Phenoloxidase System of the Blowfly, Calliphora erythrocephala

Abstract. *In* Calliphora *larvae, an inactive precursor of the enzyme phenoloxidase is present in the hemolymph. A special activator enzyme is localized in the cuticle. When phenoloxidase precursor is allowed to react with the activator, it yields a soluble enzyme having the characteristics mainly of a diphenoloxidase. If, however, this conversion takes place in the presence of mitochondria, the phenoloxidase becomes bound to the mitochondria and the resulting preparation shows equally mono- and diphenoloxidase activity.*

The enzyme phenoloxidase is normally present in insects not as an active enzyme, but as a complex system consisting of an inactive precursor or "pre-enzyme," an activator enzyme, and also possibly other components (see *1*).

Activation of the enzyme precursor, in order to yield the active phenoloxidase, can occur (i) after injury, or (ii) at the onset of puparium formation, a process which is under the control of the hormone ecdysone. In view of the great interest in this activation process as it constitutes one of the biochemical effects of ecdysone (*2*) and because of the interest in the sclerotization process itself (*3*), we have studied the components of the system in more detail. We have found that the activator protein is located in the cuticle of the larvae, while the phenoloxidase precursor is present mainly in the hemolymph.

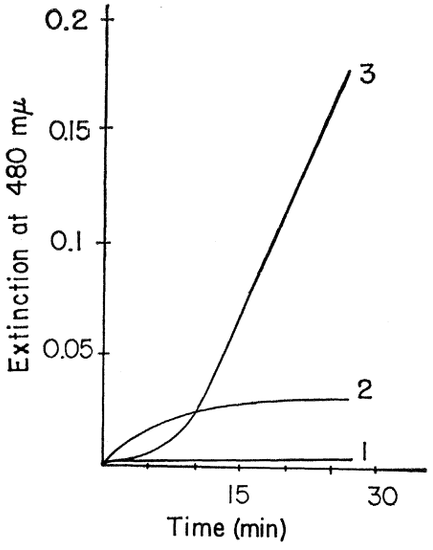

Fig. 1. Localization of the components of the phenoloxidase system in *Calliphora* larvae. Hemolymph (curve 1), cuticle extract (curve 2) and hemolymph + cuticle extract (curve 3) were incubated at 20°C with 5 ml of a 0.02 percent DL-DOPA [3-(3,4-dihydroxyphenyl) alanine] solution in 0.06*M* phosphate buffer, p*H* 7.0. The extinction at 480 mμ was measured in an Eppendorf self-recording photometer.

In order to obtain hemolymph, a small incision was made in the front part of a *Calliphora* larvae (6 to 7 days old), the animal was squeezed slightly, and the drops were collected. When the hemolymph was kept at room temperature and tested for phenoloxidase activity, it was found to be inactive (Fig. 1). Cuticle was prepared either by cutting the larvae into halves under water and removing the inner organs, or by squeezing them tightly between blotting paper. Homogenates of cuticle showed a rapidly appearing but low phenoloxidase activity (Fig. 1). When mixtures of homogenized cuticles and hemolymph were kept at 25°C, phenoloxidase activity developed within 10 to 20 minutes (Fig. 1). The time needed for the activation process depended on the amount of cuticular homogenate, while the final activity was proportional to the amount of hemolymph. This period is conveniently expressed as "half activation time." The fat body showed no activity at all when assayed either alone or after it had been incubated with hemolymph, cuticular homogenate, or both.

The nature of the activation process of the enzyme precursor is not well understood; it may be a limited proteolysis (*4*). In addition to the conversion of the enzyme precursor into the active enzyme, the activation process may result in a binding of the soluble phenoloxidase precursor to subcellular particles, as shown by the experiments in Table 1.

For these experiments, hemolymph (containing phenoloxidase precursor) was incubated with a preparation of the activator enzyme. The supernatant of a cuticular homogenate that had been centrifuged at 105,000*g* for 60 minutes was used as activator enzyme preparation. The incubated mixture of phenoloxidase precursor and activator enzyme was then centrifuged at 105,000*g* for 60 minutes. The whole phenoloxidase activity was found in the supernatant. If, however, whole homogenates of *Calliphora* larvae were allowed to develop phenoloxidase activity and were then centrifuged at 105,000*g* for 60 minutes, most of the activity was in the sediment. When mitochondria from the fat body, isolated in the usual way by centrifugation of fat-body homogenates in sucrose solution, were incubated with hemolymph, then recovered and subjected to the activator enzyme, no activity was found. If, however, the mitochondria were incubated with enzyme precursor plus activator (that is, hemolymph plus cuticular supernatant), the activity produced was easily obtained in the sediment after centrifugation. That is, the active enzyme sedimented with the mitochondria.

The soluble phenoloxidase was resistant to freezing and thawing, but the activity of the mitochondria-bound enzyme, particularly its monophenoloxidase activity, was diminished when subjected to such conditions. When the thawed mitochondrial suspension was centrifuged, the sediment (mitochondrial debris) had no activity and the supernatant retained some diphenoloxidase activity. Similar effects were evident after treatment with desoxycholate or with ultra-high-frequency sound.

The bound phenoloxidase exhibited monophenoloxidase and diphenoloxidase activity. The monophenolase activity was enhanced by hydrogen donors, for example, reduced nicotinamide adenine dinucleotide (*5*). The soluble preparation has been found to have only negligible monophenolase activity and high diphenoloxidase activity (see also *6*).

Table 1. Soluble and particle-bound phenoloxidase activity of different *Calliphora* preparations. The measurement was performed as described in the legend for Fig. 1. The activity was expressed in photometer units (100 units represent an extinction change of 0.1). The activity of the preparations was measured before as well as after centrifugation at 105,000*g* for 60 minutes.

Extract	Activity		
	Before	After	
		Supernatant	Sediment
Whole homogenate	600	80	500
Hemolymph + cuticular extract	200	180	0
Hemolymph + mitochondria	0	0	0
Hemolymph + cuticular extract + mitochondria	240	55	170

The binding of the phenoloxidase to the mitochondria thus has a significant influence on the catalytic properties of the enzyme. We assume that the binding results in some coupling with the hydrogen transport system of mitochondria, thereby potentiating the monophenoloxidase activity. Some conflicting data in the literature concerning the monophenolase and diphenolase activity of various preparations can be reevaluated in light of these findings.

C. E. SEKERIS
D. MERGENHAGEN
Physiologisch-chemisches Institut der Universität, Munich, Germany

References and Notes

1. N. H. Horowitz and M. Fling, in *Amino Acid Metabolism*, W. McElroy, D. McElroy, B. Glass, Eds. (Johns Hopkins Press, Baltimore, 1955), p. 207; P. Karlson and A. Schweiger, *Z. Physiol. Chem.* **323**, 199 (1961).
2. P. Karlson, *Gen. Comp. Endocrinol.*, Suppl. No. 1, p. 1 (1962).
3. M. G. M. Pryor, in *Comparative Biochemistry*, M. Florkin and H. S. Mason, Eds. (Academic Press, New York, 1963), vol. 4, p. 371.
4. A. Schweiger and P. Karlson, *Z. Physiol. Chem.* **329**, 210 (1962).
5. C. E. Sekeris and D. Mergenhagen, unpublished results.
6. P. Karlson and H. Liebau, *Z. Physiol. Chem.* **326**, 135 (1961).
7. We thank P. Karlson for his continued interest in our work and Frau G. Behrens for skillful technical assistance. The Deutsche Forschunsgemeinschaft has given us financial aid.

9 March 1964

Dynamics of Motion Perception in the Desert Locust

Abstract. *The torque produced by the neck muscles of a locust mounted coaxially in an illuminated cylindrical striped drum follows small sinusoidal oscillations of the drum. Peak-to-peak oscillations of 0.03 degree (± 0.02 degree) at 0.1 cy/sec elicit measurable responses. Several features of this visual response to drum oscillation can be expected theoretically on the basis of a formal neural mechanism similar to that inferred by Hassenstein and Reichardt for constant-velocity motion perception in the bettle eye.*

If a locust is mounted horizontally by the sternum in the center of a coaxial cylindrical striped drum, the head will rotate about the roll axis so as to follow angular motion of the drum. If the head is attached to a torque meter of high mechanical input impedance, torques about the roll axis may be measured which vary consistently with the drum motion, illumination, and pattern.

This reflex has been studied in adult males of the desert locust *Schistocerca gregaria* (Forskål) and in nymphs of the grasshopper *Eutropidacris cristata* (L.). The experimental arrangement consisted of a Plexiglas cylinder, 10 cm in diameter, the inner surface of which was covered with translucent white tracing or construction paper bearing equally spaced black stripes (the pattern spatial period, λ, being either 7 degrees or 45 degrees). The cylinder was rotated either at constant velocity or with small, nearly sinusoidal angular oscillations, and illuminated from the outside by either d-c incandescent or a-c fluorescent lamps. No effect of the 120-cy/sec component of the fluorescent lamps was discernible in comparison with d-c illumination.

Torque was measured as follows. The frons of the head was connected by means of wax and a steel rod to a block clamped onto a taut steel band aligned with the longitudinal body axis of the locust. The torsion of the band produced by the locust's head torque was registered as motion of a light beam reflected from a mirror on the block. The image of a 6-volt, long-coiled lamp filament was cast by the mirror on the mutual boundary of two juxtaposed 1- by 2-cm silicon solar cells. The differential voltage output of the two cells under these conditions was nearly a linear measure of head torque over a range of at least ± 2 kdyne-cm with a sensitivity of 25 mv/kdyne-cm. The torsional compliance of the taut band and connecting rod was about 0.04 degree/kdyne-cm. Since most torques measured were less than 500 dyne-cm, motion of the head about the roll axis was generally less than 0.02 degree. The visual feedback normally available to the locust eye was therefore reduced to this extent. The torque meter, d-c amplifier, and stylus drive provided constant gain and negligible phase shift from d-c to 6 cy/sec.

The pronotum of the mounted locust was waxed firmly to the thorax. The head was attached to the torque rod and drawn forward slightly, away from sensory hairs on the pronotum (*1*). Responses were measured with luminance at the outer drum surface of from 10 to 500 lux. Transmission of the diffusing white stripes was from 7 percent to 20 percent for the various papers used. Controls on the response to sinusoidal oscillation included plain white paper in place of the striped pattern, removal of drum illumination, and anesthetization of the insect; all of these conditions eliminated the response. Further, the response may readily be observed by eye in the head motion of a hand-held locust (visual feedback is in this case available to the locust visual system).

Clear following of sinusoidal drum motion was measured at drum frequencies of from 0.0014 cy/sec to 4 cy/sec; it cannot yet be excluded that slight unevenness in the mechanical drive contributed to the response at very low frequencies. Records of torque measured under constant-velocity (CV) rotation and sinusoidal-angle (SA) drum oscillation are illustrated in Fig. 1. After an initial transient, the steady-state response to either input assumed approximately the time behavior of that input. No appreciable adaptation from the level of response attained after 10 to 20 seconds has been noted for CV or SA stimuli lasting several minutes.

The SA response was less sensitive than the CV response to imperfections in the pattern such as the "joint" of the paper cylinder; a sheet of plain white paper used as a control "pattern" induced considerable CV torque but no detectable SA torque. The SA head response was also more reproducible than other optokinetic responses of the locust—for example, isometric yaw torque during tethered flight in a laminar windstream (*2*).

In order to use standard linear harmonic description (*3*) toward analysis of such a system, one must determine the extent to which nonlinearities affect input-output measurements. Several important nonlinearities exist in the neck-torque visual response to SA input. First, if the head is twisted at all when attached to the torque rod, asymmetry can appear in the records. Second, saturable, logarithmic, or other nonlinear processes certainly exist in the sensory, neural, and muscular systems which take part in the response. Although harmonic distortion in the output has not been measured, the curves of torque amplitude versus small SA-input amplitude in Fig. 2 indicate an approach to linearity at peak-to-peak inputs smaller than 0.15 degree for a frequency of 0.1 cy/sec.

If a threshold for the response exists, Fig. 2 shows that it occurs below 0.03