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Puparium Formation in Flies: Contraction to Puparium Induced by Ecdysone

Abstract. *Larvae of the fly* Calliphora erythrocephala *(Meigen) were deprived surgically of their ring glands at an age prior to the appearance of ecdysone in the blood, and then injected with ecdysone. They contracted into the typical barrel-shaped puparium, before the onset of tanning. This proved that ecdysone controls the puparial contraction as well as tanning.*

Pupariation (*1*) in flies proceeds in two steps: (i) the larva contracts to the barrel shape of the puparium, and (ii) the cuticle becomes dark and hard by a process of phenolic tanning induced by a hormone (*2*), ecdysone. Ecdysone appears in the blood of larger flies (for example, *Calliphora, Phormia, Sarcophaga*) about 15 hours before the beginning of the puparial contraction, a process which lasts about 30 minutes. The effect of ecdysone on tanning has been studied frequently (*3*), but the control of the puparial contraction has been ignored.

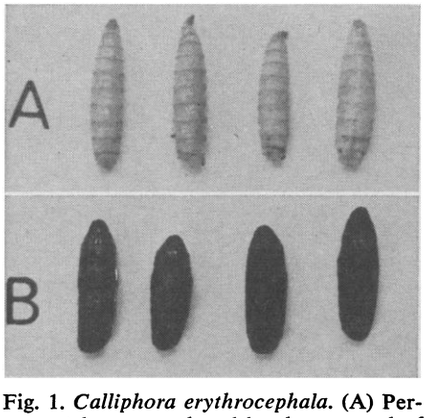

Fig. 1. *Calliphora erythrocephala.* (A) Permanent larvae produced by the removal of the ring gland in mature larvae. (B) Puparia in contracted state formed after the injection of ecdysone into permanent larvae.

Ecdysone originates from the ring gland situated above the brain. It was long considered impossible to study the effect of extirpating the gland because of its close proximity to the brain and the intrinsic difficulties of operating on a small soft-bodied organism. However, Possompès (*4*) reported a method by which the ring gland was removed without causing other damage to the larva; he showed that puparium formation was prevented when the extirpation was accomplished before a critical period but that normal puparia were formed when ring glands were subsequently implanted into operated specimens. This operation was much improved by Berreur (*5*).

The existence of a technique for the extirpation of the larval ring gland in larvae, and the availability of pure ecdysone now makes it possible to investigate the effect of ecdysone on contraction to the puparium as distinct from its effect on tanning. The fact that implantation of ring glands produces normal puparia (*4*) does not by itself prove that ecdysone alone is involved in the process.

The ring glands were removed (*5*) from 5-day-old larvae of *Calliphora erythrocephala.* This operation resulted in "permanent larvae" with otherwise normal activity. Four or five days later each larva was injected with 1 μl of a saturated aqueous solution of ecdysone (Schering). Dosages were calculated from the stated solubility of ecdysone in water (300 μg/ml). Only a few puparia had been formed 12 hours after the injection (Table 1). Those which had not pupariated were injected a second time 24 hours after the first injection. A large percentage of pupariation resulted overnight. Altogether 29 puparia were formed out of 39 permanent larvae injected with ecdysone, whereas only one puparium ensued from 30 control larvae which had received two injections of water.

Permanent larvae side by side with puparia formed from permanent larvae after the injection of ecdysone are shown in Fig. 1. The larva had contracted into the typical barrel shape with rounded ends and straight walls, exactly as in normal puparium formation, proof that the function of ecdysone is not limited to tanning but applies also to the preceding puparial contraction.

It was puzzling to note that two injections of ecdysone at intervals of 24 hours were required for inducing pupariation. According to Ohtaki *et al.* (*6*) ecdysone is rapidly inactivated in mature fly larvae so that at any time prior to puparium formation the ecdysone titer in the hemolymph is too low to induce pupariation. This suggested that pupariation is induced not so much by accumulation of ecdysone in the hemolymph beyond a critical concentration, but by a cumulative effect of small doses on the cuticle. It would appear that in our experiment ecdysone was quickly inactivated after a single dose, and a second injection 1 day later found the cuticle in a more receptive state.

Table 1. The effect of the injection of synthetic ecdysone into mature larvae of *Calliphora erythrocephala* which had been deprived of their ring glands (permanent larvae) on the formation of the puparium. A second injection was applied 24 hours after the first in those larvae which by then had not pupariated. The controls received two injections of water.

Larvae injected (No.)	First injection ecdysone (μg/larva)	Puparia formed after 12 hours (No.)	Second injection ecdysone (μg/larva)	Puparia formed after 12 hours (No.)	Puparia in controls (No.)
10	0.3	2	0.6	4	1/10
10	0.6	2	0.6	5	0/10
19	0.6	2	0.6	14	0/10

The puparia resulting from such injections were opened at suitable intervals to check on pupal and adult development in the absence of the ring gland. Almost all specimens pupated but only a few evaginated the head to the "phanerocephalic" state, a process achieved by strong contractions of muscles (*7*) which are obviously weakened in operated specimens. At this point, irrespective of head evagination, development comes to a stop. According to Shaaya and Karlson's (*8*) curve of ecdysone titer during fly development, by the time the pupal molt takes place ecdysone has disappeared, to reappear again to initiate adult development.

In the absence of the ring gland, injected ecdysone carries development exactly to the state reached with the first natural release of ecdysone, but no further development is possible (*9*). Pupae lacking ring glands are therefore virtually identical with diapausing pupae in which the second production of ecdysone is delayed (*10*). Ecdysone also controls calcification in an aberrant case of puparium formation where hardening is not due to tanning but to calcification (*11*).

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Taste Nerve Fibers: A Random Distribution of Sensitivities to Four Tastes

Abstract. *The numbers of rat glossopharyngeal and chorda tympani fibers responding to one, two, three, or four taste stimuli of different quality (sodium chloride, hydrochloric acid, quinine, and sucrose) and to each of the six possible pairs of these stimuli can be predicted if there are four independent sensitivities randomly distributed among innervating fibers.*

Mammalian chorda tympani taste neurons respond differentially to the quality of taste stimuli; that is, they respond more to some substances than to others. Substances to which a single neuron may respond can be classified into two categories: (i) those of similar quality (NaCl and LiCl) and (ii) those of dissimilar quality (NaCl and sucrose). Substances of similar quality will elicit responses in the same set of neurons, but substances of dissimilar quality may or may not (*1*). For example, a neuron which responds to NaCl will also respond to LiCl, but it may or may not respond to sucrose. It is typical for a single chorda tympani neuron to respond to stimuli of very different quality.

Many individual rat chorda tympani fibers respond to both NaCl and HCl (salty and sour substances); very few respond to both sucrose and quinine (sweet and bitter substances) (*2*). But the anterior tongue, which the chorda tympani innervates, is highly sensitive to salt and acid but poorly sensitive to sucrose and quinine. The posterior of the rat's tongue, innervated by the glossopharyngeal nerve, is more sensitive to quinine and sucrose (*3*). There are probably more receptors sensitive to these stimuli in the foliate and circumvallate papillae in the back of the tongue than there are in the fungiform papillae in the front. If the combinations of sensitivities to different qualities were probabilistic, dependent only upon the numbers of receptors sensitive to each quality in a receptive field, there should be more combinations of sensitivities to bitter and sweet stimuli in glossopharyngeal fibers.

Sensitivities of 27 rat glossopharyngeal taste fibers were determined (*4*). The animal was anesthetized with sodium pentobarbital, the back of the tongue was exposed surgically, and the nerve was dissected free and cut centrally. After removal of the sheath, groups of fibers could be separated and placed upon silver-silver chloride wick recording electrodes and their responses amplified. The criterion for a single fiber response was uniformity of recorded spike amplitude. Stimulus solutions flowed into the lumen of the circumvallate or foliate papilla through a small pipette (0.1 mm in diameter).

Test stimuli were 0.3*M* NaCl, 0.01*N* HCl, 0.001*M* quinine hydrochloride, and 0.3*M* sucrose. The stimulus intensities produce about 50 percent of the total nerve's maximum response to these common representatives of the four taste qualities (salty, sour, bitter, and sweet). A fiber was classified as responding to a stimulus if there were at least a 50 percent increase in response rate during the first 5 seconds of stimulation. Fibers which responded to several stimuli usually did not respond equally well to all; however, response rate increased at least 500

Table 1. Numbers of single fibers responding to each combination of two tastes.

Combination (x, y)	$(p_x)(p_y)$	Predicted	Observed
Glossopharyngeal fibers			
NaCl, HCl	(.6)(.6)	9.7	9
NaCl, quinine	(.6)(.4)	6.5	6
NaCl, sucrose	(.6)(.4)	6.5	7
HCl, quinine	(.6)(.4)	6.5	8
HCl, sucrose	(.6)(.4)	6.5	6
Quinine, sucrose	(.4)(.4)	4.3	3
Chorda tympani fibers			
NaCl, HCl	(.8)(.7)	14.0	14
NaCl, quinine	(.8)(.4)	8.0	8
NaCl, sucrose	(.8)(.2)	4.0	4
HCl, quinine	(.7)(.4)	7.0	6
HCl, sucrose	(.7)(.2)	3.5	2
Quinine, sucrose	(.4)(.2)	2.0	2

Table 2. Numbers of single fibers responding to 1, 2, 3, or 4 tastes; T represents the number of fibers in sample.

Responses (n) (No.)	Predicted ($P_{(n)} \cdot T$)	Observed
Glossopharyngeal (T = 27)		
1	7.1	8
2	11.2	12
3	7.1	5
4	1.7	2
Chorda Tympani (T = 25)		
1	5.4	5
2	11.3	13
3	7.2	6
4	1.0	1