THE STRUCTURE OF THE RING-GLAND (CORPUS ALLATUM) IN NORMAL AND LETHAL LARVAE OF DROSOPHILA MELANOGASTER

BY BERTA SCHARRER AND ERNST HADORN

DEPARTMENT OF ANATOMY, UNIVERSITY OF CHICAGO, AND DEPARTMENT OF ZOÖLOGY, UNIVERSITY OF ROCHESTER

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In a recent paper (Hadorn, 1937a) it was shown that puparium formation in *Drosophila melanogaster* is brought about by a hormone. As the place of origin of this pupation hormone, an endocrine gland was described lying in the neighborhood of the brain. As long as no homology to other organs of that type in insects was found, this gland was called "ring-gland," as it is identical with the "Ring" of Weismann who in 1864 described it as a supporting structure for the dorsal blood vessel of *Calliphora vomitoria*.

Experiments with this ring-gland had the following results:

(1) Compared with normal larvae the puparium in the Drosophila mutation "lethal-giant" (*lgl*-) is formed later or not at all. This process can be accelerated by implantation of a mature normal ring-gland to *lgl*-larvae, but not by any other tissue (for instance brain) from genetically normal larvae.

(2) If ring-glands from normal larvae ready for pupation are implanted into younger normal larvae those will show a premature puparium formation. The degree of acceleration depends upon the time of injection and also upon the number of glands injected, three being more effective than one. The same accelerative effect can be obtained with mature normal ring-glands of *Drosophila melanogaster* in larvae of the more slowly developing *Drosophila hydei* and in lethal male crosses of the combination *Drosophila melanogaster* × *Drosophila simulans* (Hadorn, 1937b, Hadorn and Neel, 1938).

In the present paper the histological structure of the ring-gland was studied, the following questions being of particular interest:

(1) Is there histological evidence for the secretory activity of the ring-gland cells?

(2) Do ring-glands of old and young normal larvae (temporarily or permanently) show a different appearance indicating perhaps a functional cycle?

(3) Can normal ring-glands be differentiated from lethal ones?

(4) Is the ring-gland of Drosophila homologous with the corpora allata found in other insects?

1. *Material.*—As seen in table 1, normal and lethal larvae (*lgl*-) of *Drosophila melanogaster* were preserved at different ages in Bouin, Zenker-formol

or Champy. The material was embedded in celloidin, paraffin sections being used only as control material. The 5 μ serial sections (Rubaschkin method) were stained with Heidenhain's iron hematoxylin, Mallory-azan or van Gieson.

TABLE 1

AGE OF LARVA	GENETIC CONSTITUTION OF MATERIAL	NUMBER OF INDIVIDUALS INVESTIGATED
1 day	normal + *lgl*-larvae together	5
1 day + 19 hours	normal + *lgl*-larvae together	4
2 days + 22 hours	normal larvae	4
	lgl-larvae	4
3 days	normal + *lgl*-larvae together	3
3 days + 16 hours	normal larvae	4
	lgl-larvae	3
3 days + 22 hours	normal larvae	6
	lgl-larvae	2
4 days + 4 hours	normal larvae	4
	lgl-larvae	1
4 days + 16 hours	normal larvae	4
	lgl-larvae	1
4 days + 18 hours	normal larvae	4
	lgl-larvae	1
4 days + 22 hours	*lgl*-larvae	6
5 days + 5 hours	*lgl*-larvae	5
5 days + 20 hours	*lgl*-larvae	2
6 days	*lgl*-larvae	4

2. *Appearance of the Ring-Gland of a Normal Larva Ready for Pupation.*—As shown before in a diagram (Hadorn, 1937a, p. 480, Fig. 2), the ring-gland is located dorsally between the two hemispheres of the cerebral ganglion. Its longitudinal diameter is 200 μ, the largest transverse extension 130 μ and the depth 30 to 40 μ. As described in other Diptera by former investigators (Giacomini, 1900, Snodgrass, 1924), the "ring" is anchored in its position by tendinous structures. Tracheae penetrate and pass through the ring-gland; the cells surrounding them (imaginal bud cells, Burtt, 1937) can easily be differentiated from the gland cells. Two longitudinal tracheae arising from the cerebral hemispheres take their way forward and outward within the ventral part of the ring-gland. As

in Calliphora (Burtt, 1937), these two tracheal stems are connected by a transverse trachea. Significant is the fact that the ring encircles the aorta, the main part of the gland tissue being closely attached to the dorsal part of the vessel, whereas ventralward the ring becomes much thinner and ends more or less incompletely. The aorta itself can be traced from the heart to its termination in front of the ring-gland. The cells forming the thin wall of the vessel are thickened at those points where the oval-shaped nuclei are located. Sometimes one can observe in suitably stained sections that these cells contain a few delicate myofibrils showing a transverse striation; but only the part of the aorta posterior to the ring seems to be contractile. According to Giacomini (1900) the anterior portion of the dorsal vessel is incomplete in *Eristalis tenax*, consisting only of the upper half, whereas the lower lip ends immediately in front of the ring. The same is true in Drosophila; here this dorsal portion of the vessel is exceedingly thin, containing only comparatively large nuclei and ending in a membranous structure. Sometimes blood cells are found in the vessel especially in its frontal enlarged region.

To establish the relationship between ring-gland and stomatogastric nervous system is more difficult in Drosophila larvae than in other less tiny Diptera. One can trace the recurrent nerve passing forward and forming a small ganglion (evidently homologous to the median ganglion of Lowne, 1890–95, and Burtt, 1937), which is in contact with the anteroventral portion of the ring.

The cells forming the ring-gland are of two different types which can be differentiated by their size as well as by their position. The main part of the ring is built by large cell elements (about 15–40 μ in diameter) which are very characteristic. Their cytoplasm is deeply staining and shows a more or less granular structure. In some of the cells vacuoles of different size and irregular form can be found. The cell boundaries are easily distinguished in most of the preparations. The large nuclei of those elements are round or oval and have diameters from 10 to 17 μ; they contain one or two nucleoli (about 3 μ in diameter). The chromatin is arranged in more or less distinct chromosome-like bodies as, for instance, in Chironomus (Burtt, 1937). Thus these cells are similar in their general appearance to the elements of the salivary glands.

The cells of the smaller type form the central part of the ring-gland surrounding the anterior region of the ring-hole, and thus lying in the direct neighborhood of the dorsal vessel. Especially in its anterior part, this cell group shows a very intimate contact with the thin dorsal lip of the aorta. The area occupied by these smaller cells is about 60 μ long (from the ring-hole oralward in the anterior direction), 30 μ wide and 30–40 μ deep. In some preparations (azan) a very delicate connective tissue membrane can be observed surrounding this cell group as well as the whole ring. In a full

grown ring-gland of a mature larva one may count about 20 cells of that type. The smaller cells of the ring are in their general aspect similar to its larger elements. Their nuclei are round (5–6 μ in diameter) and have one nucleolus. As in the large ring cells, the nucleolar membrane is distinct. The cell diameters are from about 8 to 20 μ. The cytoplasm, being less vacuolated, stains somewhat deeper than that of the large cells. Nerve cells as described by Burtt (1937) in *Calliphora vomitoria* have not been found in the ring-gland of Drosophila. No direct histological evidence could be brought forward until now for a glandular activity of the ring-gland cells (i.e., presence of granules or colloid substances in the cytoplasm, etc.). But the general appearance of those cells (affinity of the cytoplasm to stains, vacuolization, large size of nuclei and their large amount of chromatin) do at least not speak against a secretory activity.

3. *Homology of Ring-Gland and Corpora Allata.*—Already in the first paper on Drosophila (Hadorn, 1937a) the question of a homology between ring-gland and corpora allata has been discussed. At the same time Burtt (1937) working with *Calliphora vomitoria* concluded that Weismann's ring represents a modified corpus allatum. He did not, however, prove this assumption by an embryological study, but he gave a comparative survey emphasizing the great variability in shape, size and position of the corpora allata not only within the insects but even within the Diptera. As yet the embryological development of the ring-gland in Drosophila has not been studied, this small species not being the most favorable object among the dipterous insects. But on the basis of a histological study of different postembryonic stages we came to the same result in Drosophila as Burtt in Calliphora. Among further morphological correspondences between ring-gland and corpora allata, we may mention their position in the body and relationship to other organs (especially to the dorsal vessel and to the tracheal system) and the histological properties of their elements (affinity to plasma stains, granular cytoplasm containing vacuoles, appearance of the nuclei, etc.).

There is one point more to be discussed concerning the two different cell types in the ring-gland of Drosophila. As mentioned above, the smaller cell elements have a well circumscribed position within the ring, being even separated from the large gland-cells by a fine membrane. Therefore the question arises whether this well defined central cell group forms a special part of the corpus allatum or must be separated from it as a different structure. It is very probable that the small ring-cells represent the corpora cardiaca (Pflugfelder, 1936–37, p. 47; esophageal ganglia, Burtt, 1937; pharyngeal ganglia of other investigators). Several reasons speak in favor of such an assumption:

(1) The name of corpora cardiaca was proposed by Pflugfelder for the "pharyngeal ganglia" of other authors, because of their intimate re-

lationship to the blood vessel system; in some insects (for instance in Nabis, Rhynchota) the corpora cardiaca represent even a modified and thickened wall of the heart or aorta, respectively. In Drosophila the close contact between the small ring-cells and the thin membrane of the dorsal vessel is obvious.

(2) Another reason for discarding the former name of pharyngeal or esophageal ganglia is that, at least in part of the insects, their purely ganglionic nature can no longer be maintained. Pflugfelder (1937a and b) showed that for instance in *Dixippus morosus* part of the cells of the corpora cardiaca contain (secretory or excretory?) osmio- and fuchsinophile substances. The small cells of the Drosophila ring have a glandular appearance rather than a nervous.

(3) The position of the corpora cardiaca in a number of insects (for instance in Syromastes, Rhynchota) is closely oralward of the corpora allata, with only a thin membrane separating these structures from each other. One may easily assume that in Drosophila the corpora allata on the basis of their special development have grown around their corpus cardiacum, thus building an even more intimate connection. The thin membrane found between the smaller and larger ring cells has already been mentioned.

(4) From other insects we know that the cell elements of the corpora cardiaca are smaller than those of the corpora allata.

(5) There has not been found as yet another organ in cyclorraphous insects which could be homologized with the corpora cardiaca.

In this connection we may ask what conditions we find in the other cyclorraphous insects. In the ring of Lucilia (we have studied only two specimens to date) we found also two cell types of different size and appearance, but they are not so separated from each other as in Drosophila. According to Burtt (1937, p. 21, Fig. 6) the same seems to be true for *Calliphora vomitoria*. Whether these smaller cells scattered in the ring-glands of Lucilia and Calliphora correspond to the group of small elements in the Drosophila ring cannot be decided at the present. In the same way the question of a homology between the small ring-gland cells and the corpora cardiaca will remain uncertain as long as we do not have a more detailed study of those organs in different groups of insects.

4. *Development of the Ring-Gland in Normal and Lethal Drosophila Larvae.*—In young larvae (age: 1 day, 1 day and 19 hours) the ring-gland elements (diameter of nuclei 3 μ) cannot yet be differentiated with certainty from the surrounding tissues, especially from the salivary glands. Normal larvae aged 2 days and 22 hours show already a typical ring. The ring-gland cells in this stage as well as in 3 day old larvae are to be distinguished from those of older ones by their size, the diameters of the nuclei being only from 6 to 8 μ. The smaller cells are already present in the center of the ring, but they do not differ so much in size from the large ones as later. In

three day old larvae the diameters of the small cell nuclei are from 4 to 5 μ. At the age of 3 days and 16 hours the ring-glands as well as their elements have reached their full size (Fig. 1) and now do not differ from the oldest normal stages described above.

Accordingly we may state that the growth of the ring-gland during the larval development of Drosophila takes place by an increase in the size of the cells, but not by a considerable increase in their number. That is why mitoses are to be found only exceedingly rarely in the ring-gland cells.

Also in the *lgl*-larvae the ring-gland has reached its final size at the age of 3 days and 16 hours but there is one striking difference in contrast to normal larvae. The ring-glands of *lgl*-larvae are considerably smaller than the normal. Even in 5–6 day old *lgl*-larvae (Fig. 2) the length of the ring is only about 120 μ, the width 70 μ and the depth 30 μ. As we may expect from what was said about the growth of the normal ring, the comparative smallness of the lethal ring-gland is due to the fact that the cells are smaller than normal. The nuclei of these large ring cells do not reach greater diameters than from 7 to 10 μ. In this connection it must be emphasized that an *lgl*-larva is as a whole not smaller than a normal one of the same age. Besides the size there have been observed no other marked differences between normal and lethal ring-gland cells, at least not with the technique used and on the basis of the present material. The small cell type in a lethal ring-gland does not differ in size from that in normal ones, neither are those cells less in number.

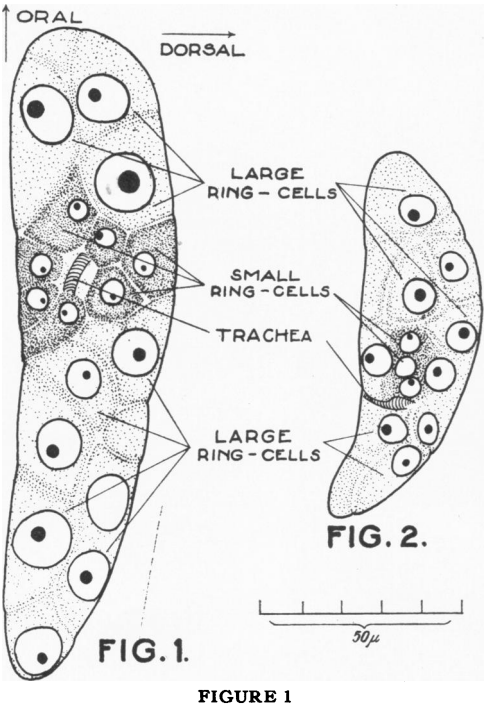

FIGURE 1

Paramedian sagittal section of ring-gland (corpus allatum) of a normal Drosophila larva (age 3 days and 16 hours). Camera lucida drawing.

FIGURE 2

Corresponding section of a lethal (*lgl*-) larva (age 5 days and 5 hours).

From the experiments of Hadorn (1937a) it was concluded that an *lgl*-

ring-gland does not furnish enough hormone to bring about puparium formation at the right time. The histological statements reported above may explain this fact. An *lgl*-ring-gland never grows to full size and apparently does not as a rule reach the hormone producing phase. It remains rather at a stage typical for a young normal larva of about 3 to $3^1/_2$ days. Unpublished transplantation experiments of Hadorn and Neel show that it is impossible to promote puparium formation with unripe glands even if they are of genetically normal constitution.

We are, however, not allowed to assume that an *lgl*-gland does not produce any hormone at all, since many of the lethal larvae later may form puparia. It is rather possible that an *lgl*-ring-gland, in spite of its underdeveloped histological structure, furnishes a small amount of hormone which reaches the necessary threshold only with great retardation.

A more detailed discussion of the literature will be given in another paper.

5. *Summary.*—The finer structure of the ring-gland (Weismann's "Ring") in *Drosophila melanogaster* is described. For the secretory activity of the ring-gland cells (pupation hormone) as suggested by Hadorn (1937) no histological evidence could be established. The ring-gland, or at least its greater part (large cells), is homologous to the corpora allata found in other insects. The question of a homology between the small ring-cells and the corpora cardiaca (Pflugfelder) is discussed. During the larval development of Drosophila the growth of the ring-gland (corpus allatum) takes place by an increase in the size of the cells. In the grown-up *lgl*-larvae the ring-gland as well as its cell elements is considerably smaller than normal. It is therefore concluded that the retardation in the puparium formation of the *lgl*-larvae is caused by an underdevelopment of their corpora allata.

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