

*Bolm. Zool., Univ. S. Paulo*

2:159-167, 1977

## COMPARATIVE MORPHOLOGY OF THE ENDOCRINE BRAIN AND RETROCEREBRAL COMPLEX OF THE HONEYBEE WORKER AND QUEENS (HYMENOPTERA-APINAE).

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### ABSTRACT

A study of the endocrine brain complex (brain neurosecretory cells, *corpora cardiaca* and *corpora allata*) in the adults of workers and queens at various ages were made. The features of these structures, when stained with chromium hematoxylin-phloxin indicate an increased activity of the workers neurosecretory cells after the 15th day of age, which is accompanied by similar changes in the *corpora cardiaca* and in the *corpora allata*.

In fully active fertilized queens the functioning of these structures remains unchanged, but, when egg laying decline the endocrine complex shows histological signs of degeneration, and in the virgin queens they show histological signs of hypofunctioning.

### RESUMO

Foi estudado o aspecto das células neurosecretoras da *pars intercerebralis* e dos *corpora cardiaca* e *allata*, em material obtido de rainhas e operárias de *Apis mellifera* em diferentes idades. O material foi corado com hematoxilina crômica-floxina, verificando-se uma degranulação das células neurosecretoras das operárias após os 15 dias de idade com correspondente aumento da atividade dos *corpora cardiaca* e dos *corpora allata*.

Nas rainhas, com exceção da rainha virgem e da rainha fecundada, com 3 anos de idade, a atividade desse sistema endócrino manteve-se constante, fato que parece indicar a atuação dos hormônios produzidos na vitelogenese.

### INTRODUCTION

*Apis mellifera* is a bee with two morphologically and physiologically well defined female castes. The differentiation between workers and queens starts with the first larval instar. This instar can be divided into two periods: an early one in which the larva may become either a worker or a queen depending upon its food intake, and later, one where the castes are already defined. Several papers report the food influence on the caste (see Wilson, 1971 for a review) and in the endocrine system differentiation (Canetti et al., 1964; Joly, 1966; Herrmann, 1969). On the other hand the queen development is detectable as a raise in the juvenile hormone titre in the hemolymph (Wirtz, 1973) this hormone effect in differentiation being proved by the induction of queen-like individuals from worker larvae treated with it or its bioanalogues (Wirtz & Beetsma, 1972). Thus, the caste differentiation is the result of differential juvenile hormone production.

The juvenile hormone is produced by the *corpora allata*, a component of the retrocerebral endocrine complex.

The endocrine complex in bees is composed of the neurosecretory cells in the *pars intercerebralis*, the *corpora cardiaca* and the *corpora allata*, all interconnected by nervi and having functions similar to those of the hypophysarium system of vertebrates (Hanström, 1942). The brain neurosecretion controls functions of the *corpora cardiaca* and *allata*. These functions are well studied for the larval stages, but for the adults their activity is quite controversial mainly concerning the workers.

As these organs remain present in the adults, this phase of bee life seems to be appropriate for comparative studies, since here the caste activities are well determined and many differences exist between the workers and the queens role in the colony.

A previous paper (Cruz-Landim & Höfling, 1972) showed histophotometric differences among the queens, the workers and the drones in all retrocerebral complex components. These differences were mainly correlated to the reproductive activities of these individuals. Gast (1967) verified that the queen honey-bee substance inhibits the *corpora allata* development in young workers, but not in the aged ones.

In attempting to probe further into the dimorphic changes in *Apis* females, we considered exploring the histological aspects of the endocrine system in these insects.

#### MATERIAL AND METHODS

Imagos of workers and queens with various ages were dissected in saline solution and the brain complexes were isolated. These specimens were fixed in 10% neutral formalin, embedded in paraffin and cut at 7  $\mu\text{m}$ . The sections were stained with chromium hematoxylin-phloxin.

Were studied workers 0, 10, 15, 20, 30 and 40 days old, virgin queens and fertilized queens with 1 and 6 months and 1, 2 and 3 years old.

#### RESULTS AND DISCUSSION

In a middle section of the brain complex of the honeybee, one can distinguish the neurosecretory cells, which are in the *pars intercerebralis* as a strip of cells situated in the protocerebrum (Figs. 1 and 4). From these the *nervi corpus cardiacum* stretch ventrally where they split on both sides of the oesophagus — going in the caudal direction (Fig. 2). The nervi leave the brain and penetrate the *corpora cardiaca* located dorso-laterally to the oesophagus (Figs. 3 and 5). Some of the nervi axons leave the *corpora cardiaca* in the ventro-caudal direction and penetrate into the *corpora allata*, situated caudally to the *corpora cardiaca*, latero-ventrally to the oesophagus (Figs. 3 and 5). Thus the neurosecretory cells in the *pars intercerebralis* are connected by their axons, first to the *corpora cardiaca*, and second to the *corpora allata*. The neurosecretion passes through these axons to both organs referred above (Fig. 2).

The neurosecretory cells from workers show an almost constant amount of secretion granules until the first 15 days of age and a decrease in them on the

20th, 30th and 40th days, (Fig. 1). The degranulation of the cytoplasm, resultant from the secretion liberation, is seen mainly around the nucleus. A slight increase in cell volume is seen in workers with 0 and 10 days of age. The secretion discharge is interpreted as a signal of activity and its storage as a signal of activity blockage, thus the worker cells are apparently less active until the 15th day and more active after this age.

In queens the main differences refer to cell volumes which increase from the virgin queens to the 2 years old ones. The virgins do not have granules in the neurosecretory cells and the 3 years old queen have small cells.

The *corpora cardiaca* and *allata* were stained lightly by this method, but some granules were observed in the corpora cardiaca of 15 and 20 days old workers. Judging for the nuclei, morphology, the *corpora allata* seem more active in 20 days old workers than in the others ones (Fig. 3d), which is in accordance with the possible stimulatory functions of the brain hormone.

The *corpora allata* from queens are more developed and present signs of more activity (dispersed nuclear chromatin) than those from workers. Many authors (Cassier, 1967; Pan & Wyatt, 1971) have pointed out the *corpora allata* play a role in vitellogenesis, which justify the condition presented in the queen. The *corpora cardiaca* are very similar in both castes, but the 3 years old queen had the *corpora cardiaca* and *allata* on a regression phase which also is in accordance with the controlling function of the neurosecretory cells over these organs.

### CONCLUSIONS

As shown above the workers and queens present differences in the histological aspects of the endocrine retrocerebral complex. The origin of these differences occurs during the larval development due to food quality and quantity effects. The further differences have not yet their causes well known because the extension of the control of the queen over the workers is not completely established and the target organs of the hormone produced in the retrocerebral complex are not determined in both castes.

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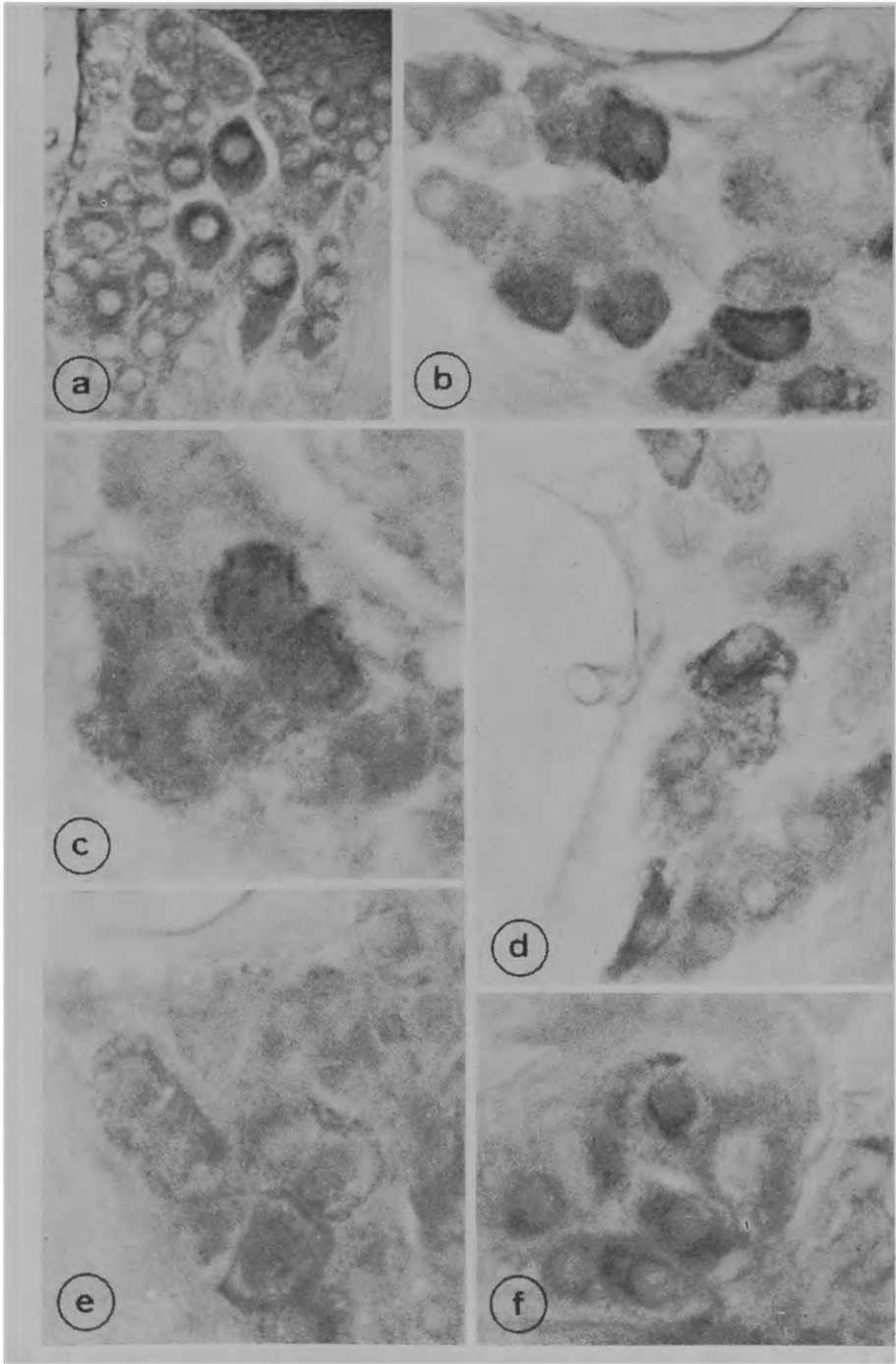


Fig. 1 — *Pars intercerebralis* from *Apis mellifera* worker: a — Worker 0 days old; b — 10 days old; c — 15 days old; d — 20 days old; e — 30 days old; f — 40 days old.

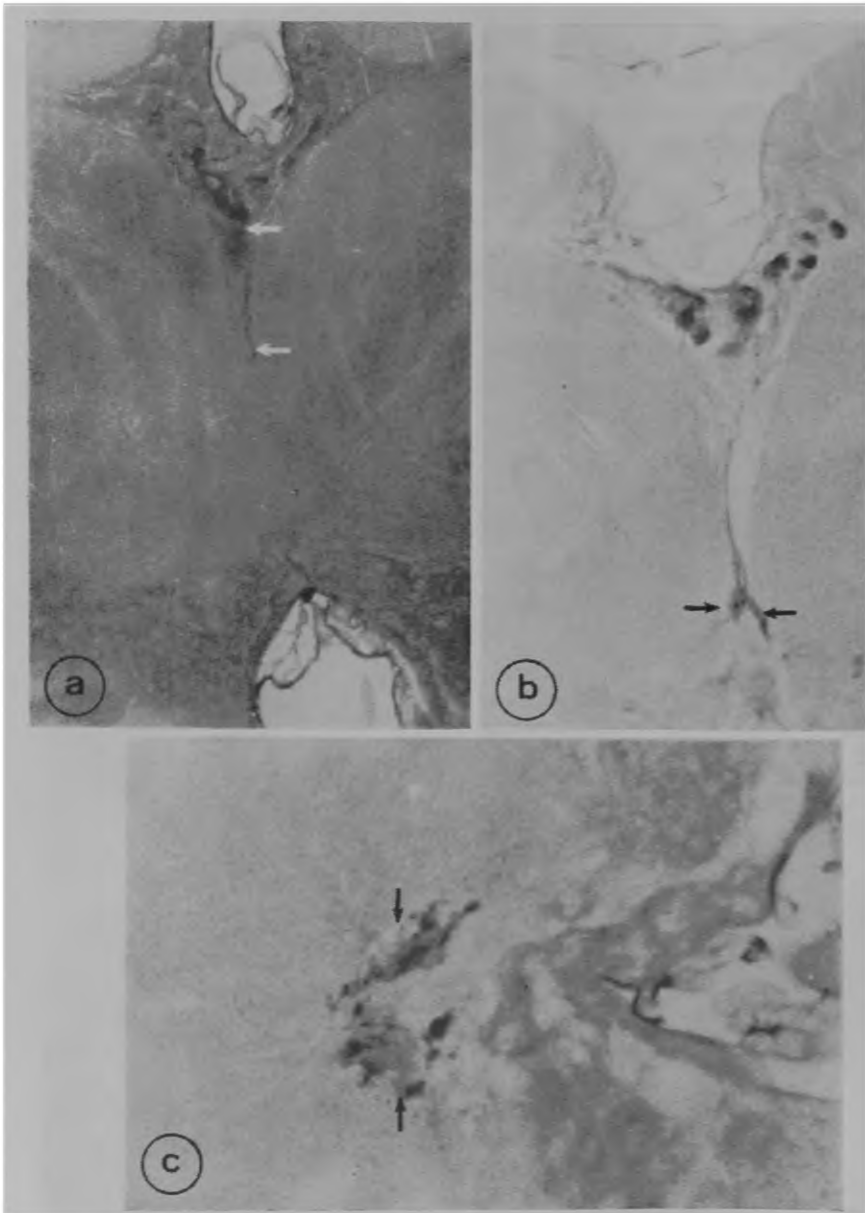


Fig. 2 — Nervi corpus cardiacum leading secretion from the *pars intercerebralis* to the *corpora cardiaca*. The fotos show the sequence of secretion way through the axons.

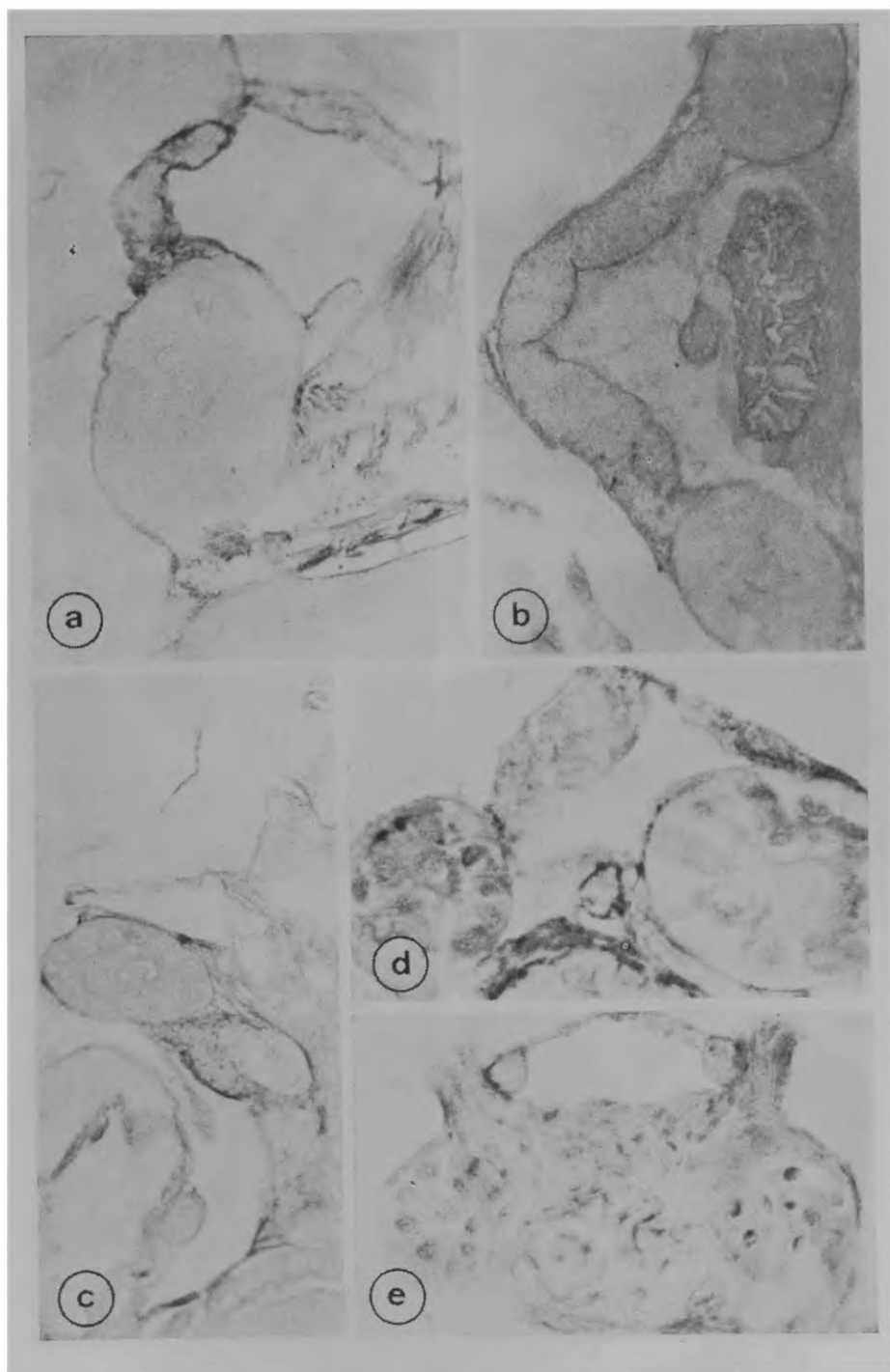


Fig. 3 — Workers *corpora cardiaca* and *corpora allata*. a — 0 days old; b — 10 days old; c — 15 days old; d — 20 days old.

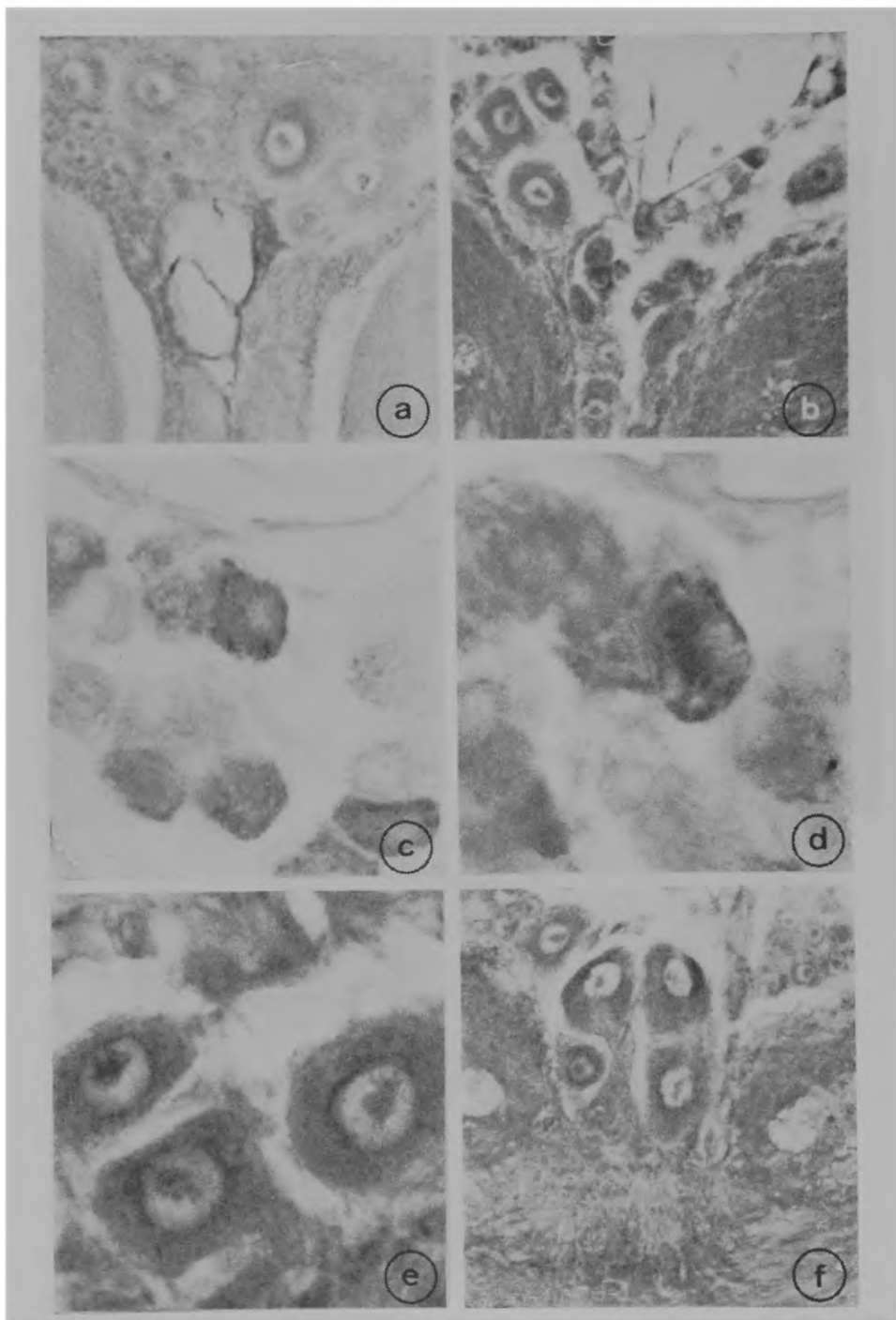


Fig. 4 — *Pars intercerebralis* from queens: a — virgins queen; b — fecundated queen 1 month old; c — 6 months old; d — 1 year old; e — 2 years old; f — 3 years old.



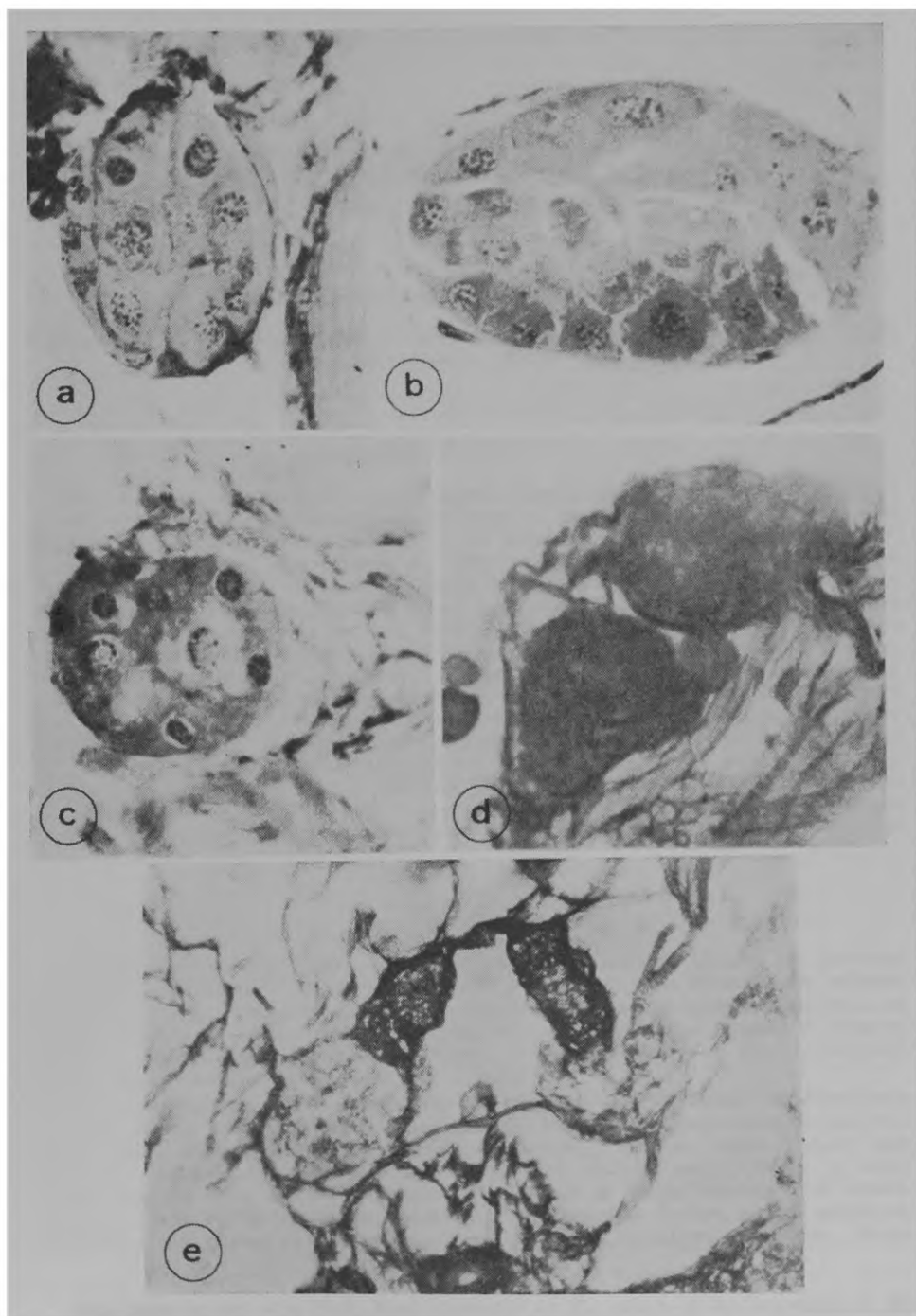


Fig. 5 — Queens *corpora cardiaca* and *corpora allata*.

