Technical Papers

Cytochrome *c* Oxidase Activity During the Metamorphosis of *Drosophila virilis*

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The cytochrome *c* oxidase activity during the metamorphosis of the diapausing giant silkworm *Platysamia cecropia* has recently been investigated by Williams (*1*). He found that the activity of this system during pupal life follows a characteristic U-shaped curve and pointed out that rise of the oxidase activity and release of the hormone that causes the termination of the diapause coincide in time. From this evidence, he suggested that the observed metabolic changes in the cytochrome system may be part of the mechanism by which the hormone breaks diapause. Thompson (*2*) observed in adult male and female blowflies (*Calliphora*) a slight decrease in the O_2 consumption after transplantation of 3 corpora allata from adult donors into adult male and female hosts. A possible effect of the corpus allatum hormone on the cytochrome system is thus indicated. Experiments by Wolsky (*3*) point in the same direction. He noticed that certain adult structures failed to develop after the cytochrome oxidase in *Drosophila* had been inhibited with carbon monoxide. Sacktor (*4*) has studied the cytochrome oxidase activity during the pupal life of houseflies and obtained a U-shaped curve similar to that found in *P. cecropia*. Despite these several lines of evidence, the nature of the relationship between the cytochrome oxidase system and the various hormonal factors concerned in growth and metamorphosis is not clear. Since the humoral situation that governs metamorphosis in Drosophila is quite well known, the present study was undertaken in the hope of learning something more about the supposed relationship between hormone and cytochrome oxidase activity.

Two types of experiments were performed on males of *D. virilis* Sturtevant. In the first, cytochrome oxidase activity was determined for the pupal period, and for adults 1–4 days old, with the results given in Fig. 1. The determinations were made with the Beckman model DU spectrophotometer, following the method of Sacktor (*5*). In another series, for activity determinations on operated animals, Sacktor's modified method (*6*) was employed, using an optimum phosphate buffer, a higher temperature, and a more dilute homogenate. Thus the data given in the normal curve (Fig. 1) and the results of paired experimental determinations given below are not directly comparable. The data in Fig. 1 are based on 119 pairs of specimens. The oxidase activity of each pair was determined in duplicate. The first measurements were made on old last-stage larvae (circle) and show that the oxidase activity at this time is higher than in the newly formed prepupa. Fig. 1 shows that oxidase

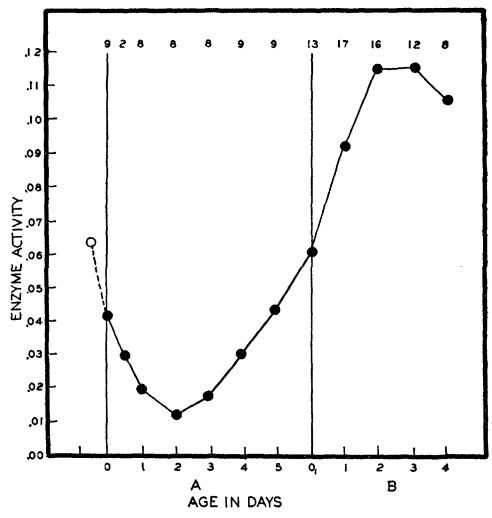

FIG. 1. Cytochrome *c* oxidase activity of *Drosophila virilis* during the pupal period and the first four days of adult life. Abscissa: *A*, pupil period; *B*, adult period; *O*, puparium formation; O_1, emergence of fly. Ordinate: cytochrome *c* oxidase activity $\frac{\Delta \log [Cy\ Fe^{++}]}{\Delta t}$. Numbers above indicate numbers of pairs used for the determination of each age group.

activity decreases steadily during early pupal life, reaching its lowest value in the 2-day-old pupa. At this time a drastic change occurs which results in a progressive increase in the oxidase activity. On emergence of the fly, the activity has reached a point well above that prevailing at the beginning of pupal life. In the young adult fly, the activity of the enzyme continues to rise and reaches its maximal height in the 2-day-old fly, where it remains for the following two observations.

Efforts to alter the oxidase activity of young adult flies through humoral unbalance led to the following experiments. Twenty pairs of male flies 3 hr after emergence were divided into two equal groups. The flies of one group were then decapitated in such a way as to remove the corpora allata and corpora cardiaca with the head. The flies of the other group were also decapitated, but here the corpora allata and cardiaca were left in the headless individuals. If these operations are performed carefully, the headless flies live for 4 days or longer. The oxidase activity of both groups was determined 2 days after the operation. The enzyme of the group with glands removed had an activity of $0.085 \pm 0.0051 \frac{\Delta \log [Cy\ Fe^{++}]}{\Delta t}$, and that of the group with glands in place, $0.091 \pm 0.0053 \frac{\Delta \log [Cy\ Fe^{++}]}{\Delta t}$. Evidently there is no significant difference between the two groups. Moreover, the observed enzyme activity in both decapitated groups is as high as expected of normal 2-day-old flies. The

presence of the corpus allatum and cardiacum is hence not necessary for the rise of the oxidase activity customarily observed after emergence.

In another series of experiments, 3 ring glands from mature larvae were transplanted into a male host 3 hr after emergence. Ten pairs of such individuals were available for analysis. The transplanted gland is a compound structure containing, in addition to corpus allatum and cardiacum, a third group of cells that furnish the growth and differentiation hormone. As controls, 10 pairs of flies of the same age and sex were injected with Ringer's solution. These individuals do not contain a source of the growth and differentiation hormone, since the portions of the ring gland that produce it have degenerated. The oxidase activity was determined in both groups 1 day after the operation. In the group bearing the transplant, the enzyme activity was found to be 0.108 ± 0.0092 $\frac{\Delta \log [\text{Cy Fe}^{++}]}{\Delta t}$ and that for the controls 0.115 ± 0.0094 $\frac{\Delta \log [\text{Cy Fe}^{++}]}{\Delta t}$. These results show that, as in decapitated animals, the enzyme activity in specimens with transplants has increased to a value normal for flies of this age, and that there is again no significant difference between the operated and control groups of animals. It will be noted that values obtained in the last experimental group are somewhat higher than those of the decapitation experiments, although the determinations with transplants were made on flies only 1 day old and should therefore be lower than those of the 2-day-old flies. The explanation for this is that the flies used in the first group were headless and came from cultures containing smaller flies. There is always some variation in the size of the flies. The pairs used for comparison were therefore taken from the same culture bottle in all cases and in addition were matched for size. This procedure allows a better comparison between animals in these experiments.

It is tempting to compare the cytochrome *c* oxidase activity curve of flies with that of the *P. cecropia* moth. In the housefly (*4*) and in Drosophila the curves are very similar. The shape of the U is different in *P. cecropia,* for here the low point of the curve is not transient but lasts throughout the period of diapause. This difference is of interest. Shortly before pupation, the prothoracic gland hormone is active in both forms and causes the animal to pupate. In *P. cecropia,* the titer of prothoracic gland hormone is apparently only high enough to cause pupation but not the ensuing events of metamorphosis. The already low hormone titer continues to decrease after pupation, and it is for this reason that the animal goes into diapause. A new burst of hormone is needed to start the pupa on its way to adult development. When this occurs, the cytochrome oxidase activity rises. Thus the decline of the oxidase curve seems to be somehow related to the period of the decreasing hormone titer, and the rise of the oxidase to the period when the hormone concentration increases in the animal. In flies, on the contrary, such a relationship cannot be established. As in *P. cecropia,* however, the oxidase activity is high at pupation and declines thereafter. The suspected activity of the prothoracic gland follows this part of the oxidase curve very well, for it, too, is high at pupation and then falls off. In the 2-day-old pupa of Drosophila, the prothoracic gland is partially degenerated and, as experiments have shown (*7*), has lost its capacity to produce hormone. Thus in flies the subsequent rise of the oxidase is definitely not associated with an increase in hormone production. The negative results obtained here by introducing prothoracic gland hormone through the transplantation of larval ring glands seem therefore not astonishing.

As far as the corpora allata are concerned, they are active in young flies (*8*), but it is not known how soon before emergence of the fly their activity starts. The experimental evidence shows that removal of this gland from young adult flies does not prevent the normal rise of enzyme activity.

Without further speculation, we must state that it is impossible at this stage of investigation to draw any conclusions as to the directness of action of the hormones on the cytochrome oxidase system in flies.

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Thin Films of Supersaturated Solutions for Detecting, Counting, and Identifying Very Small Crystalline Particles[1]

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A simple technique has been devised for making thin films of supersaturated solutions of a wide variety of crystalline solids. It has been found that these thin films are a useful tool for detecting, counting, and identifying very small crystalline aerosol particles.

Films of solution supersaturated with respect to a given crystalline substance are prepared in the following way. Two miscible solvents are chosen in which the given substance is readily soluble, one of these solvents being quite volatile and the other nonvolatile. An unsaturated solution is prepared by dissolving the substance in the volatile solvent. This solution and the nonvolatile solvent are each put in burettes, so that

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