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SECRETORY ACTIVITY AND ENDOCRINE REGULATION OF MALE ACCESSORY GLANDS IN THE BLOOD-SUCKING BUG *PANSTRONGYLUS MEGISTUS* (HEMIPTERA: REDUVIIDAE)

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The epithelial cells of Panstrongylus megistus *male accessory glands (MAG) present ultrastructural characteristics of a secretory cell. Their secretory products are accumulated in the lumen of the four MAG lobes.*

During the first 8 days of adult life a strong secretion activity occurs, accumulating enough material to produce the first spermatophore. Cerebral neurosecretions as well as juvenile hormone are both involved in MAG secretory activity regulation. Juvenile hormone seems to be the responsible for the stimulation of most protein synthesis in male accessory glands. Cerebral neurosecretion seems to be necessary to stimulate juvenile hormone production and release by the corpus allatum. Furthermore, neurosecretion is required for some polypeptides synthesis by MAG. Although topic application of precocene II to adult males does not reproduce the same effects on MAG as does allatectomy, this compound causes strong reduction on male reproductive capacity.

Male accessory gland secretions of insects play multifunctional roles not only in the transference, activation and protection of the gametes but also, in a number of species, by inducing certain types of female behaviour and by stimulating ovarian development and oviposition.

The research on accessory gland secretions, considered, 20 years ago, to be in its infance (Leopold, 1976) has, in recent years, attracted an increasing interest. However, our knowlege about accessory glands themselves and specially about the origin, chemical nature and function of their secretory products remain at almost the same level we had before, at least in most Insect groups.

In our laboratory we have been studying some structural, developmental and functional aspects of the male accessory glands (MAG) of *Panstrongylus megistus,* an important Chagas' disease vector in Brazil.

P. megistus male has a pair of accessory glands, each one consisting of four lobes: anterior, dorsal, internal and tubiform. A mesodermal rudiment goes under a differentiation process to form the MAG 4 lobes, after the blood meal given in the 5th nymphal instar. Eight to ten days after the blood meal, the four lobes are already apparent, but they remain coated together by a thin conjunctive sheet which disappears some days before imaginal molt (IM). Ten days after blood meal, the development consists of the lobe rudiment elongation until just prior to the IM, which occurs 22-25 days after blood meal (Regis & Schuetz, unpublished data).

After IM, the wall of each MAG lobe presents basically the same histological architecture, that is the same general pattern observed in most of the studied insect species. The main tissue consists of a cilindrical monolayer epithelium resting on a thick, well developed basement membrane, coated by two layers of muscle cells. A rich tracheolar system guarantees oxygene supply. A number of tracheoles run between the basement membrane and the muscle cells, as well as among muscle cells themselves and sometimes between the plasmatic membrane of two epithelial cells. The epithelial cells, very thin and tall in empty glands, are tightly appressed to one another in their lateral surface. Well developed interdigitations at the apical surface probably contribute to maintain epithelium integrity. In the anterior lobe, most of the epithelial cells show identical morphological ultrastructure. Few cells presenting different morphological aspects were observed within the epithelial layer at the anterior lobe. At the moment, studies are being per-

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formed in an attempt to localize those cells in the other lobes. In active glands, epithelial cells are clearly polarized. The apical pole presents a large number of secretory vesicles, some Golgi complex and endoplasmatic reticulum. Thick and irregular microvilli rise from the cell free apex. The basal pole is very richly endowed with RER, which presents, sometimes, a labyrinthic form. Concentration of a large number of mitochondria, and the plasmatic membrane deeply infolding at the basal edge suggest a strong absorptive activity. The ultrastructural morphology is the one generally expected for cells which specialize in producing "export" protein (Regis, Furtado, Coutinho & Coutinho, in preparation).

Accumulation of secretory material in the lumina of MAG lobes starts on the first day after IM and grows until the 8th day. Consequently MAG become swollen, the glandular area grows on this period going from 4.4 to 10.8mm^2 in unfed males and from 4.4 to 12.2mm^2 in fed males (Fig. 1). The quantity of material accumulated over this period in both unfed and fed male is enough to promote the first spermatophore formation. In fact, in this species, first mating generally takes place about 8 to 10 days after IM (Regis et al., 1985).

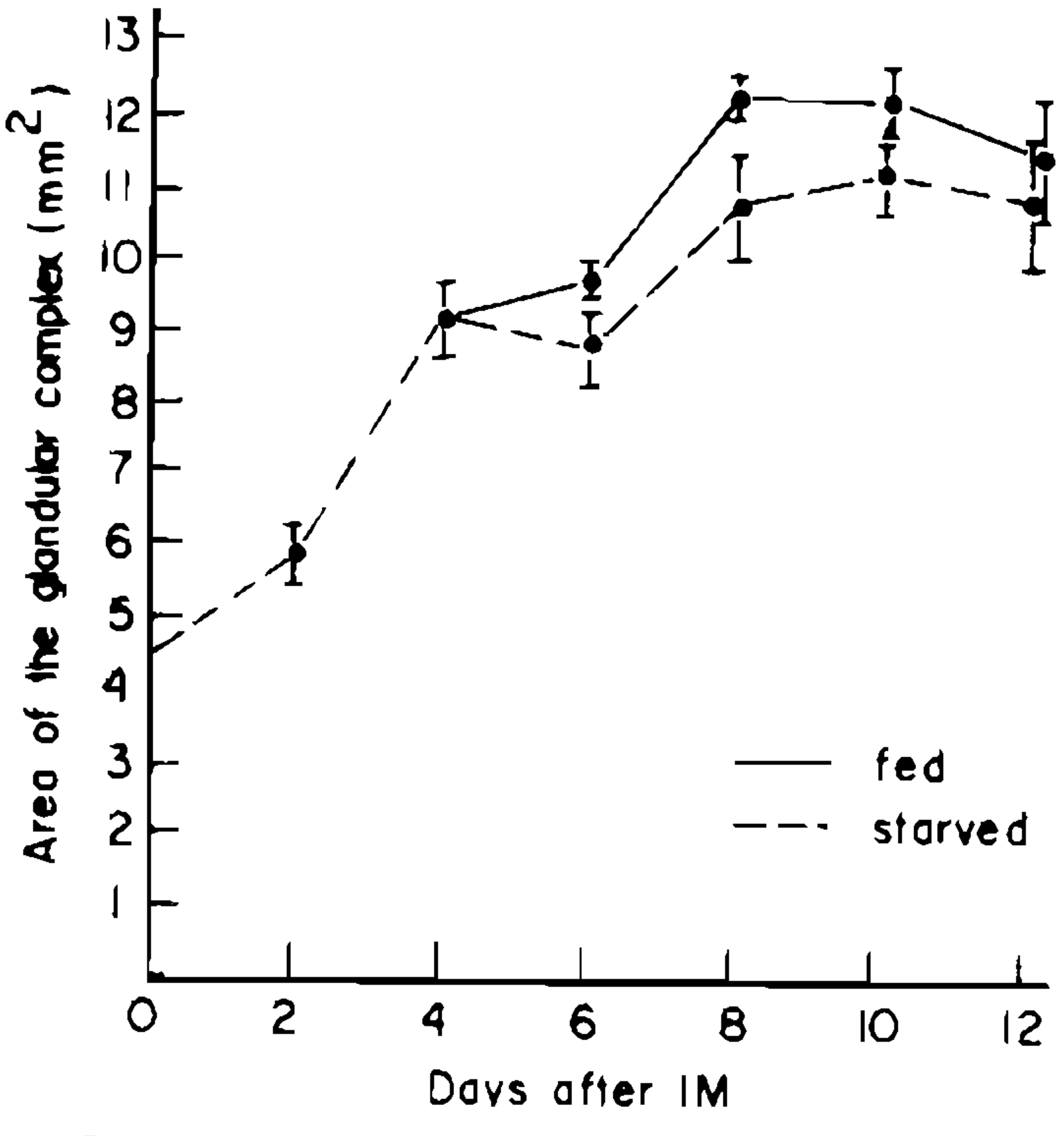

Fig 1: MAG area of unfed and fed adult *Panstrongylus megistus* (Regis et al., 1985).

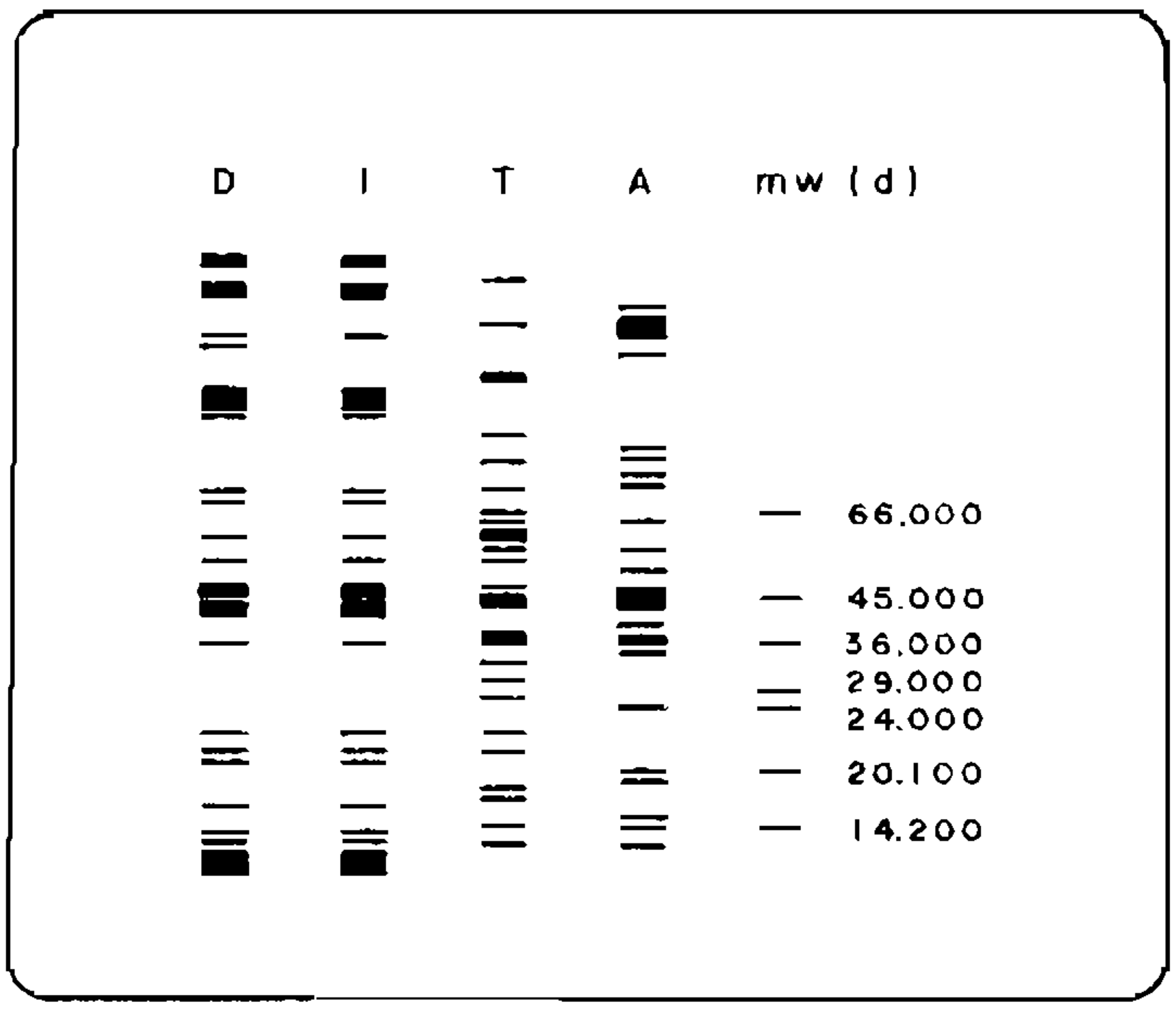

Fig. 2: Electrophoretic protein pattern scheme of accessory secretions from 8 days old males (SDS – PAGE Comassie blue). Secretion from anterior (A), dorsal (D), internal (I) and tubiform (T) MAG lobes of *Panstrongylus megistus* (Furtado et al., unpublished data).

Analysis of the accessory secretion protein pattern by SDS-PAGE shows that dorsal and internal lobes produce the same type of secretion, whereas those from anterior and tubiform lobes have different protein patterns (Fig. 2) (Furtado, Gomes, Regis & Brito, unpublished data). When the anterior lobe secretion is mixed, *in vitro*, with those obtained from both dorsal and internal lobes, they form a fibrilar material presenting a band pattern closely similar to that seen in the spermatophore (Rhem & Regis, unpublished observations).

These knowlegments about secretory epithelium structure, storage dynamics and protein pattern of MAG secretions, allow us to analyse the consequences of juvenil hormone (JH) or cerebral neurosecretion (CNS) lack, in order to understand endocrine regulation of MAG secretory activity. Interferences on neuroendocrine system, like parsectomy, allatectomy or JH application was carried out soon after IM. Parsectomy and allatectomy were perfomed as earlier described by Furtado (1979) and Regis (1978). 20μg of JH III (Calbiochem) diluted in acetone was topically applied on dorsal abdominal integument, half an hour after surgery. The effects of such interferences were evaluated by optical and electronic microscopy, gel electrophoresis and MAG area measurement. Structural changes in the secretory epithelium and strong reduction of secretion accumulation in the MAG lumina of both parsectomized and allatectomized males, demonstrate that brain neurosecretion as well as JH are involved on MAG secretory activation process. JH involvement on MAG regulation has been shown in many studied insect species. Involvement of both JH and neurosecretion has also been shown in *Rhodnius prolixus* (Barker & Davey, 1981, 1983).

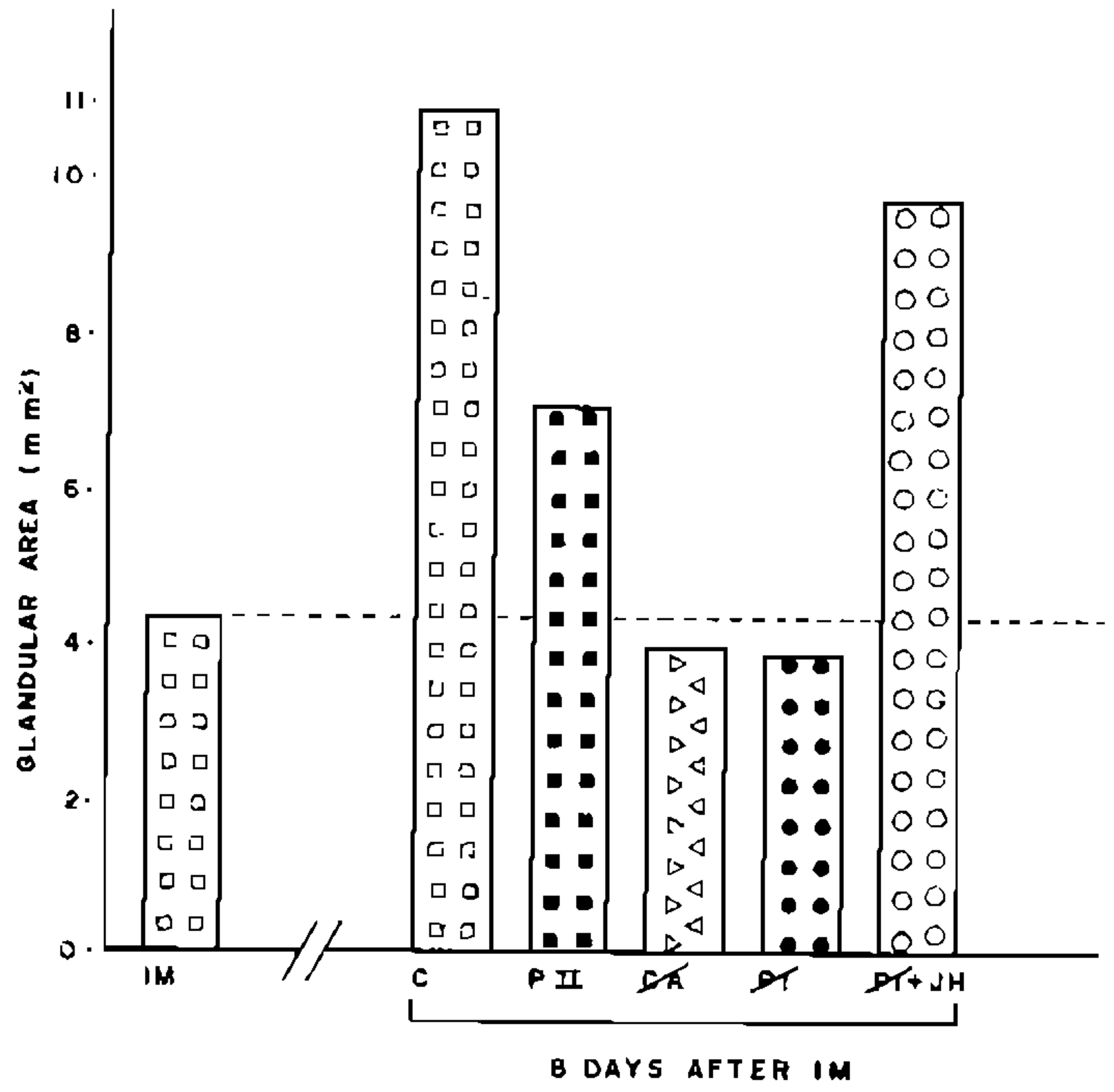

Fig. 3: MAG area of *Panstrongylus megistus*, IM = imaginal moult; C = control; ~~CA~~ = allatectomy; ~~PI~~ = parsectomy, JH = juvenile hormone (Regis et al., unpublished data).

Our results also allow us to analyse some details of the relationship between hormones and accessory glands activity.

Secretion accumulation can not be detected by measuring glandular area in parsectomized or allatectomized males (Fig. 3). However, eletrophoretic analysis of disrupted glandular complexes, shows that two important secretion components, of aproximately 40.000 and 45.000 daltons (which occur in secretion of each MAG lobe in control group), are also present in parsectomized as well as in allatectomized males. Furthermore, in allatectomized males MAG, small molecules, which molecular weight ranges below 14.200 daltons (mainly originated from dorsal and internal lobes), are also produced. These data suggest that (a) synthesis of 40.000 and 45.000 dalton bands seems to be neither JH nor CNS dependent; and (b) synthesis of small molecules (polypeptides) possibly results from a direct action of CNS on MAG.

One interesting result is that the treatment of parsectomized males with JH-III causes reappearence of most bands, when protein pattern is compared with those of parsectomized non-treated and normal control males. Quantitative evaluation by measuring glandular area shows that secretion accumulation in parsectomized JH-treated males reaches a level approaching that of normal control. This reversion of parsectomy effects by JH strongly suggest that (a) in parsectomized males there is no circulating JH, and (b) JH directly stimulates synthesis of most of the accessory secretion proteins.

There is, perhaps, a relationship between the occurrence of two kinds of direct stimulation – by either JH or CNS – and the existence of at least two different cellular types in MAG secretory epithelium.

Theoretically, on quantitative basis, both allatectomized and parsectomized males are incapable to produce spermatophore and to undergo semen transfer.

Based on the important role played by JH on MAG stimulation and also on the fact that MAG differentiation occurs along the last larval instar – therefore, in presence of very low JH level – it would be interesting to investigate the effects of precocenes on MAG. We have studied these effects by examining MAG morphology of precocious adults resulting from precocene treated 4th stage nymphs, as well as by analysing MAG activity of males treated by precocene II (200 or 300μg/specimen), 0 to 5 hours after IM. Precocious adult MAG show very strong abnormalities. Among more than a dozen examined specimens only one of them had the four lobes differentiated, but those lobes were not anatomically normal. Besides disturbance of lobe differentiation, MAG common excretory ducts of precocious adults were not completely differentiated, and in some cases there were only vestiges (Regis & Schuetz, unpublished data).

MAG secretory activity in precocene treated adults is far higher than that of allatectomized males. Compared with that of control group, secretion accumulation in treated male glands is reduced to about 50%, whereas in allatectomized males it is not possible to detect any storage, by using the same evaluation method. The electrophoretic pattern of treated male secretions presents, 8 days after IM, five bands which are absent in CA deprived males of the same age.

In order to know if the secretion accumulated in MAG lumen of precocene treated males is enough to produce spermatophore and to promote sperma transfer, their reproductive capacity was observed by forming couples with no treated females. Among such couples, 50% was able to copulate, but some of those did not produce spermatophore. Among those males which were able to form spermatophore, some of them were not able to actually transfer gametes to the female spermathecae and only 21% of the total sample, observed until 20 days after IM, was capable to produce offspring (Cavalcante & Regis, in preparation).

Although precocene II, even in the highest dose used, does not reproduce, in *P. megistus,* the effect of allatectomy on MAG activity, this compound can cause a significant reduction in the male reproductive capacity.

The important role played by JH on MAG activation and the possibility of using very simple methods to evaluate the effect of this hormone lack on accessory secretion production, make MAG a good model to test anti-allatotropins and antijuvenile hormones effectiveness. On the other hand, due to MAG important and multifunctional role for the reproductive success – one among the very efficient survival strategies óf Insecta – those structures can be a potential "Achillis ankle", at least for some Insect groups.

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