THE EFFECTS OF THE CEPHALIC COMPLEX UPON THE EYE DISCS OF DROSOPHILA MELANOGASTER

(CONDENSED TITLE: EFFECT OF BRAIN UPON EYE DISC)

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Many insects reach adult stage after passing through some metamorphic processes such as larval and pupal stages, which are hardly seen in other animals. A number of investigations in the field of physiology of metamorphosis showed that some of these metamorphic developments such as molting, pupation, or emerging were accelerated or inhibited by a qualitative or quantitative difference of hormones secreted from the central nervous system and other endocrine organs. In Drosophila, the destruction of many larval organs takes place with the organization of adult structures from the imaginal discs during the post larval and whole pupal stages. It stands to reason that the presence of the cephalic complex— involving the brain hemispheres, the ventral ganglion, and the ring gland—is very significant in the development of the imaginal discs.

In the last century, Rabl-Rückhard (1875) observed the blind worker of the African ant, Typhlopone, which was found to have no optic ganglia. Holmgren (1909) reported that reduction in the optic ganglion of the worker caste of the termite, Eutermes chaquimayensis, was accompanied by a corresponding reduction of the compound eye, and that the soldiers of this species have no eyes at all and the optic ganglia were completely absent.

In Drosophila melanogaster, Kafka (1924), who studied the development of the compound eye, noticed that the optic ganglion of Bar mutant was seen to be less than half as great in diameter, compared with that of the normal fly. Richards and Furrow (1925) also reported that the inner glomeruli of eyeless mutant was contracted into a more or less shapeless mass. A similar but detailed study on heterozygous Bar females, Bar males, homozygous Bar females, and double-Bar males of D. melanogaster was reported by Power (1943), who found that reduction in ommatidia of the compound eye was correlated with reduction in the inner glomerulus, middle glomerulus and external glomerulus. Power (1946) also showed that there was a dependence of one sensory area upon another, for the normal volumetric development of the antennal glomeruli occurred only when there was full development of the optic glomeruli.

As described above, many studies state that the character of the compound eye of adult insect is correlated with the cephalic complex. Therefore the size of the eye controlled by certain genes seems to accept some genic actions through the cephalic
complex—organs secreting the metamorphic hormones—, for the compound eye of adult insect is formed during the post larval and whole pupal stages, under the hormonal control of the cephalic complex. In other words, the gene controlling the character of the eye may express the primary action on the cephalic complex, then the secondary on the eye disc.

To ascertain the genuineness of an application of this principle, the extirpation and transplantation methods of the eye disc or the cephalic complex seem to be useful, but they do not give any definite evidence, because of the remaining and mixing of the substance secreted from the cephalic complex in the body fluid.

In the present work, a morphological mutant strain concerned with the eye of *D. melanogaster* was used to culture their eye disc in the synthetic medium, which was devised by Kuroda (1954 a, 1954 b, 1954 c, 1954 d), investigating the direct effect of the cephalic complex cultured together.

**Materials and method**

A wild strain (*Oregon*), and an eye mutant strain (*Bar ; 1-57.0*), of *D. melanogaster* were used as donors for culture.

These were isogenic strains made through several generations of one to one matings. About twenty females and twenty males of the abult flies were put in a glass tube (3 cm in diameter by 12 cm in length), in which a slide glass placing a tracing paper soaked in yeast solution was inserted. Eggs newly laid on this tracing paper were gathered and sterilized with 0.05% HgCl₂ solution dissolved in 70% ethyl alcohol for 30 minutes, then transferred to autoclaved bottles containing food. The components of the food mixture were malted rice, sugar, peptone, yeast powder, and

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agar. Third-instar larvae grown at 25°C under these sterile conditions were transferred to sterile physiological salt solution (Carlson's formula, 1946) on a depression slide, and were dissected to take out their eye-antennal discs and the cephalic complexes under a binocular microscope in a glass sterilizing chamber. The eye-antennal discs and the cephalic complexes were cultured at 25°C in hanging drops by the cover slip method. The components of the synthetic medium used for culture are shown in Table 1.

These substances were dissolved separately into ten groups, making the basic stock solutions, which were stored at 0°C. Appropriate mixtures of these stock solutions with pH adjusted to 7.2 were sterilized by filtration through a Seitz filter.

Experimental results

1. Comparison of the eye-antennal disc cultured alone with that cultured together with the cephalic complex.

The eye-antennal disc taken out from the mature third-instar larva (95 hours old after hatching at 25°C) of the Oregon strain was observed to grow after culturing in the synthetic medium for 17 hours. At the beginning of the culture (Fig. 1a), the antennal disc anterior was clearly distinguishable from the eye disc posterior, some concentric folds in the former disc, and both lateral and anterior folds in the latter disc being observed. In the posterior half portion of the eye disc near the eye stalk, the cell groups that would differentiate to the ommatidia, the so-called "cell clusters" were observed as still undifferentiated state. After 17 hours, the concentric folds in the antennal disc showed thickening and forming more or less winding layers, whereas the "cell clusters" in the eye disc were observed to differentiate strikingly (Fig. 1b).

When the eye-antennal disc of the Oregon larva was cultured together with the cephalic complex of the Oregon strain in the same preparation, more marked increase and higher differentiation were observed in the eye disc. In comparison with the eye disc at the beginning of the culture (Figs. 2a, 3a), after 17 hours' culture (Figs. 2b, 3b), thickening of the concentric folds in the antennal disc was observed as well as on culturing the eye-antennal disc alone, whereas both lateral and anterior folds in the eye disc showed thickening in width. In the posterior portion of the eye disc near the eye stalk, the ommatidial cell layers that had differentiated from the "cell clusters" were very clearly observed. In this portion, the appearance of the compound eye was almost completed. The peripheral cells of the disc was not observed to loosen as done on culturing the eye-antennal disc alone, a firm connected state of the disc tissue being maintained.

More than two cephalic complexes of Oregon larvae brought more pronounced growth and differentiation on the eye disc of the Oregon larva.
2. Comparison of the effect of the cephalic complex of Oregon with that of Bar upon the eye-antennal disc of Oregon.

The results obtained in the culture of the eye-antennal disc of the Oregon together with the cephalic complex of the Oregon were as described above (Figs. 2, 3). When the eye-antennal disc of the Oregon was cultured together with the cephalic complex of the Bar, compared with the eye-antennal disc at the beginning of the culture (Figs. 4a, 5a), after culturing for 17 hours (Figs. 4b, 5b), the growth and differentiation in the antennal disc were observed as done with the cephalic complex of the Oregon, whereas the growth in the eye disc was markedly different from culturing with the cephalic complex of the Oregon. The eye disc showed an increase towards the antero-posterior axis rather than the lateral axis, and consequently a wide winding form. The "cell clusters" were observed to form some ommatidial cell layers also in this case. These results seem to indicate that even the eye disc of the Oregon larva performed the development of the eye disc of the Bar larva, when the former was cultured together with the cephalic complex of the Bar larva.

3. Comparison of the effect of the cephalic complex of Oregon with that of Bar
The eye-antennal disc of the Bar larva is smaller in size than that of the Oregon larva, and especially in the posterior portion of the eye disc of the Bar, less "cell clusters" was observed (Fig. 6a). When the eye-antennal disc of the Bar was cultured for 17 hours together with the cephalic complex of the Oregon (Fig. 6b), the posterior eye disc showed a striking increase in size, and the ommatidial cells that had differentiated from the "cell clusters" were observed to form the compound eye. These experimental results show that even the eye disc of the Bar larva performed the development of the eye disc of the Oregon larva, when the former cultured together with the cephalic complex of the Oregon larva.

In the eye-antennal disc of the Bar larva cultured together with the cephalic complex of the Bar larva, compared with that at the beginning of the culture, after 17 hours, the growth in the antennal disc was marked, whereas the anterio-posterior growth in the eye disc was hardly recognized. Thus the eye disc showed the more characteristic developmental growth and differentiation of the Bar strain in the presence of the cephalic complex of the Bar larva.

Discussion and conclusion

It was reported that when the eye-antennal disc as connected with the cephalic complex was cultured, the growth and differentiation of the eye disc was more pronounced than culturing the eye-antennal disc alone (Kuroda, 1954b). To solve that the promotion of the growth and differentiation of the eye disc is due whether to the nervous stimulus of the cephalic complex, or to its hormonal substances, the eye-antennal disc was cultured together although unconnected with the cephalic complex in the same preparation, comparing with culturing the eye-antennal disc alone. When the eye-antennal disc was cultured together with the cephalic complex of the Oregon larva or the Bar larva, it showed more thickening of the folds in the eye disc and more differentiation to the ommatidial cells from the cell clusters. Since on culturing the eye-antennal discs alone of the Oregon and the Bay strains, in the respective discs the growth as the development of the Oregon and the Bar strains was observed, it was suggested that the growth and differentiation of the eye disc had been determined in the mature third-instar larva (Kuroda, 1954d). In the present work, however, it was found that the cephalic complex of the Oregon larva had an effect of increasing in the ommatidial-forming portion as towards the development of the Oregon strain even upon the Bar eye disc as well as upon the Oregon eye disc, whereas the cephalic complex of the Bar larva had an effect of performing as the development of the Bar strain to the Oregon eye disc as well as the Bar eye disc. Therefore the eye disc taken out from the mature third-instar larva and cultured alone is assumed to perform the development of the donor strain by the remaining effect of the cephalic complex.

By the additional culture of the cephalic complex, however, the growth and
differentiation of the eye disc is placed under the control of that cephalic complex. In the cephalic complex one of the organs concerned with promoting the eye disc seems to be the so-called "ring gland" that is located dorsocephally between the two brain hemispheres of the larve and that controls, by hormonal action, molting, metamorphosis, organ growth, and apparently also general metabolic activities in *Drosophila* (Hadorn and Neel, 1938; Bodenstein, 1943a, 1943b, 1944; Vogt, 1942a, 1942b, 1943). Hormone secreted from the "ring gland" is assumed to be controlled by the "adenotropes" hormone secreted from the neurosecretory cells of the brain.

The brain and the "ring gland" of the cephalic complex cultured together with the eye disc in the synthetic medium seem to give the hormonal effect directly or indirectly to the development of the eye disc. Therefore it is assumed that the genic action appears first on the brain and the ring gland, which then influence the growth and differentiation of the eye disc.

In the present work, the attention was directed towards the appearance of only eye-antennal discs cultured together with the cephalic complexes, to investigate the effects of the latter upon the former. It is, however, possible to assume that the detectable change might occur also in the cephalic complex itself. The interrelation between the eye-antennal disc and the cephalic complex is in anticipation of the future work.

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**Summary**

1. The eye-antennal discs from the mature third-instar larvae (95 hours old after hatching at 25°C) of *D. melanogaster* were cultured in *vitro* in a synthetic medium, to investigate some effects of the cephalic complexes cultured together.

2. In comparison with culturing the eye-antennal discs alone, the culture of the eye-antennal discs together with the cephalic complexes showed more pronounced growth and differentiation of the eye discs.

3. The cephalic complexes of *Oregon* larvae were found to have effects of promoting growth and differentiation of the *Oregon* strain upon the eye discs of *Bar* larvae as well as those of *Oregon* larvae.

4. The cephalic complexes of *Bar* larvae showed to have effects performing the development of the *Bar* strain upon the eye discs of *Oregon* larvae as well as those of *Bar* larvae.

5. Those facts suggest that the genic action appears first on the brain and the "ring gland" in the cephalic complex, which then influence the growth and differentiation of the eye disc.
Literature cited


