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## Effect of Brain Hormone from *Bombyx mori* on Metamorphosis of *Calliphora erythrocephala*.* (26589)

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Neurosecretion of the brain of insects is known to stimulate the prothoracic gland to function(1-9). Possible direct action of the brain hormone(9) in induction of metamorphosis has been tested in a series of experiments.

*Methods.*† Mature larvae of *Calliphora erythrocephala*(10) were ligated at the seventh segment and were observed for 24 hr at 25°C. Those with pupation of the segment anterior to the ligature were selected for use in the bioassay. A second ligature was tied posterior to the first and the larva divided between them. After mounting the posterior segment on a glass needle passing through the second ligature, appropriate dilutions of brain hormone and ecdysone, alone and in combination, were injected into respective larval segments. The ligature was useful in closing the anterior end of the specimen after injection and removing it from the needle of the microsyringe. After 24 hours at 25°C, the larval segments were then scored for pupation. Controls included the original bioassay of the brain hormone and ecdysone in which degree of activity was verified in terms of concentration; segments without treatment; and those injected with sesame oil, the solvent for the brain hormone. The results appear in Table I.

*Results.* Experience with the bioassay indicates that it is qualitative for a single con-

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TABLE I. Effect of Ecdysone and Brain Hormone on Pupation of *Calliphora erythrocephala*.

| Injection | | | Isolated abdomens | | |
|---|---|---|---|---|---|
| Sesame oil | Ecdysone, γ | Brain hormone, γ | No. pupated | Total No. | % pupated |
| | | | | 60 | 0 |
| + | | | | 20 | 0 |
| + | | 38 | | 10 | 0 |
| + | | 75 | | 20 | 0 |
| + | | 150 | | 20 | 0 |
| | 7 | | 6 | 20 | 30 |
| | 15 | | 1 | 10 | 10 |
| + | 29 | | 11 | 20 | 55* |
| + | 7 | 75 | 14 | 20 | 70* |
| + | 15 | 150 | 16 | 20 | 80* |
| + | 29 | 150 | 10 | 10 | 100* |

* Positive.

centration, and tests are considered positive when 50% or more of the specimens pupate. Dosages of ecdysone of 29 γ yielded positive bioassays; lesser amounts did not. Negative results were obtained when brain hormone up to a dose of 150 γ was injected. However, addition of 75 γ of brain hormone to 7 γ of ecdysone and 150 γ of brain hormone to 15 γ of ecdysone gave positive bioassays despite negative results with these amounts of ecdysone alone. Although not indicated in the Table, control studies were carried out simultaneously with the bioassays.

Ecdysone(11,12) was prepared from pupae of *Bombyx mori* preserved in methanol, and previous bioassay showed the sample of extract used to be active. Brain hormone† was prepared from pupal brains of *Bombyx mori* dissected free without inclusion of corpora cardiaca and corpora allata. Methanol-acetone-ether extraction yielded a crude but active extract. With Dr. Jiro Kirimura, it was found that injected, artificially diapausing, brainless pupae (Dauer-pupae) of the silkworm became imagines in 3 to 5 weeks when the head of each was injected with 0.2 mg of the oily hormone.

*Discussion.* Williams(5-8) was able to show that a hormone is secreted in the brain of insects which activates the prothoracic gland to secrete hormone which promotes growth and differentiation. This has now been confirmed in *Lepidoptera* by Ichikawa and Nishiitsutsuji(1), Kobayashi(2) and others(13-18). The results of the present work suggest that the brain hormone not only functions in this way but also acts directly on the tissues in conjunction with ecdysone. These 2 pathways of action are illustrated diagrammatically in Fig. 1.

Ichikawa and Nishiitsutsuji-Uwo(19) utilized larvae of the *Eri* silkworm, *Philosamia cynthia ricini*, during the fifth instar and found that 40% of the isolated abdomens became moths following implantation of 3 brains, but whether a subthreshold amount of hormone from prothoracic gland was contained in the isolated abdomens is unknown. Similar uncertainty attaches to the report of Kobayashi and Yamashita(4). They implanted 2 fresh larval brains into the fifth abdominal segment of 61 silkworm larvae at the fifth instar. The abdomen was isolated 2 hours after pupation, and 2 of the 61 animals emerged as moths 34 to 35 days following pupation. This could not be repeated when 10 larval brains were implanted into each of 11 abdomens.

The results obtained by using purified brain hormone and ecdysone suggest that hormonal extracts of the brain have two functions. The first is its tropic function on the prothoracic gland and the second is its direct, synergistic action with ecdysone on induction of metamorphosis. Whether separate brain hormones are responsible for the two functions respectively is a matter of conjecture.

*Summary.* Appropriate extraction of brains and pupae of *Bombyx mori* yielded active brain hormone and ecdysone respectively. When various concentrations of these 2 hormones were introduced alone and in combination into isolated larval abdomens of *Calliphora erythrocephala,* pupation occurred

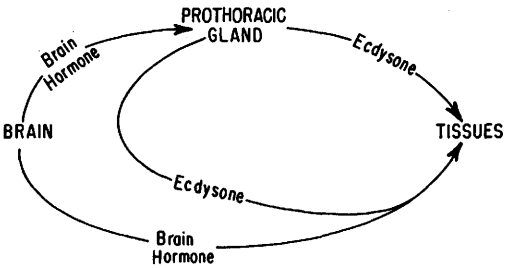


FIG. 1.

when brain hormone was added to concentrations of ecdysone which would not induce pupation alone. (Pupation was not induced by brain hormone alone.) Brain hormone therefore may have a direct action on the tissues when acting synergistically with ecdysone in addition to its tropic action on the prothoracic gland.

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## Inhibition of Growth of Streptococci by Adenine.* (26590)

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Bernheimer *et al.*(1) described a synthetic medium for cultivation of *Streptococcus pyogenes,* specifically strain C203S. For several years we have used this medium with success for cultivation of strains C203 and C203S. Recently, however, we experienced difficulty in growing 2 other strains of *S. pyogenes* (S23 and C203U) in the same medium and had occasion to delete adenine and uracil from the medium. The 2 strains which had earlier failed to grow now showed growth comparable to that of strain C203S. On further testing it appeared that the inhibition of strains S23 and C203U was due to adenine. The inhibition could be reversed in the normal growth period by addition of hypoxanthine, xanthine or guanine or their corresponding nucleotides. When adenine was present with uracil in the medium growth eventually occurred but the lag phase was prolonged to several hours. A somewhat similar phenomenon was observed by Pierce and Loring(2) using a pyrimidine deficient mutant of *Neurospora crassa.* The growth of this organism could be inhibited by adenine and yeast adenylic acid. In this case however the reversal of inhibition was brought about by the pyrimidines cytidine or uridine. They further found that guanine caused a similar though less marked inhibition of growth of the mold.

Strain C203U, which is inhibited by adenine, is a mutant of strain C203S which is not. The only reported difference between the 2 strains is that C203U fails to produce streptolysin-S (Bernheimer)(3).

*Methods.* The organisms used were strains C203S, C203U, S94, and S23 of *S. pyogenes.* These organisms were kindly supplied by Dr. Cornelia A. Eddy, Dept. of Microbiology, Tulane Univ. School of Medicine. The bacterial stock cultures were carried on Pia egg slants at 4°C, transferred monthly. All inocula were prepared in heart infusion broth incubated at 37°C. Incubation time varied

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