AN ACCELERATING EFFECT OF NORMAL "RING-GLANDS" ON PUPARIUM-FORMATION IN LETHAL LARVAE OF DROSOPHILA MELANOGASTER

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In several groups of insects it has recently been shown that hormones are concerned in moulting, pupation and metamorphosis (for literature, see Bodenstein 1936, Wigglesworth 1937). In connection with these problems the mutation "lethal-giant" in Drosophila melanogaster is of a special interest, since here there is a cessation of development at the beginning of pupation (Hadorn 1937). This paper deals with the question of whether it is possible to influence the process of puparium-formation of lethal larvae by injecting into them different tissues from genetically normal individuals. Since the study of this problem has led to the finding of a special "pupation-gland" the experiments may contribute not only to the physiology of lethals but also to the general physiology of insects.

1. Material.—The gene lethal-giant (lgl-Chrom. 2, 8±, found and located by Doctor C. B. Bridges, to whom I am grateful for unpublished information and for stocks) is kept in the balanced stock lgl cn bw sp²/Cy. In such a balanced stock three types of fertilized eggs are formed: (1) Cy/Cy, (2) lgl cn bw sp²/Cy and (3) lgl cn bw sp²/lgl cn bw sp². Individuals, homozygous for the Curly-chromosome (Cy) die either in the egg or as small larvae. Individuals homozygous for lgl grow through the three larval instars. The combination of cinnabar (cn) and brown (bw) in homozygous condition is characterized by almost colorless Malpighian tubes (Beadle 1937). On the basis of this character the homozygous lgl larvae of the third instar can be distinguished and separated from the non-lethal heterozygotes of the same culture (lgl cn bw sp²/Cy), which have yellow Malpighian tubes. Eggs were collected at two-hour intervals, so that the age of the larvae of any experimental series was known within this limit.

2. Puparium-Formation in Untreated lgl Larvae.—When a larva of normal genetic constitution is ready for pupation, it everts its anterior spiracles and ceases to move. The cuticle then becomes hardened and darkened and develops the characteristic pupal shape. Within such a puparium the real pupa develops by evagination of the imaginal discs.

In larvae homozygous for lgl, puparium-formation may occur in a morphologically normal manner. But since the imaginal discs have degenerated at this time, no pupae can be formed within the puparia. We may call such a formation a "pseudopupa" (Hadorn 1937). With respect to pu-
parium-formation, lethal and normal larvae differ considerably in the time at which this process takes place. Normal larvae, in this case also the heterozygotes, pupate during the 5th day after the egg is laid ($25^\circ$C.). The $lgl$ larvae never start to form puparia before the beginning of the 7th day and there is a great variability among individuals of the same age. The second curve in figure 1 shows a percentage-distribution of a group of 220 individuals. Only 50% of them have formed puparia at $8^{1/2}$ days. On the 11th day 10% are still in the larval stage. Some of these form puparia later, while others never do so at all. In such cases the larval life may be extremely long (up to 25 days). The shape of the puparium is not always normal. There may be incomplete eversion of the spiracles, or flattening of the dorsal head skin may not occur.

3. Influence of an Implanted Genetically Normal “Ring-Gland.”—Though puparium-formation of Calliphora has been shown to be dependent on a hormone secreted somewhere in the head (Fraenkel (1935)), in the present case the question arises as to whether the delay in puparium-formation observed in the lethals is due solely to a lack of the necessary hormone or to a more general retardation involving both the acting and the reacting organs. An attempt was therefore made to find an organ of normal constitution which could act as a source of a pupation hormone. Of the different tissues tested by transplantation experiments none but a small body which is located dorsally between the two hemispheres of the “brain”
(supra-oesophageal portion of the central nervous system) had an accelerating effect of the formation of puparia in lethals.

This organ was described first by Weismann (1864) in his classical work on the postembryonic development of the Muscidae; he called it the "Ring" and suggested that it might function as a supporting structure for the "dorsal vessel" which goes through the ring. Many later investigators do not mention the ring at all. Lowne (1890-1895) states that in Calliphora the ring grows rapidly in "resting larvae" preparing for pupation.

In *Drosophila melanogaster* I have found this ring to be similar in shape to that reported by Weismann for *Calliphora vomitoria*. Figure 2 shows its typical form and location with respect to the larval central nervous system. A detailed description of its structure and development cannot be given here. But it may be stated that the ring has a characteristic histology which makes it easy to identify in sections. The cells and nuclei are large and the cytoplasm deeply staining. The pattern formed by the tracheae which penetrate the ring is so characteristic that the latter may be located

![Diagram of Ring-Gland, Tracheae, and Hemisphere](image-url)
without difficulty. It is easily separated from other tissues by means of dissecting needles.

Since it has been shown that the ring has a glandular function, the term 'ring-gland' may be used so long as no homology to other structures in other insects has been established.

**Experiments.**—Using the method of Ephrussi and Beadle (1936) ring-glands from normal larvae were transplanted to a total of 161 lgl larvae. Hosts and donors were taken from the same 2-hour group; the donors were almost ready for puparium-formation. The glands were injected into the posterior portion of the larvae. Cultures were kept at 25°C. The experiments were run in rather small series. The time after operation at which puparium-formation for the different individuals occurred was noted and this was compared with the time of puparium-formation in non-injected control series of lgl larvae from the same 2-hour group (table 1, a and b). Observations were made every four hours during the day. Table 1 shows

<table>
<thead>
<tr>
<th></th>
<th>Number of Individuals</th>
<th>Interval in Hours Between Time of Implantation and Puparium-Formation of First Puparium</th>
<th>Percentage of Individuals in 50%</th>
</tr>
</thead>
<tbody>
<tr>
<td>(a) lgl with implanted normal ring-gland</td>
<td>161</td>
<td>14</td>
<td>15-23</td>
</tr>
<tr>
<td>(b) lgl controls without implant</td>
<td>220</td>
<td>12</td>
<td>30-79</td>
</tr>
<tr>
<td>(c) lgl with various implants, exclusive of normal ring-gland</td>
<td>123</td>
<td>13</td>
<td>41-80</td>
</tr>
</tbody>
</table>

first the data for the time when in the different series the first puparium was observed. The time given for the untreated control series (b) is measured from the time when the experimental series (a) were injected. The difference between the two means is 46.4 times its standard error and there is no overlapping even for the maximal range. The table shows further the figures for the time interval which elapsed before puparium-formation took place in 50% of the individuals in each series. Here again the difference between the two means is statistically significant (36.5 times its standard error). Curves 1 and 2 in figure 1 demonstrate percentage-distributions of all series. It can be seen that 50% of the injected lethals formed puparia before any of the 220 controls started. From the later course of the curves it can be seen that 75% of the injected larvae had formed puparia at 7½ days after egg-laying, while the untreated controls required 9½ days to reach the same percentage of puparia. For the last 25%, the difference between the two groups is smaller. Although puparium-formation took place in certain series in 100% of the cases, however, for the injected
group as a whole at the 11th day 5% of larvae were still present. Some of these formed a puparium later; others died as larvae in the manner described for the non-injected lethals. As there is always a possibility that the operation may not be successful, we can assume that some or possibly all of the individuals which formed puparia later or not at all, did not contain an implant.

4. **Implantation of Other Tissues.**—Since in Lepidoptera the "brain" is supposed to be the source of a pupation hormone (Kopeč 1922; Kühn and Piepho 1936), it is important to test its effect on *lgl* larvae. In 36 such individuals a "brain" (hemispheres) **without the ring-gland** was injected with the result that no accelerating influence whatsoever was observed. The injected larvae formed puparia even later than the average of the non-injected *lgl* controls. Together with the "brain" was injected either the eye-antennae-disc or the sub-oesophageal part of the central nervous system, so that we also know that these structures have no influence.

In a second group of 42 individuals the implant consisted of a large portion of the *fat body containing an ovary*. Here again no acceleration of puparium-formation was observed. The same lack of effect was found for *salivary glands* (22 cases).

The data reported above show that the acceleration of puparium-formation is not a general and non-specific effect of any tissue of normal constitution, but is due to a specific activity of the ring-gland. The question now arises as to whether the presence of a supernumerary ring-gland, regardless of its genetic constitution, causes the effect. This question was answered by injecting *ring-glands from lethal* donor larvae into lethal hosts. Since in a total of 23 individuals no acceleration was observed we conclude that the host and implanted *lgl* ring-glands together either do not furnish a sufficient amount of hormone or do not produce any at all during the time when the normal gland is active. In table 1 and curve 3 of figure 1, all the different injected series which did not contain a genetically normal ring-gland are grouped together. A comparison with the data and curves of the two other groups shows that, where anything other than a normal ring-gland is injected, puparium-formation occurs even later than in the untreated controls. This is true for most of the single series as well as for the group as a whole. It is obvious that, whatever the influence of the operation as such may be, it would not accelerate puparium-formation but rather retard it. Therefore, it is concluded that the accelerating effect obtained with normal ring-glands is entirely the result of an activity of the injected cellular material.

5. **Discussion.**—The experiments show that the retardation of the process of puparium-formation characteristic of the homozygous *lgl* larvae is not due to an inability of the reacting material (larval skin) to undergo
changes typical for pupation. The puparium can be formed in a lethal larva after a genetically normal ring-gland has been present for 15–23 hours. The retardation in the untreated lethals therefore seems to be the consequence of the absence of some substance normally secreted by the ring-gland.

A detailed histological study is needed to determine whether there are temporary or permanent differences between ring-glands of normal and lethal larvae. At present we can say that there is no indication of cellular degeneration in a lgl ring-gland at the end of the 5th day, while at this time the imaginal discs of lgl larvae have completely degenerated.

Since most of the untreated lethal larvae are able to form puparia, although this process is greatly retarded, the difference between the action of the lgl gene and its normal allele, so far as the process of puparium-formation is concerned, is perhaps quantitative. This difference may be of such a nature that the amount of hormone which is necessary for puparium-formation reaches the required threshold at different times in the two classes of larvae. However, we do not know whether the ring-gland of the lethals begins to secrete later in development than does a normal ring-gland or whether it becomes functional at the same time but produces the substance in a smaller amount. In either case the threshold would be reached later than in normal larvae. On the basis of such a quantitative interpretation an implanted normal ring-gland would supply to the lethal host enough hormone to bring it to threshold concentration earlier than in the untreated lethals.

Since the imaginal discs of lgl larvae were quite degenerate at the time normal ring-glands were implanted, no development beyond the puparium-formation was possible; we therefore do not yet know whether the ring-gland normally has any other activity besides its influence on puparium-formation.

Fraenkel (1935) showed by means of constriction experiments that only those parts of a Calliphora larva which contained the "brain" region pupate when the operation is made during a certain larval stage. Bodenstein (1936) briefly reported similar results obtained on Drosophila. Fraenkel concluded that either the "ganglion" (central nervous system) or something in its immediate neighborhood furnished the pupation hormone. Since the experiments reported in the present paper prove that for Drosophila the ring-gland and no other parts of the brain region can accelerate puparium-formation, it is probable that the ring-gland is also the source of the pupation hormone in Fraenkel's experiments.

Although Fraenkel claims "there exists no organ in the fly larva which can be homologized with the corpora allata in other insects," it should be emphasized that the ring-gland of Drosophila to a certain extent agrees in location and histology (Nabert 1913) with the corpora allata in different
groups. An embryological study is necessary to decide whether the two organs are homologous.

Summary.—Lethal larvae of the mutation \textit{igl} in \textit{Drosophila melanogaster} form puparia, but this process is greatly retarded as compared with normal larvae. It is shown that puparium-formation can be accelerated by transplantation of a normal ring-gland to lethal larvae. There is therefore evidence that the ring-gland is an organ of internal secretion and produces a pupation hormone.

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\textit{FREQUENCY OF INDUCED BREAKS IN CHROMOSOMES OF DROSOPHILA MELANOGASTER}

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One of the effects of x-radiation of living cells is the production of chromosome breaks. When such a break takes place detectable genetic changes frequently occur at the point of breakage. This coincidence is so high that the suggestion has been offered that a break in a chromosome may result from a change in a gene.\textsuperscript{1} To test this hypothesis a study has been made of the frequency of breakage of the \textit{Y}-chromosome and the autosomes of \textit{Drosophila melanogaster}. Genetic data suggest that the \textit{Y}-chromosome of this species is essentially inert as compared with the \textit{X}-chromosome and the autosomes. Cytological studies show that the \textit{Y} is heteropyknotic during interphase and prophase stages of mitosis\textsuperscript{2} and that it is represented in salivary gland nuclei by a small mass of heterochromatin composed of very few bands.\textsuperscript{3} If a change in a gene is responsible for a break in a chromosome or favors the occurrence of such breaks, it is to be expected that the