

JUVENILE HORMONE III AND NUTRITION EFFECTS ON SPERMATOGENESIS IN THE 4TH INSTAR NYMPHS OF *PANSTRONGYLUS MEGISTUS* (HEMIPTERA: REDUVIIDAE)

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When 4th instar nymphs of Panstrongylus megistus are fed with a saturant blood meal, there is an intense proliferation of the spermatogonia. At the end of the intermoult, the older spermatogonial cysts differentiate into 1st primary spermatocyte cysts. In the nymphs deprived of the blood meal this evolution is not observed, but a small growth of the testicular follicles occurs, due to a few mitotic divisions. This growth is observed at least, until 25 days after ecdysis. Since day 15, an autolytic process starts in the older spermatogonial cysts. The presence of exogenous juvenile hormone III (JH III) does not promote the development of the germ cells in the fasting insects. There is only a small growth of the testicular follicles and the autolytic process is also observed.

In the precocious adults obtained by allatectomy or precocene II treatment, germ cells are observed in all developmental stages, except packed and elongated spermatozoa bundles.

It seems probable that the juvenile hormone (JH) plays an inhibitory role on meiotic divisions in P. megistus, since they do not occur during the 4th instar, but are present in the testicular follicles of the precocious adults.

Key words: *Panstrongylus megistus* – hemiptera – juvenile hormone – mitosis – meiosis – nutrition – spermatogenesis

Since Fukuda (1944) suggested that the development of the germ cells, in the male and female *Bombyx mori*, was controlled by hormones, several papers have been published, whose goals were to show how this control is realized.

The JH plays an inhibitory role on the spermatogenesis of *B. mori* and maintains the *Periplaneta americana* testes in a juvenile state, i. e., retards their development (Takeuchi, 1969; Blaine & Dixon, 1970; Yagi & Fukushima, 1975). The allatectomy does not influence the spermatogenesis in *Schistocerca* and *Rhodnius prolixus* because it seems not to be under the control of the corpora allata (Wigglesworth, 1936; Cantacuzène, 1967). However, the JH appears to stimulate the production of sper-

matids and the spermiogenesis in *Dysdercus cingulatus* (Ambika & Prabhu, 1978).

The juvenoid compounds, hydroprene and methoprene, cause alterations in the spermatogenesis of *Spodoptera littoralis* and *Corcyra cephalonica* (Gelbic & Metwally, 1981; Deb & Chakravorty, 1981).

In *Rhodnius*, exogenous JH in the early 5th instar, inhibits the spermatogonial mitosis and the spermatocyte meiosis (Dumser & Davey, 1974). The presence of this hormone in the last instar of *Locusta migratoria* does not affect directly the spermatogenesis but inhibits the spermioducts differentiation (Szöllösi, 1975).

The chromenes were isolated by Alertsén (1955) from plants of the genus *Ageratum* and synthesized by Huls (1958). The biological activity of these compounds in insects was discovered by Bowers et al. (1976) and then

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denominated precocenes. These substances are cytotoxic to the corpus allatum. They cause gland atrophy and inhibition of the JH synthesis, simulating a gland ablation (Unnithan et al., 1977, 1980; Bowers & Martinez-Pardo, 1977; Bowers et al., 1982; Azambuja et al., 1981).

It is thus possible to observe, as shown above, that JH III exerts either a stimulatory or an inhibitory role or does not intervene at all in spermatogenesis of the insects. Its role in a blood-sucking bug is thus interesting to investigate further, specially in connection with the role of the blood meal, which is of importance for the development and reproduction of the blood-sucking bugs. The effects of JH III and nutrition on 4th instar nymphs of *P. megistus* spermatogenesis are the subject of the experiments reported in this paper.

MATERIALS AND METHODS

Insects – The blood-sucking bugs *P. megistus*, were obtained from a colony maintained in the Laboratory of Entomogenesis, Department of General Biology, Federal University of Pernambuco. The insects were kept in incubator at 28 ± 1 °C and 70-80% RH, and fed once on guinea pigs.

Nutrition effects – To observe the effect of the nutrition on the germ cell development, fed and unfed 4th instar nymphs were used. Fifteen starved nymphs in each group were dissected at 5, 10, 15, 20 and 25 days after moulting. In the other group, a blood meal was offered 10 days after moulting, and the dissections were carried out 5, 10 and 15 days after the blood meal. As in the 1st experiment 15 insects were used at each time.

Unfed nymphs plus JH III – In this experiment the behaviour of the germ cells in the absence of the blood meal, but in the presence of the JH III, was observed. We applied topically, with a Hamilton microlitre syringe 1 or 5, or 10 μg JH III/insect, on the abdominal tergites of the 4th instar nymphs, at the 5th day after ecdysis. The JH III (Sigma Chemical Company) was dissolved in acetone (1 and 10 μg JH III/ μl acetone). The insects were dissected 15 days after drug application. The controls received 1 μl acetone/insect (groups treated with 1 or 10 μg JH III), or 0,5 μl acetone/insect (group treated with 5 μg JH III).

Allatectomy – Fourth instar nymphs were allatectomized as described by Furtado (1977), 24 h after the blood meal. The control insects (sham-operated groups) were submitted to the same surgery, without removing the gland.

Treatment with precocene II – On the abdominal tergites of the 4th instar nymphs, were applied topically 200 μg precocene II/insect (Sigma Chemical Company) dissolved in 2 μl acetone, 24 h after blood meal. The controls received 2 μl acetone/insect.

The precocious adults obtained by the treatment and the control insects were dissected 24 h after ecdysis.

Histological techniques – The testicular follicles were dissected in Ringer saline and fixed in Bouin's fluid for 2 h at room temperature. The material was embedded in agar-paraffin; sections were cut 5 μm thick and stained with hematoxylin-eosin, and mounted in Canada balsam.

RESULTS

Development of the germ cells during 4th instar in fed and unfed insects – In the nymphs of *P. megistus*, the testes are already totally structured, but not developed, in the early 4th instar, as may be observed at the 5th day after ecdysis. At this moment, the germarium fills almost completely the testicular follicle (Fig. 1). When the blood meal is offered at the 10th day after ecdysis, the spermatogonia undergo mitosis continuously. This may be detected by the increase of the length of the follicles and mainly by the number of spermatogonia within the cysts (Fig. 3). At the end of the intermolt period, the older spermatogonial cysts differentiate in 1st primary spermatocyte cysts (Fig. 4). Therefore, in normal nutritional and hormonal conditions, an active proliferation of the spermatogonia, 1 of the 3 successive phases of the spermatogenesis dynamic process, occurs through the 4th instar. This proliferation was not observed in the unfed nymphs, where only a relative growth of the follicle took place. In addition there was not spermatogonial development, like that seen in the fed insects. Twenty-five days after ecdysis the follicles presented some new cysts in mitosis and others, older ones, with pycnotic nuclei (Fig. 2).

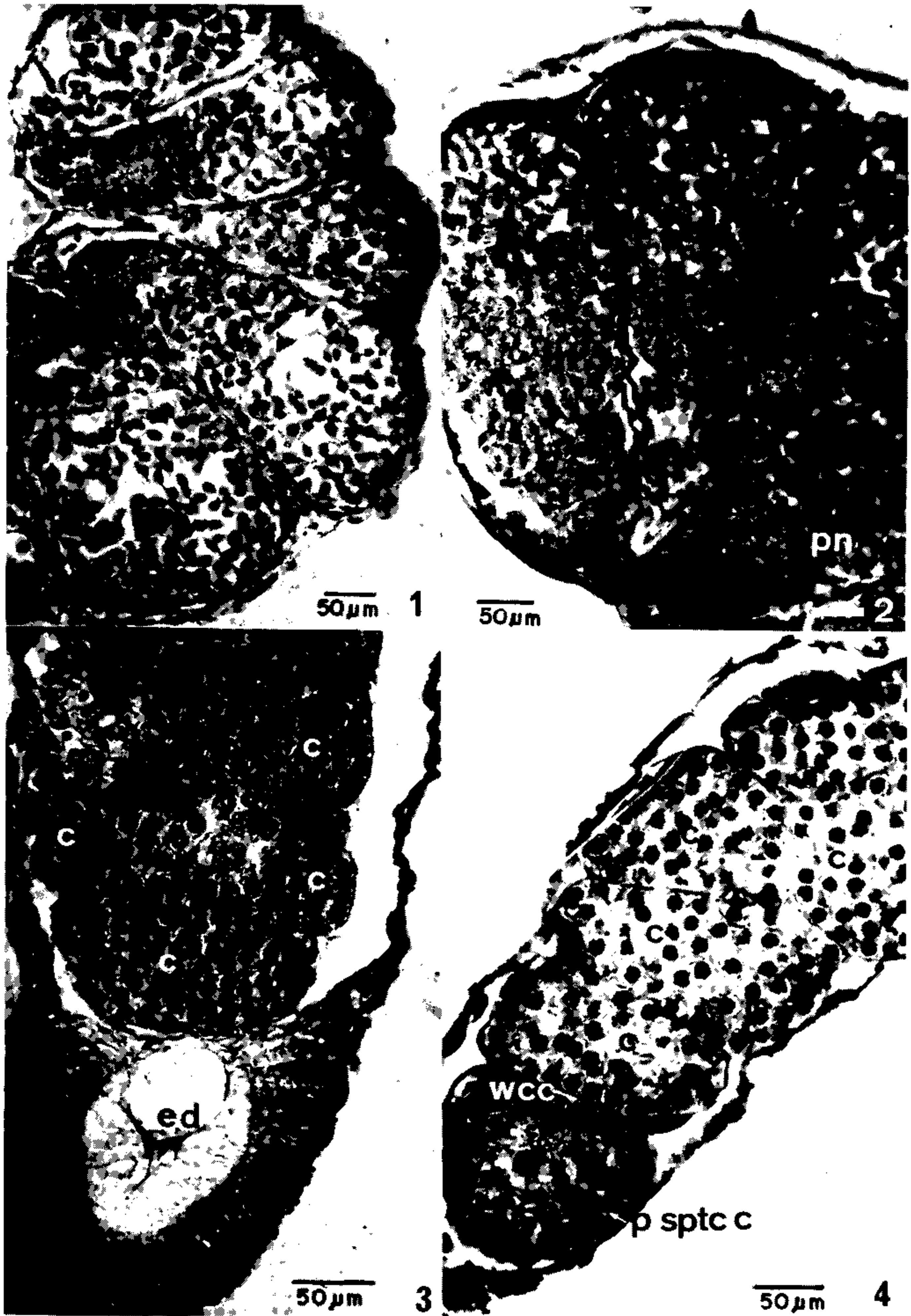


Fig. 1: testes of the 4th instar nymph, 5 days after ecdysis. Longitudinal section showing 4 of the 7 testicular follicles at the beginning of the spermatogonia proliferation phase. Fig. 2: longitudinal section of the testicular follicle of the unfed 4th instar nymph, 25 days after ecdysis, where are seen cysts with pycnotic nuclei (pn). Fig. 3: longitudinal section of the testicular follicle of the 4th instar nymph, 10 days after blood meal. Cysts with different number of spermatogonia. Cyst (c); efferent ductus (ed). Fig. 4: longitudinal section of the testicular follicle of the 4th instar nymph, 15 days after blood meal. Cyst (c); primary spermatocyte (p.sptc.c); wall cell cysts (wcc).

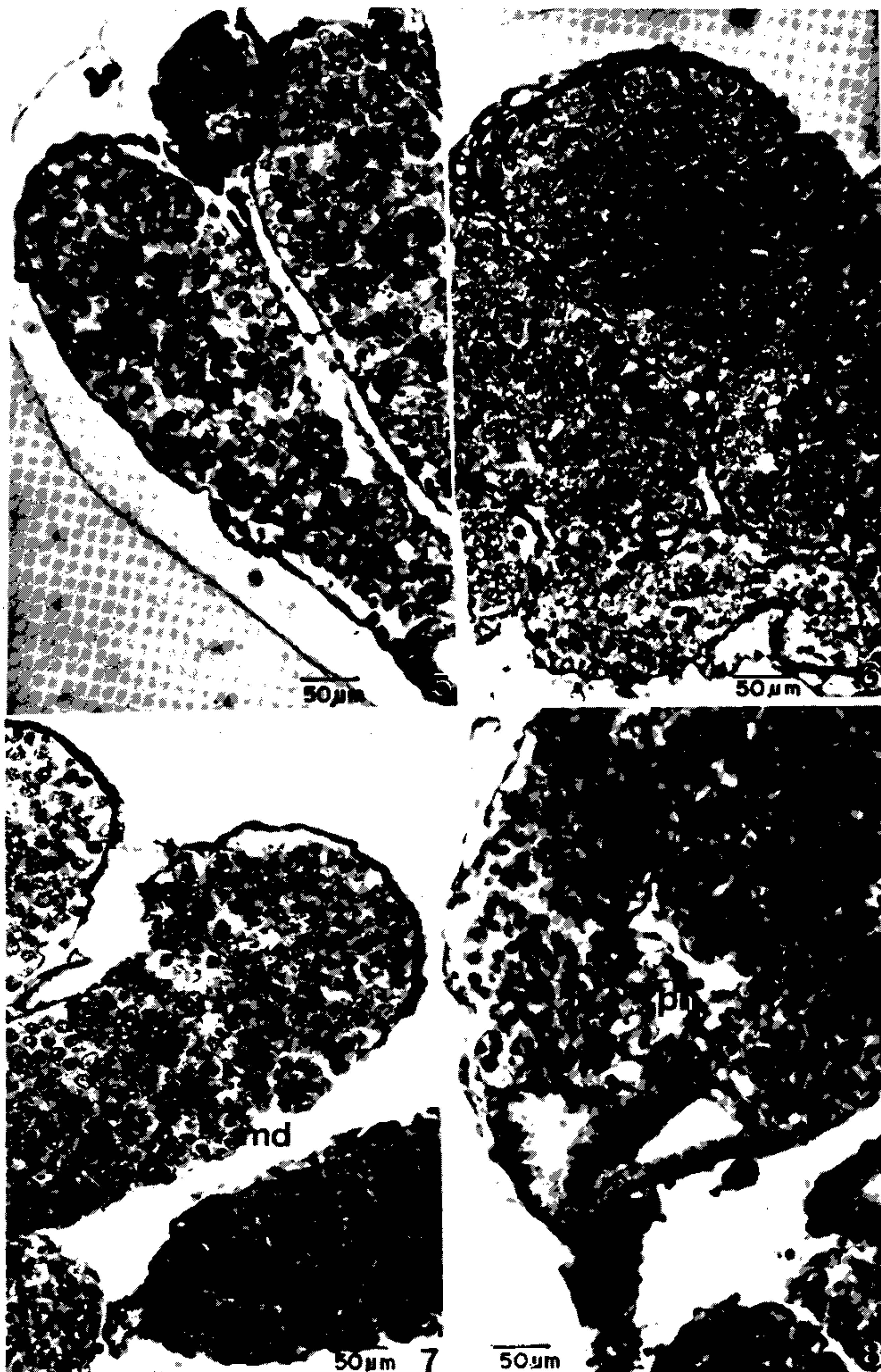


Fig. 5: longitudinal section of the testicular follicle of the unfed 4th instar nymph, that received $1 \mu\text{g}$ JHIII. Spermatogonia cyst with pycnotic nuclei (pn). Fig. 6: longitudinal section. Testicular follicle of the unfed 4th instar nymph that received $5 \mu\text{g}$ JHIII. Fig. 7: longitudinal section. Testicular follicle of the unfed 4th instar nymph that received $10 \mu\text{g}$ JHIII. Apical region. Cyst in mitotic division (md). Fig. 8: longitudinal section. Testicular follicle of the unfed 4th instar nymph that received $10 \mu\text{g}$ JHIII. Basal region. Pycnotic nuclei (pn).

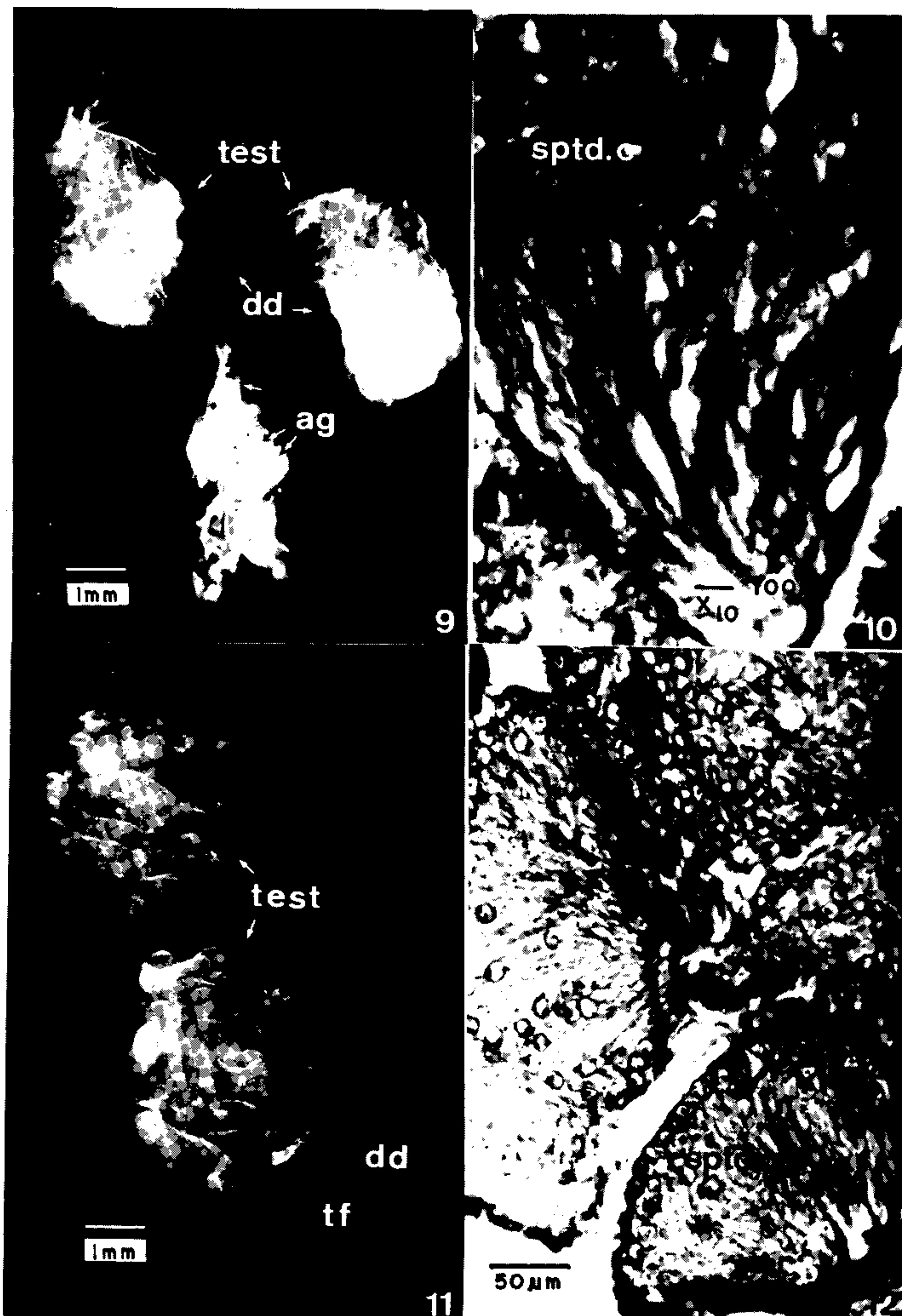


Fig. 9: reproductive system of the precocious adult obtained by allatectomy (Ca). Accessory glands (ag); deferent ductus (dd); testes (test). Fig. 10: developing spermatid cysts of a precocious adult, obtained by allatectomy. Spermatid cyst (sptd.c). Fig. 11: gonad of the precocious adult obtained by treatment with precocene II. Deferent ductus; testicular follicle (tf); testes (test). Fig. 12 developing spermatid cyst of a precocious adult obtained by treatment with precocene II. Spermatid cyst (sptd.c).

Development of the germ cells in unfed nymphs in the presence of exogenous juvenile hormone III – The testicular follicles of the unfed 4th instar nymphs that received topically 1 μg JH III 5 days after ecdysis, were dissected 15 days after application of the drug. They did not present growth (Fig. 5). The follicles of the nymphs that received 5 μg each (Fig. 6), were longer than those of nymphs treated with 1 μg . The follicles of the nymphs that received 10 μg showed a slightly larger size (Fig. 7), and mitotic divisions were observed. A complete development did never occur. An autolytic process was observed to take place with most frequency in the insects that received 5 and 10 μg JH III. The cysts in autolytic process were often seen in the basal region, among the oldest cysts which contain a great number of cells (Fig. 8). It is important to observe that autolytic process does not occur normally in the 4th instar nymph, but only latter in the 5th instar.

Allatectomy – Fourth instar nymphs were allatectomized 24 h after the blood meal. Some nymphs did not moult, up to 2 months, and 3 were not able to escape from their old cuticle. Similar results have been obtained by Azambuja et al., 1981, and Garcia et al., 1987. All of them were male precocious adults, which testicular development was studied. Abnormalities in the gonad morphology were not observed (Fig. 9). The histological analysis of the testicular follicles showed the occurrence of spermatogonia, spermatocyte, and spermatid cysts in different stages of the spermiogenesis (Fig. 10), as well as a high frequency of cysts with pycnotic nuclei, like in normal 5th instar nymphs. Although these cysts have been observed more frequently among the primary and secondary spermatocyte cysts, they were often seen among the spermatid cysts. Packed or elongated spermatozoa bundles were not seen. The growth of the maturation zone was revealed by the space occupied by this zone within the testicular follicle. This growth is directly related to the number of primary and secondary spermatocyte cysts. Therefore, the 4 zones where the germ cells are distributed, in accordance with their developmental stage, were observed, i. e.; the germarium, the growth zone, the maturation zone and the differentiation zone. The stage of development reached by germ cells of the precocious adults resembles that of the 5th instar nymphs 7 to 10 days after ecdysis.

The results suggest an inhibitory effect of the JH on the maturation of the germinal cells, since, in 4th instar nymphs meiotic divisions do not occur, while in the testicular follicles of the precocious adults, we observed spermatocytes I and II, as well as elongated spermatid bundles.

Treatment with precocene II – After treatment of 80 4th instar nymphs with precocene II, 42 precocious adults were obtained. Among these, 20 males were observed. Disturbance in the gonad morphology was not observed (Fig. 11). The histological study of the testicular follicle showed germinal cells in all evolutive stages, except the stage of spermatozoa bundles. Spermatid cysts were seen in different and advanced stages of spermiogenesis (Fig. 12). An intense autolytic process was observed. The control insects performed a normal ecdysis to the following instar. The histological analysis of their follicles showed only primary spermatocyte cysts, whose presence is characteristic of the 5th instar nymph in the early intermoult. These results are similar to those obtained after allatectomy and show that in the absence of the JH, proliferation, maturation and some steps of the process of differentiation occurred normally.

DISCUSSION

Spermatogenesis is a dynamical process where three distinct phases are identified: i) spermatogonia multiplication, ii) maturation and iii) spermiogenesis.

The 4th instar testicular follicle of *P. megistus*, is characterized by an intense proliferation of the spermatogonia. This proliferation occurs by mitotic divisions whose number is constant in each species. Therefore, the number of the mitotic divisions undergone by the spermatogonia is limited. The results presented in this paper show that some divisions occur in the absence of the blood meal, at least until 25 days after the ecdysis (Fig. 2). These divisions, that result in a small growth of the follicle, may be explained as produced by the nutritive reserves of the early instar; this was suggested by several papers which show the role of the quantity of blood ingested in the previous instar in fasting resistance, as well as, in the number of eggs layed by unfed females of *Triatoma brasiliensis* (Costa & Perondini, 1973; Perondini et al., 1975).

According to Baehr (1973) and Baehr et al. (1978), fasting *R. prolixus* is not submitted to any endocrine stimulation, the rate of JH being very low, almost zero. Then, it is possible, that the mitotic spermatogonial divisions which occurred in the unfed 4th instar nymphs of *P. megistus* were realized in the absence of JH. It is therefore probable, that the small growth observed in the follicles of the nymphs that received 5 or 10 μg JHIII, is caused more by the mobilization of nutritive reserve than by the action of the JH itself. The JHIII appears, however, to be as morphogenetic as gonadotrophic, at least, in some insects (De Kort et al., 1982).

The increase of the fasting period causes the autolysis of the spermatogonia, mainly in the older cysts, a fact also observed by Dumser (1980) in unfed nymphs of *R. prolixus*. The autolysis occurs even in the presence of the JHIII which may be seen in the Figs 5 and 8.

The differentiation of the older spermatogonial cysts, in the 1st cysts of the primary spermatocytes, at the end of intermoult, suggests the existence of a direct temporal relation between the duration of the intermoult period, the sequence of the mitotic divisions of the spermatogonia and their differentiation into primary spermatocytes. According to Dumser (1980), "the ability to maintain the level of differentiation within the germ line in phase with its somatic development, argues strongly in favour that there must exist rate regulating mechanisms or regulatory points within the development sequence".

The results obtained by allatectomy or by treatment with precocene II, show that the absence of JH during the 4th instar of *P. megistus* does not modify the development sequence and the differentiation of the germ cells; the reached stage was similar to that seen in the 5th instar nymphs at 7 days after ecdysis (Schuetz, in preparation). Alterations observed in the JH titers were shown not to modify the developmental sequence and differentiation of the germ cells of *B. mori* (Takeuchi, 1969). The proliferation of the germ cells is related with the number of mitotic divisions undergone by the spermatogonia. This number is genetically determined (Loeb & Birnbaum, 1981).

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