

The Structure of the Corpus Cardiacum of *Locusta migratoria*

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With one plate (fig. 1)

SUMMARY

The corpus cardiacum of *Locusta* consists of a syncytium containing nuclei of two sizes. The most obvious cytoplasmic inclusions are spheroid bodies, mostly about 0.6μ in diameter, which are easily seen in the living tissue, especially by phase-contrast microscopy. They do not appear in routine microscopical preparations, but are blackened by the classical 'Golgi' techniques. They give positive reactions for phospholipines. The cytoplasm also contains very numerous minute granules, which appear to be mitochondria.

INTRODUCTION

WHILE the structure and physiology of the corpora allata of insects are fairly well known, those of the corpora cardiaca have received comparatively little attention. Recent researches on the neurosecretory cells of the insect brain tend to suggest that the corpora cardiaca have important roles to play in the endocrine system of the insect (Scharer, 1952; Thomsen, 1952).

Ontogenetically the corpora cardiaca must be considered as ganglia (Hanstrom, 1948), but their histology shows that they function as secretory organs (review by Hanstrom, 1941).

The orthopteran corpora cardiaca have been studied by de Lerma (1933, 1937), Pflugfelder (1937), Cazal and Guerrier (1946), and Cazal (1948). The organs are variously described as being syncytial, or as consisting of nervous and secretory cells or chromophobe and chromophil cells, with a considerable amount of eosinophil and fuchsinophil secretion. Cazal and Guerrier (1946) record the finding of numerous granular mitochondria and Cazal (1948) mentions 'dictyosomes' also in the chromophil cells. The presence of chromaffin granules and the diphenolic nature of the secretion have been reported by Cameron (1953).

MATERIAL AND METHODS

Adults belonging to both sexes of *Locusta migratoria* were kindly supplied by the Anti-Locust Research Centre, London. Three batches of two dozen each were obtained and studied.

In dissections, the pronotum and the vertex of the head of the live locust are cut away and after the animal has been pinned in a dish of saline (0.8 per

cent. sodium chloride with 0.2 per cent. of 10 per cent. calcium chloride), the region behind the brain is stretched. When the large transverse tracheal trunk behind the brain is cut, the corpora cardiaca come into view as bluish-white elongated bodies, intimately connected to the anterior end of the aorta. The whole tissue is cut out carefully as one piece and transferred either to fresh saline for the examination of live tissue, or else to a fixative. The tissue in saline remains without any noticeable structural change for about an hour.

The following methods were used in the investigation of the minute structure and composition of the glands.

Fresh tissues. Freshly dissected corpora cardiaca were examined in saline under the ordinary, phase-contrast, and polarizing microscopes. The following dyes were used supravitaly: neutral red, methylene blue, janus black, and dahlia violet. Vital staining by injection of the dyes was not successful.

Whole mounts. Whole mounts of the glands were made by fixation in 10 per cent. formalin and staining in Mayer's acid haemalum.

Routine sections. Sections of material fixed in Bouin's fluid, Zenker's fluid, and 10 per cent. formalin were stained in Heidenhain's iron haematoxylin.

Methods for showing cell boundaries

(a) Herxheimer's Giemsa-tannin method (1915).

(b) Recklinghausen's silver nitrate method (1862) for intercellular substances, adapted as follows. Freshly dissected corpora cardiaca were washed in distilled water for 1 minute and then treated in 0.25 per cent. silver nitrate in the dark for 6 minutes. They were then washed in distilled water, mounted on a slide in distilled water, and pressed slightly with a clip; the preparation was exposed to light until brown.

(c) Smith's Lyons blue/picric acid method (1912).

For mitochondria. Altmann's acid fuchsin according to Metzner's method (1928) after fixation in osmium tetroxide solution and postchroming.

Classical 'Golgi' techniques

(a) Weigl's Mann-Kopsch method (1910).

(b) Kolatchev's method (1916).

(c) Aoyama's method (1929).

For spheroids and lipochondria

(a) Baker's Sudan black method (1944) after fixation in formaldehyde-saline, formaldehyde-calcium, or Champy's fluid.

(b) Thomas's modification (1948) of the above for paraffin sections.

(c) Thomas's method of studying 'Golgi elements' by treatment in 1 per cent. osmium tetroxide and examination from time to time (1947).

Histochemical methods. Of the many tests tried, the following were the most useful in throwing light on the chemical composition of the structures studied.

(a) Baker's acid haematein and pyridine extraction tests for phospholipines (1946).

- (b) Cain's Nile blue method (1947) for acidic lipides.
- (c) Cain's plasmal reaction (1949).
- (d) Baker and Jordan's unpublished modification of the pyronin/methyl green method for nucleic acids.
- (e) Lison's chromaffine test; the Vulpian reaction; Sevki's method; the hexamine silver method of Gomori; all these for chromaffin and argentaffin granules (Pearse, 1953).
- (f) Gomori's chrome alum/haematoxylin-phloxine method (1941) for lipofuscins.
- (g) Schmorl's method (Pearse, 1953) for lipofuscins.
- (h) Schultze's method for cholesterol (see Pearse, 1953).

RESULTS

Nuclei (see fig. 1, A). The numerous nuclei were especially clearly seen in the thin, flattened parts of the living gland. Most of them were spherical or subspherical, and measured on the average 8μ in diameter in life. Scattered in the gland, but especially in the vicinity of the nerves, were larger, ovoid nuclei, measuring about 12μ in greater diameter, on the average. The smaller nuclei contain much chromatin and stain dark blue with pyronin/methyl green. The larger nuclei contain much less chromatin, which appears to be distributed in the form of threads staining pale blue with pyronin/methyl green. The two kinds of nuclei are shown in fig. 1.

Ground-cytoplasm. By phase-contrast this appears to be slightly heterogeneous in life, with faint striations. It stains pale pink with pyronin/methyl green.

Cells or syncytium? Cell-limits could not be seen during life by ordinary or phase-contrast microscopy. None of the methods intended to show cell-limits in fixed preparations succeeded in doing so in the corpus cardiacum, though Smith's Lyons blue/picric acid method (1912) was very successful in showing them in various other tissues. It appears that the corpus cardiacum of *Locusta* is a true syncytium.

Spheroids. The most obvious cytoplasmic inclusions are spherical or subspherical objects, which are easily seen in life, especially by phase-contrast microscopy (see fig. 1, B). The majority are about 0.6μ in diameter, though a fairly large proportion range up to about twice this diameter (fig. 1, D), and some even to 2μ . They appear bluish and are easily distinguished from the fat-droplets that sometimes get loose from the fat-body and become entangled in the surface of the gland, for the latter are yellow and always larger.

The spheroids show a perfectly black rim and pale interior under positive phase-contrast, as though they were provided with a cortex of high refractive index (fig. 1, B).

Supravital staining with dahlia violet at 1:10,000 colours the rims of the spheroids bluish-violet and their centres red or reddish-violet. The rim appears to be of slightly uneven thickness.

The other vital dyes tried did not stain the spheroids. Strangely enough, 0.01 per cent. neutral red is lethal to the syncytium, for the nuclei become strongly coloured in less than 15 minutes. The dye was tried at 0.003 per cent., at which concentration the nuclei were unaffected, but so also were the spheroids.

In classical Golgi preparations, made by the methods of Weigl, Kolatchev, and Aoyama, the spheroids became enveloped in a deposit of osmium or silver. In Weigl preparations they showed a clear interior and a thick black rim (much thicker than the rim seen by phase-contrast or after vital staining by dahlia). In some cases two or three spheroids were artificially joined together by a deposit of osmium. The most life-like appearance was given by the Kolatchev technique, post-osmication being restricted to 4 days at 34° C. In Aoyama preparations the spheroids usually appear to be blackened all through (fig. 1, c), though sometimes the silvering is restricted to their outer edges. Those who give the name of 'Golgi apparatus' to cytoplasmic inclusions that are not seen in routine microscopical preparations but are blackened by the classical Golgi techniques, will apply it to the spheroids of the corpus cardiacum.

The process of blackening by osmium could be well studied by treating the freshly dissected glands with 1 per cent. osmium tetroxide and examining at intervals. This method of Thomas's showed the gradual blackening of the edges of the spheroids, leading to the ultimate picture seen in the classical Golgi preparations. Recklinghausen's method also proved useful, for reduced silver appeared round the spheroids and displayed them very clearly.

When the organ was fixed in formaldehyde-calcium and coloured with Sudan black, dirty blue-black patches were seen in the cytoplasm. The sudanophil material presumably represented the spheroids.

The spheroids reacted positively to Baker's acid haematein test, but negatively after pyridine-extraction. They therefore may be taken to contain phospholipine. They are coloured blue by Cain's Nile blue method, a result that would be expected if they contained phospholipine. They also reacted positively to Cain's version of the plasmal reaction.

Tests for cholesterol, chromaffin material, and glycogen gave negative results.

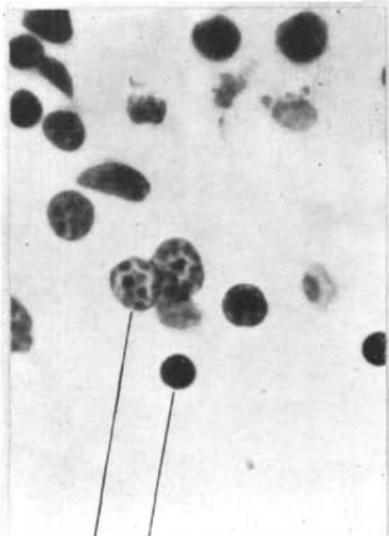
The spheroids showed no birefringence between crossed polaroids.

It seems probable that the spheroids represent the endocrine secretion of the gland.

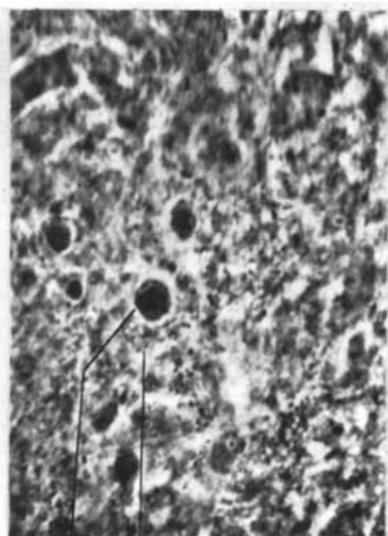
FIG. 1 (plate). Photomicrographs of the corpus cardiacum of *Locusta migratoria*.

- A, a pyronine/methyl green preparation, showing the two kinds of nuclei.
 B, a fresh preparation in saline, showing the spheroids, which here appear in optical section as small rings. (Positive phase-contrast.)
 C, an Aoyama preparation, showing the spheroids blackened by silver.
 D, a fresh preparation in saline, showing several of the minute granules. Most of the minute granules are considerably smaller than this and are in continual Brownian movement, so that they cannot be photographed. (Positive phase-contrast.)

10 μ



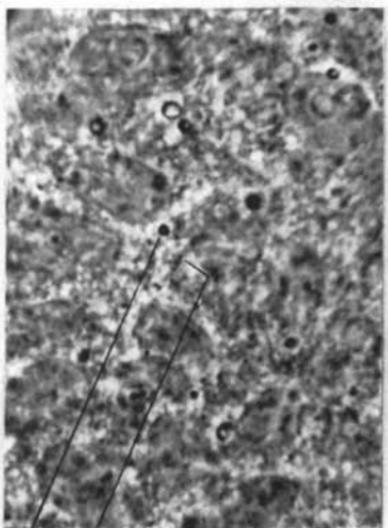
A
small nucleus
large nucleus



B
nucleus
spheroid



C



D
minute granule
large spheroid

FIG. 1
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Minute granules. The cytoplasm contains a large number of very minute granules, too small to be accurately measured. Some are larger than others. In life they are in active Brownian movement, and this, combined with their very small size, makes it difficult to photograph them in life. The larger ones, however, are sometimes immobile, and several of these are shown in fig. 1, D. When a single granule is accurately focused with phase-contrast, it appears black. These minute granules are stained bluish-violet by dahlia. They were not coloured by the other vital dyes tried, but, although unstained, they were more easily seen by ordinary microscopy after treatment with neutral red at 0.003 per cent. They were found not to be birefringent.

When the gland is fixed in a mitochondrial fixative and stained by Metzner's method, the cytoplasm is seen to contain a large number of minute red granules. These are presumably mitochondria, and it seems likely that they are the same as the minute granules seen in life.

Miscellaneous histochemical notes. Lison's test showed scattered chromaffin granules in the cytoplasm, but negative results were obtained with the Vulpian test and Gomori's hexamine silver method.

The chrome alum/haematoxylin-phloxine method of Gomori showed no positive reaction for lipofuscins in the gland, but in the vicinity of the nerves the Schmorl method revealed blue granules.

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