Effect of the *Papilio*-Brain on the Imaginal Differentiation of *Luehdorfia*-Pupa

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Concerning the mechanism of Lepidopterous metamorphosis in *Luehdorfia*, *Philosamia*, *Bombyx* and *Dictyoploca* ('51–'55), we have substantiated that a hormone from the brain takes part in the metamorphosis in terms of stimulating the prothoracic glands, the hormone of which is directly responsible for this phenomenon. On the other hand, with *Papilio xuthus* Ozeki ('54) contends that the brain of this species exserts no effect on metamorphosis, at least on emergence. He concludes this on the basis of his experimental results that the headless pupa could emerge normally when the head was ligatured just after pupation.

But from our experience with *Bombyx* it seems likely that the brain hormone had already been secreted in the body-fluid before the ligation was performed. To clarify this point, a simple experiment was carried out in which *Papilio*-brain was implanted into *Luehdorfia*-pupa in diapause. The results will be described in the present paper.

Material and method

Fifty pupae of *Luehdorfia* which had spent six or seven days after pupation, were used as hosts, and 150 larvae of *Papilio* which had reached maturity or one day before maturity were used as donors of the brains. Suboesophageal ganglia from the same donors were also implanted as a control. After being etherized, each pupa was supplied with three larval brains through a small hole made on the integument of the second thoracic segment. The hole was covered by a cut piece of integument and coated with melted paraffin. The operated pupae were kept at room temperature.

Two lots of pupae served as controls : the first consisted of 41 pupae each of which had received three suboesophageal ganglia as described above, and the other was comprised of 50 pupae which were left without any operation.

As mentioned in our previous papers ('51, '55), the pupal integument of *Luehdorfia* is so hard that it is impossible for us to tell from outside whether or not imaginal differentiation is occurring within. Therefore, all of the pupae were dissected 52 days after the operation (July 22).

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Results and remarks

Twenty-three out of the fifty pupae which had received three brains were alive at the time of dissection, and ten of them (43%) had developed to the adult form. Adult organs such as legs, eyes, antennae and wings were completely formed and coloured in black except for the wings which were yellow. The black pattern of wing appeared in two cases. This appearance of wing suggested that the two pupae were in the state ready for emergence.

The remaining 13 pupae which were alive upon dissection were all in a non-differentiating state, as were the dead specimens.

Out of the 41 control specimens which had received three suboesophageal ganglia, 33 were alive upon dissection, but none of them had undergone imaginal differentiation.

Forty-eight out of the fifty non-treated pupae survived but they did not show any sign at all of imaginal development. It is needless to remark upon the development of the two dead specimens.

The data are arranged in Table 1.

Table 1. Implantation of larval brains and suboesophageal ganglia from *Papilio* into *Luehdorfia*-pupae.

	No. of specimens	Results on dissection		
		dead	alive	
			non-diff.	adult form
3 brains	50	27	13	10
3 suboesophageal ganglia	41	8	33	0
non-treated control	50	2	48	0

From this result, although the occurrence of differentiated pupae is not so high as our expectation, it may be stated that the neurosecretory cells in the brain of *Papilio* can release the prothoracicotropic hormone and that they are able to function in the *Luehdorfia*-pupa. We make this statement because it is impossible for the prothoracic gland of *Luehdorfia*-pupa in diapause to release the metamorphosis-promoting hormone unless it is stimulated by the hormone from the brain.

Next, several experiments were carried out, using prepupae and pupae of *Papilio*, in which extirpation of the brain or ligation of the head resulted in the production of adult forms. This result appears, at first sight, to support Ozeki's

argument that the brain takes no part in the metamorphosis of *Papilio*, but it really does not mean that the brain is not involved in this process.

A similar result was also obtained with *Bombyx* which has no diapause in the post-embryonic life. However, when the brain of *Bombyx* was implanted into the diapausing pupa of *Luehdorfia*, the implanted brain actually induced the imaginal differentiation of the latter ('51). It must be noted here that *Papilio* used in the present experiment was of non-diapausing generation, and that the pupa was destined to experience a short pupal stage like the pupa of *Bombyx*. In these species the prothoracic glands may have already been stimulated before pupation by the brain hormone so as to produce the metamorphosis-promoting hormone. Indeed, from the results of Bounhiol's ligation experiments with *Bombyx* ('38), Andrewartha ('52) inferred that the neurosecretory cells in normally feeding larvae produce their hormone about 24 hours after the gut has been finally emptied. This consideration may hold true in *Papilio* also. Therefore, it seems reasonable to consider that even if the brain is removed before or just after pupation of these species, the activated prothoracic glands are able to produce their hormone, and emergence results. In short, it is impossible to conclude that, "the brain plays no part in stimulating the prothoracic gland to secrete its hormone" (Ozeki, '54). On the contrary, from the results of the present hetero-transplantation, and in the light of the above mentioned considerations it seems quite reasonable to say that the brain of *Papilio* is also concerned with metamorphosis in that it apparently governs the function of the prothoracic glands.

The implantation of suboesophageal ganglia yielded negative results, although the ganglion contains some neurosecretory cells which are very similar in appearance to those of the brain. The neurosecretory cells in the ganglion seem to have no relation to emergence, notwithstanding the fact that this ganglion has been shown to produce the substances which control the voltinism of *Bombyx* (Fukuda, '51, '52).

Summary

1) Three brains taken from the last instar larvae of *Papilio xuthus* were implanted into a diapausing pupa of *Luehdorfia japonica*.

2) Forty-three percent of the pupae thus operated developed to the imaginal form during the 52 days following the operation. Therefore, it is clear that the brain of *Papilio* secretes the prothoracicotropic hormone. It is highly probable that in *Papilio* as in other lepidopterans, the brain governs the phenomenon of metamorphosis through stimulation of the prothoracic glands.

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