic rate of the latter, one would expect ovaries to become hyperplasic very quickly.

So far histological and ultrastructural research has only been carried out on sexually mature animals, so that these hypotheses have gone unchallenged by experimental findings.

Our attempt to solve this problem has been based on observation of the morphological evolution of ovaries in specimens belonging to ex-fissiparous strains, some of which have become sexual spontaneously and others after being fed on crushed tissues of *Polycelis nigra*.

We have also tried an experimental approach to the problem, by examining the rate and mechanism of regeneration of the pre-pharyngeal region, particularly the ovary, both in ex-fissiparous specimens with hyperplasic ovaries and in normal sexual specimens, all within the "*D. gonocephala* group".

Regeneration problems in planarians have received considerable attention (for surveys see Brøndsted [2] and the symposium edited by Kiortsis and Trampusch [7]), but little is known about the rate of regeneration of female gonads. To our knowledge the most recent study is that by Grasso [3], who reported that in *Dugesia lugubris*, a species which has a very high regenerative power, ovaries began to reorganise only 16 days after excision.

Our morphological and ultrastructural examination of ovaries at various stages of reorganization has, incidentally, enabled us to confirm data given in a previous paper [6] on when metabolic changes leading to the necrosis of oocytes take place.

Materials and methods

Our observations on animals which became sexual spontaneously have been carried out on all the fissiparous strains of *D. gonocephala s.l.* reared in our laboratory which produce sexual specimens with hyperplasic ovaries.

For regeneration experiments we have used the following strains:

Ex-fissiparous specimens with hyperplasic ovaries which became sexual spontaneously

1) River Jordan (Israel) length 21 mm (1)
2) Ruisseau des singes (Algeria) length 16 mm
3) River Alcantara (Sicily) length 17 mm

Ex-fissiparous specimens with hyperplasic ovaries which became sexual after being fed on crushed tissues of P. nigra

4) Camogli (Ligurian Riviera) length 20 mm
5) Rome length 21 mm

(1) Full length measurements (mean values).

Ex-fissiparous specimens with normal ovaries

6) Castello Pino (Corsica) length 14 mm

Specimens with exclusively sexual reproduction

7) Paradojo (Corsica) length 14 mm
8) Corfù (Greece) length 14 mm

Ten animals from each population were kept at the same temperature (19–20° C), and under identical lighting and feeding conditions (*Tubifex* twice a week) for a month previous to, and throughout, the experiments. This was done to maximise our chances of obtaining similar morpho-physiological levels in all the specimens, as recommended by Brøndsted.

Each experiment was carried out three times between January and July, 1972.

The first cut was made simultaneously in all the specimens 1 mm above the pharynx after light CO_2 anaesthesia. The regeneration of the head was checked by using the reappearance of the eye as test.

A second cut was made 0.5 mm below the first in some specimens from Populations 1, 4 and 5, after 4 days (Group A), 10 days (Group B) and 20 days (Group C) respectively. By using this procedure we were sure that we were removing not only the blastema but also the area where, according to Teshirogi and Ohba [13], many mitotic neoblasts are found.

In other specimens from the same populations the pre-pharyngeal area was removed five times at intervals of 10 days.

Head and ovary regeneration was first checked in the living animals by binocular examination, and then by histological examination on sections 6–7 μ thick embedded in paraffin and stained with 0.5 % toluidine blue. The cytological study was carried out on squashed fragments of the animals stained with acetic carmine.

To count the oogonial mitoses the specimens were treated for 3–4 hours with 0.3 % colchicine.

For submicroscopic observation the same technique was used as that described in a previous paper [6].

RESULTS

Our observations show that the development of hyperplasic ovaries in unsectioned specimens is very rapid. Only 6–7 days after becoming recognizable as small clear dots, ovaries reach a length of 600–650 μ, when they are clearly visible to the naked eye (Plate I, fig. 1).

Our histological and cytological examinations showed that the ovaries consist of oogonia and young oocytes. In proportion to the very high numbers of oogonia, there were rather few mitotic divisions. When first cut, the specimens from Populations 1, 4 and 5 regenerated eyes after about 3 days (75–76 hours); 7–8 days later the ovaries were recognizable as small dots.

A typical hyperplasia was reached after 10–11 days, though the length of the ovary (400–450 μ) was lower than before the cut (900–950 μ) (Plate I, figs. 2–3). By this time the pigmentation of the whole cephalic area was completely restored, and the region itself could hardly be distinguished from the rest of the body.

Histological examination has shown that at this stage hyperplasic ovaries consisted only of oogonia and young oocytes (maximum length 18–20 μ) enveloped by a great many cells which were identifiable as differentiating neoblasts by their typical morphology and submicroscopic structure (Plate I, fig. 4).

Electron-microscope observations showed that all the germ cells were morphologically normal at this stage (Plate II, fig. 6). Much of each cell was taken up by the round nucleus, where chromatin granules were gathered in large groups; the very large nucleolus (2–2.5 μ in diameter) contained mixed granular and fibrillar components; the cytoplasm was very rich in ribosomes, which were free or in clusters. No Golgi bodies were seen; little endoplasmic reticulum was present; mitochondria were clustered near the nuclear envelope, and were often associated with small, finely granular bodies which probably came from the nucleus (Plate II, fig. 7). This feature seemed to be common to all differentiating cells in planarians [8], [10], [11].

At this stage no morphological lesions were recognizable in the nucleus or in cytoplasmic components, and the lysosomal system was almost entirely absent.

Twenty days later the ovaries were 730–770 μ in length, little less than in unsectioned specimens; many oocytes were in the vitellogenic stage and the first morphological lesions could now be seen, especially in the nucleus.

Specimens from Populations 2 and 3, which were smaller than those in Populations 1, 4 and 5, had slower regeneration rates for head and ovary. Populations 6, 7 and 8 consisted of specimens without hyperplasic ovaries; eye reappearance took about 6 days (136–140 hours) after the first cut in Population 6, and about 7 days (152–156 hours) in Populations 7 and 8. The first clusters of germ cells and neoblasts recognizable as ovaries were seen 23–24 days after the first cut (Plate I, fig. 5).

In specimens from Populations 1, 4 and 5 sectioned for the second time 4 days after the beginning of the first regeneration (Group A), the eyes reappeared after 80–82 hours; the ovaries reappeared after 9 days and became typically hyperplasic after 12–13 days. In Group B specimens neither eye-regeneration times (77–78 hours) nor ovary-regeneration times (11–12 days) were significantly different from those of specimens sectioned for the first time. There were no significant differences between the results for Group C and those for animals which were regenerating for the first time.

After five consecutive cuts at intervals of ten days the ovary has not reorganized when viewed histologically after ten more days; but in every case the ovaries did reorganize later, and were then invariably hyperplasic.

As to the specimens which become sexual after being fed on crushed tissues of *Polycelis nigra*, a higher and higher proportion lost the capacity

to regenerate the removed parts of their reproductive system (ovaries and testes) after each cut was made at intervals of 10 days; increasing regression of the unsectioned parts (copulatory apparatus) was seen, and the animals reverted to the architomic system of reproduction.

DISCUSSION

Our observations on ex-fissiparous specimens with hyperplasic ovaries sectioned 1 mm above the pharynx showed that head regeneration time (eye spot reappearance as test) is much lower (75–76 hours) than in ex-fissiparous specimens without hyperplasic ovaries (136–140 hours) or in normal sexual specimens (152–156 hours) (fig. 1), (Table I). This might be explained by the very high number of neoblasts found throughout the bodies of animals of the first type, even after they reach full development and sexual maturity (see also Grasso and Benazzi [5]).

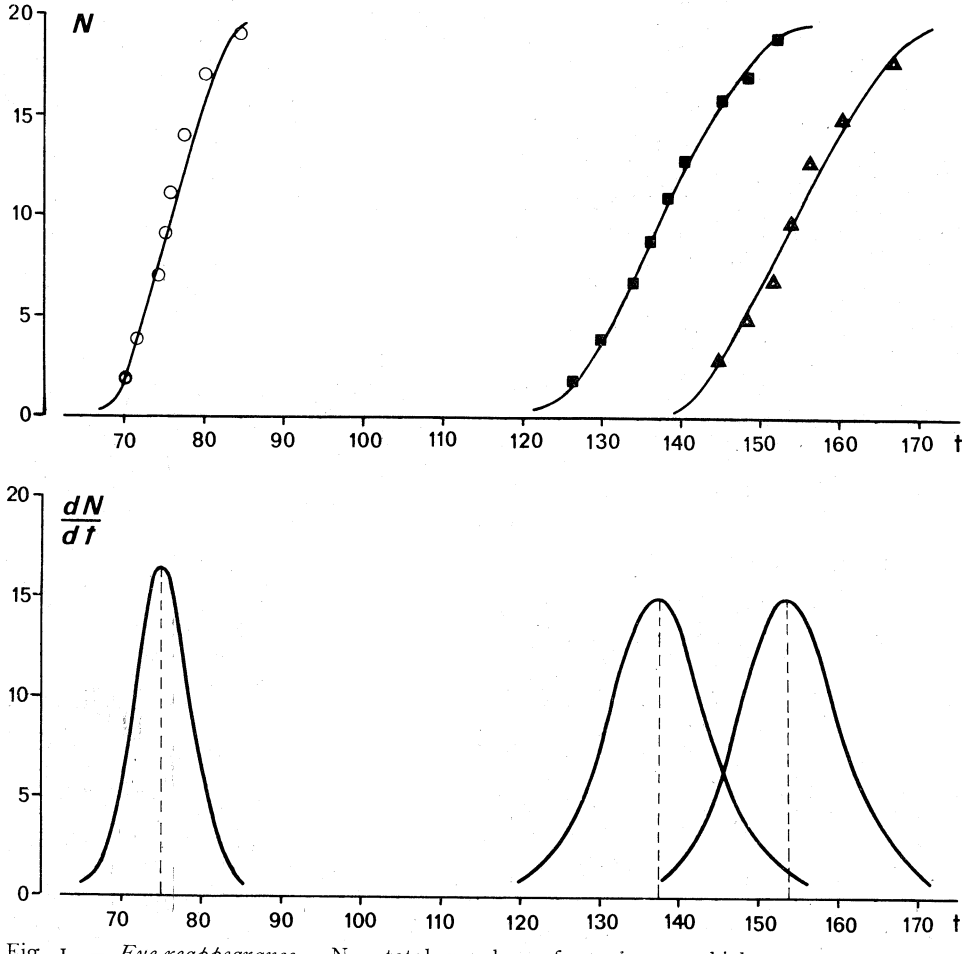

Fig. 1. – *Eye-reappearance.* N = total number of specimens which regenerate eyes at time *t*. ○ = Pop. 1; ■ = Pop. 6; ▲ = Pop. 7. Below: graph showing the mean time of eye-reappearance in Populations 1, 6 and 7 respectively.

TABLE I

Eye-reappearance after the 1st cut.

Hours	Population 1		Population 6		Population 7	
	N.	%	N.	%	N.	%
70	2	(10%)	0		0	
72	4	(20%)	0		0	
74	7	(35%)	0		0	
75	9	(45%)	0		0	
76	11	(55%)	0		0	
78	14	(70%)	0		0	
80	17	(85%)	0		0	
84	19	(95%)	0		0	
87	20	(100%)	0		0	
126			2	(10%)	0	
130			4	(20%)	0	
134			7	(35%)	0	
136			9	(45%)	0	
138			11	(55%)	0	
140			13	(65%)	0	
145			16	(80%)	3	(15%)
148			17	(85%)	5	(25%)
152			19	(95%)	7	(35%)
154			20	(100%)	10	(50%)
156					13	(65%)
160					15	(75%)
167					18	(90%)
172					20	(100%)

Ovary regeneration took place very quickly in Populations 1–5. Ovaries reappeared only 7–8 days after the first cut, and reached a length of 400–450 μ after 10–11 days. In Populations 6–8 the ovaries began to develop after 23–24 days and reached a length of 140–160 after 40 days (fig. 2).

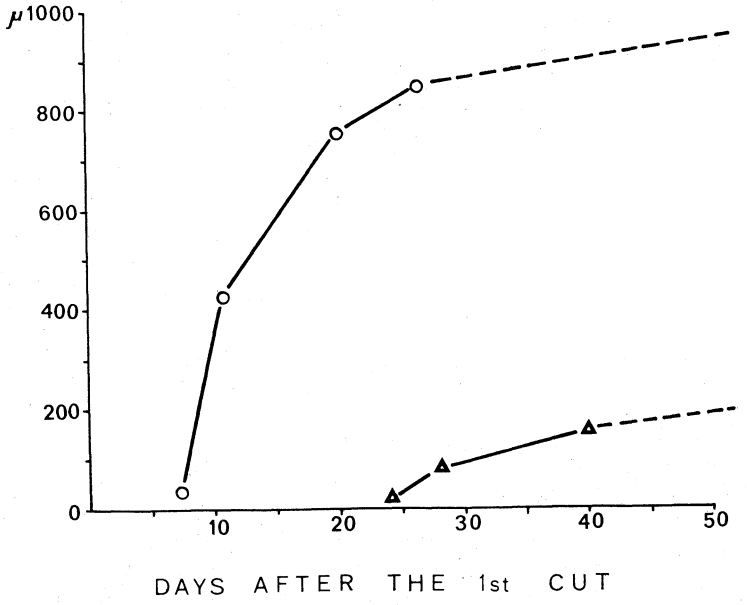

Fig. 2. – *Ovary-regeneration.* ○ = Pop. 1; △ = Pop. 7. Size of the gonads in μ plotted as ordinates against duration of experiment in days as abscissae.

In considering the origins of ovarian hyperplasia in these animals, it is worth noting that as soon as ovaries reappeared—containing only small germ cells (oogonia and pre-vitellogenic oocytes)—so did signs of hyperplasia. This finding, which is confirmed by data obtained from specimens which were not cut, appears to rule out the hypothesis that hyperplasia is determined by the prolonged presence of vitellogenic degenerating oocytes within the gonad. These oocytes do not, in fact, appear in cut specimens until 15–16 days after the first cut.

Equally, the hypothesis that an abnormally fast oogonial mitotic rhythm is responsible for the rapid increase in numbers of oocytes must be rejected on the basis of cytological data obtained from hyperplasic gonads of specimens pre-treated with colchicine.

Both head and ovary regeneration took place more slowly when a second cut, 0.5 mm nearer the pharynx, was made 4 days after the first one, whereas there was no significant difference when 10 or, a fortiori, 20 days were allowed to elapse between the two cuts.

All these data seem to suggest that it is the great demand for neoblasts during the first regeneration and their subsequent removal which causes the delays observed in the formation of the second blastema and in the differen-

tiation and growth of hyperplasic ovaries. This mechanism would also explain the delays in ovary reorganization after various subsequent cuts—the smaller the time interval between cuts, the greater the delay in ovary reorganization.

Ultrastructural examination of regenerating ovaries has confirmed that the first morphological changes are those in the nucleus. These occur when little yolk globules appear in the cytoplasm.

As to the control of ovarian hyperplasia, it must be stressed that even after the pre-pharyngeal area has been removed many times, involving repeated removal of cerebral ganglia, nerve cords and ovarian nervous plexus, each of the successive female gonads reappeared in hyperplasic form. This finding appears to confirm Benazzi's hypothesis that this hyperplasia is of genetic origin. In this connection one must stress the contrast between specimens which become sexual after being fed on crushed tissues of *Polycelis nigra* and those which become sexual spontaneously. The first lose their capacity to develop ovaries when these are repeatedly removed. Whereas the second regenerate their reproductive system however many times it is removed.

Ovarian hyperplasia did not appear in ex-fissiparous specimens of Population 6 even if they were fed on crushed tissues of *P. nigra* over a period of many months.

These findings seem to show conclusively that the neurohormonic substances which are believed to stimulate the differentiation and ripening of gonads has no direct influence on the development of ovarian hyperplasia. Ovaries develop either normally or hyperplasically according to the genetic constitution of individual specimens.

Our findings, therefore, have led us to the following conclusions:

(1) Ovarian hyperplasia is determined by the rapid differentiation of many neoblasts into oogonia, and not by a quicker mitotic rhythm of oogonia.

(2) The only result of the prolonged presence of slowly degenerating diplotenic oocytes in the ovaries is a further increase in the size of gonads; it is not the main reason for hyperplasia.

(3) Ovarian hyperplasia is of genetic origin; it does not appear to be influenced by the neurosecretory substances which control the differentiation of the gonads.

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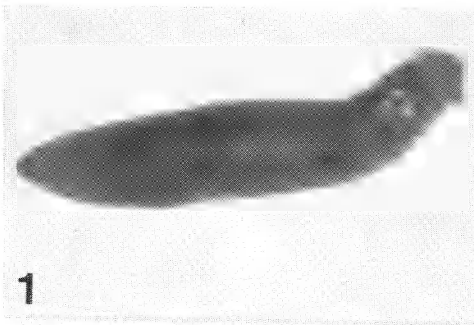

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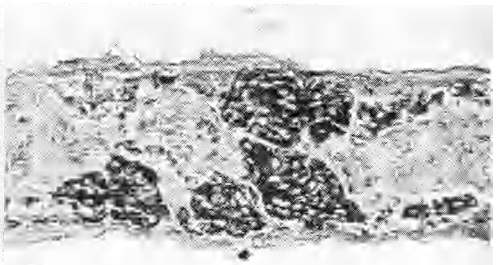

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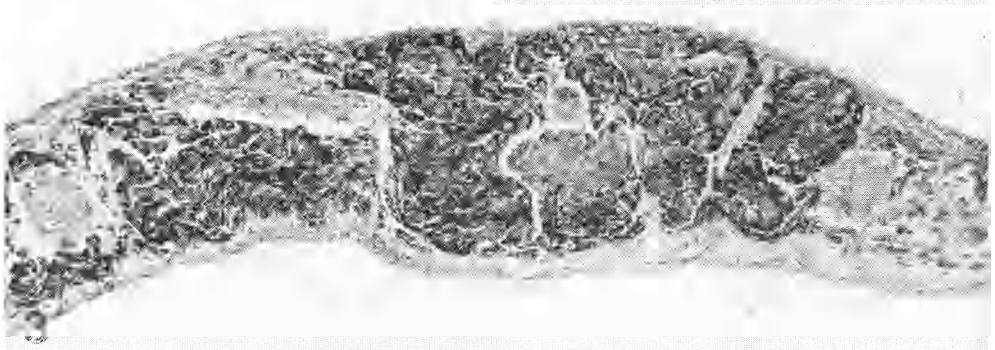

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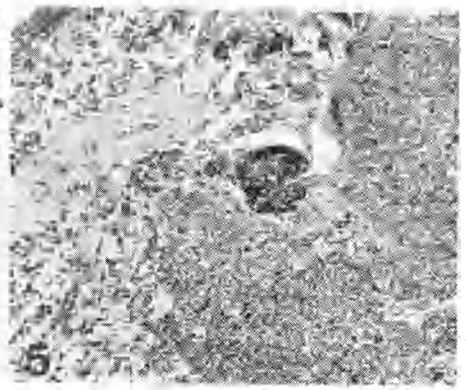

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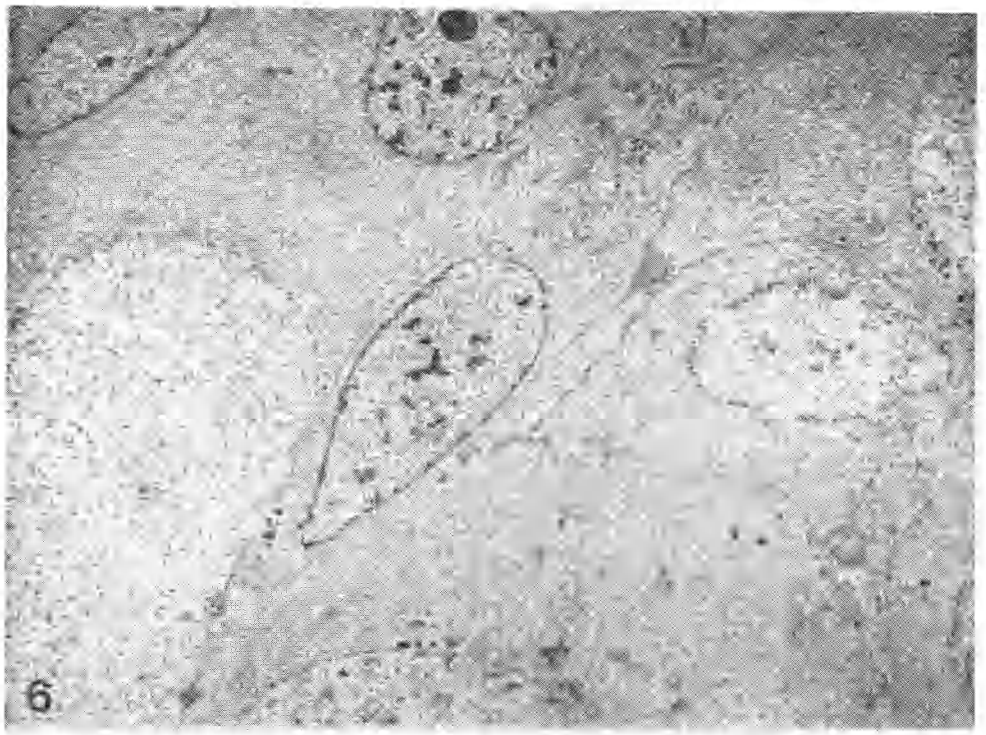

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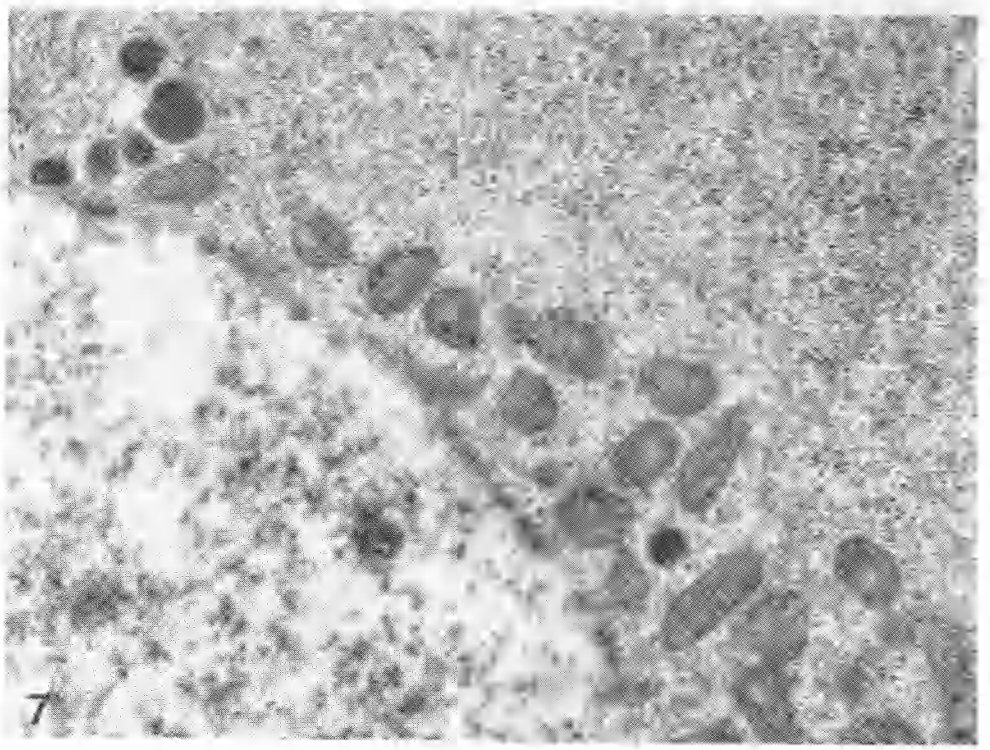

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EXPLANATION OF PLATES I–II

PLATE I

Fig. 1. – Ex-fissiparous specimen from Population 1, seven days after becoming sexual, with developing hyperplasic ovaries. (×3).

Fig. 2. – Hyperplasic ovary from a specimen of Population 1, 10 days after the 1st cut. (×100).

Fig. 3. – Hyperplasic ovary from an unsectioned, sexually ripe specimen of Population 1. (×100).

Fig. 4. – High magnification of hyperplasic ovary 11 days after the 1st cut, showing only oogonia and young oocytes. (×150).

Fig. 5. – Traces of an ovary from a specimen of Population 6, 24 days after the 1st cut. (×100).

PLATE II

Fig. 6. – Low magnification micrograph showing many oogonia and many young oocytes with normal morphology. Carnovsky—OsO_4. (×3500).

Fig. 7. – Clusters of mitochondria associated with dense bodies near the nuclear envelope. Carnovsky—OsO_4. (×23000).