Alcohol Dehydrogenase Activity During Metamorphosis of Ephestia kühniella¹

Developmental changes in the activity of the enzyme alcohol dehydrogenase (ADH) have been described in *Drosophila melanogaster*^{2, 3}. During the pupal period, when the reorganization from a larval to an adult body plan is taking place, the activity of ADH per organism has been shown to decrease in the early pupa, reach a minimum, and rise prior to eclosion, following a U-shaped curve³. Similar activity curves have been reported for several other enzymes during pupal life of various insects (reviewed in references⁴ and ⁵). In many cases these have been interpreted as being a reflection of histolysis of larval structures followed by histogenesis of adult structures. In the present communication, the pattern of change in ADH activity is described in the pupa of the meal moth *Ephestia kühniella*.

A highly inbred wild-type strain known as NCR was used exclusively. The method of rearing and staging *Ephestia* pupae has been previously described⁶. Staged pupae raised at 23–24°C were homogenized in chilled distilled water (10 individuals per ml, or approximately 100 mg of material per ml) by means of a motor-driven teflon and glass homogenizer. The extract was centrifuged in a Sorvall Refrigerated Centrifuge at $30,000 \times g$ for mixture consisting of 0.1 ml 2-butanol, 0.1 ml nicoti-



20 min. 0.1 ml of the supernatant was added to a reaction namide adenine dinucleotide (NAD)solution (10 mg/ml), and 3 ml of 0.1 M glycine-sodium hydroxide buffer at pH 9.5. The increase in absorbance of the reaction mixture per min at a wavelenth of 340 nm was measured in a Beckman DU Spectrophotometer. The light path was 1 cm, and the temperature of the reaction mixture was maintained at 30°C. An increase in absorbance of 0.001 per min as compared to a reaction mixture without added substrate (2-butanol) was taken as one unit of ADH activity. If the amount of enzyme preparation is increased in the reaction mixture, the units of enzyme activity increase in a linear fashion to at least a value of 80.

- ¹ Supported by a grant from the Biomedical Sciences Committee of the University of Maryland.
- ² G. R. DUNN, T. G. WILSON and K. B. JACOBSON, J. exp. Zool. 171, 185 (1969).
- ³ H. URSPRUNG, W. H. SOFER and N. BURROUGHS, Wilhelm Roux Arch. EntwMech. Org. 164, 201 (1970).

As shown in the Figure, during the first 6 days following pupation, a U-shaped curve is approximated. However, ADH activity drops off sharply following the 6th day, and reaches a low value on the 8th day which remains relatively constant for the duration of the pupal period. The sudden drop in activity during midpupa is in contrast with the pattern of ADH activity reported for Drosophila³. The descending portion of the curve for the first 3 days following pupation may reflect histolysis of larval structures. The subsequent sharp rise and fall in activity cannot be easily interpreted. By the 6th day the pupa as a whole is well advanced in the development of adult structures, though metamorphosis is far from complete. There is no obvious correlation between a specific morphogenic process and the peak of ADH activity on the 6th day. At this point there is no evidence that a decrease or an increase in ADH activity is due to degradation or synthesis of enzyme molecules. However, from a study on the effect of the nucleic acid base analog 5fluorouracil on the changes in ADH activity described in this communication, evidence exists that this developmental program is dependent on synthesis of nucleic acid during a specific portion of pupal life⁷.

> Changes in units of ADH activity per organism during the pupal period of *Ephestia*. Each point represents a determination on at least 30 individuals. The first point represents individuals 0-6 h after pupation. The other points represent daily intervals, but accurate within \pm 3 h (e.g., 2 days after pupation is 48 \pm 3 h, etc.). Emergence of adults occurs 13 days after pupation. The last point represents individuals still in their pupal cases, but about to emerge as adults.

Zusammenfassung. Bei Puppen der Mehlmotte, Ephestia kühniella, wurde der Verlauf der Alkohol-Dehydrogenase-Aktivität pro Tier bestimmt: anfänglich Aktivitätsabfall, erreicht am 4. Tag nach der Verpuppung ein Minimum; hierauf erfolgt ein Maximum am 6. Tag. Nach erneutem Abfall bleibt die Aktivität vom 8. Tage an auf erniedrigtem, relativ konstantem Niveau.

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- ⁴ L. I. GILBERT, in Comprehensive Biochemistry (Eds. M. FLORKIN and E. H. STOTZ; Elsevier, Amsterdam 1967), p. 199.
- ⁵ G. R. WYATT, in *Metamorphosis* (Eds. W. ETKIN and L. I. GIBERT; Appleton-Century-Crofts, New York 1968), p. 143.
- ⁶ R. B. IMBERSKI, J. exp. Zool. 166, 151 (1967).
- ⁷ R. B. IMBERSKI, Experientia, in press (1971).