References

1. A. D. Baddeley, paper presented before the British Exptl. Psychol. Society, Jan. 1962; L. M. Horowitz, *J. Exptl. Psychol.* **62**, 51 (1962); G. A. Miller, *ibid.* **56**, 484 (1958).
2. N. G. Burton and J. C. R. Licklider, *Am. J. Psychol.* **68**, 650 (1955); W. R. Garner and D. H. Carson, *Psychol. Repts.* **6**, 123 (1960); G. A. Miller and E. A. Friedman, *Information & Control* **1**, 38 (1957); C. E. Shannon, *Bell System Tech. J.* **30**, 50 (1951). See also the review by W. R. Garner, *Uncertainty and Structure as Psychological Concepts* (Wiley, New York, 1962).
3. R. S. Bogartz and E. C. Carterette, *J. Exptl. Psychol.*, in press; D. D. Kluppel, dissertation, U.C.L.A., 1962.
4. E. B. Newman and L. J. Gerstman, *J. Exptl. Psychol.* **44**, 114 (1952); E. B. Newman and N. C. Waugh, *Information & Control* **3**, 101 (1960); C. E. Shannon, *Bell System Tech. J.* **27**, 379 (1948).
5. G. A. Miller and W. C. Madow, Air Force Cambridge Res. Center, *AFCRC-TR-54-75* (1954).
6. W. R. Garner and W. J. McGill, *Psychometrika* **21**, 219 (1956).
7. Texts were the Ginn and Company basic readers: *On Cherry Street* (1st reader, 1957 revision); *We Are Neighbors* (2nd reader, level I, 1957 revision); *Finding New Neighbors* (3rd reader, level I, 1957 revision); *Trails to Treasure* (5th reader, 1957 revision). The number of different words in the vocabularies of the readers were 326 (1st), 552 (2nd), about 1088 (3rd), and 2792 (5th).
8. See R. Seibel, "*N*-Gram (sequences of letters *N* long) analysis of English for *N* from 1 through 12," Eastern Psychol. Assoc. Mtg., April 1962, for some effects of sample size.
9. G. Dewey, *Relativ* (sic) *Frequency of English Speech Sounds* (Harvard Univ. Press, Cambridge, Mass., 1923), p. 185.
10. R. Jakobson, E. C. Cherry, M. Halle, *Language* **29**, 34 (1952).

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Ecdysone: Five Biologically Active Fractions from Bombyx

Abstract. *Five fractions of the growth and differentiation hormone, ecdysone, separated from extracts of* Bombyx *have been detected by bioassay. Three of these have not been described heretofore.*

Growth and metamorphosis of insects is controlled by the interaction of several hormones. Cholesterol has been identified in one of the biologically active extracts of brain from the silkworm that activate Dauer pupae (*1*). Farnesol and its oxidation product, farnesal, isolated from *Tenebrio* (*2*) have activity similar to that of the secretion of the corpora allata, neotenin or juvenile hormone (*2*). The growth and metamorphosis hormone, ecdysone, was originally isolated as α- and β-fractions, and crystallized (*3*); its chemical structure and biological activity have been the subject of further study (*4*). This hormone increases the rate of protein synthesis in mammalian cells (*5*). Puffs in the salivary chromosomes (*6*) were originally described in *Drosophila* (*7*) and the sequence of appearance delineated later (*8*). The relationship between the appearance of the puffs, hormonal secretion, and stage of metamorphosis has been studied by a number of investigators (*9*). We have confirmed (*10*) in *Drosophila* results obtained in *Chironomus* (*11*) in which the pattern of puffing in the salivary chromosomes is altered by ecdysone.

Ecdysone was extracted (*12*) from chrysalides of *Bombyx* obtained from Japan in ton lots. The method (*3*) was changed by utilizing *Calliphora* for bioassay to determine the distribution of the crude hormone between water and several immiscible organic solvents so that higher yields were obtained. Each bioassay was performed by injecting 10 μl of hormone into the posterior segment of each of 20 ligated *Calliphora* pupae with the anterior end pupated during the antecedent 24 hours. The degree of pupation of each individual was scored as 1.0, 0.75, 0.50, or 0.0 during the ensuing 24 hours and expressed as a percentage of the number surviving. After active crude extract was obtained, it was partitioned at first with a cyclohexane-butanol-water system. An ethyl acetate-water system later proved to be more satisfactory. By using a Craig counter-current machine with 200 transfers, active material was found, not only in the tubes expected for isolation of α- and β-ecdysone, but also farther along in the series of fractions. Careful bioassay revealed five peaks of activity separable not only by a threshold of 50 percent activity in the system of weighted bioassay but as low as 30 percent as well (Fig. 1). The same was true with the cyclohexane-butanol-water system.

Apparently there are five separate fractions that can be isolated from *Bombyx* at the stage of metamorphosis when the titer of crude hormone is highest (*13*). The γ-, δ-, and ε-fractions have not been noted previously. Theoretical *K* values (partition coefficients) for the compounds yielding this peak biological activity in the ethyl acetate-water system are 0.41, 0.96, 2.2, 6.2, and 50, respectively.

Separation of the hormone or hormones that bring about metamorphosis into individual components opens additional avenues for exploring the mechanics of growth and differentiation. Initial experiments indicate that crude material alters the pattern of growth of mammalian cells as well as accelerating the rate of protein synthesis in the cytoplasm (*14*); therefore study of the relationship between the action of invertebrate hormones and differentiation may be profitably extended to vertebrates. The results of studies in progress on possible differences between the effects of the fractions isolated should be of particular interest (*15*).

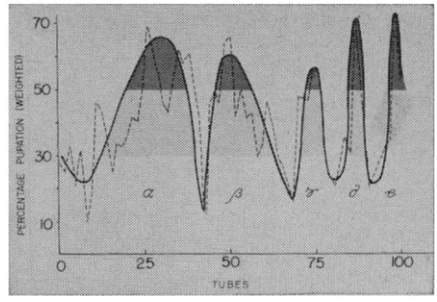

Fig. 1. Distribution of five fractions of the growth and metamorphosis hormone, ecdysone, detected by bioassay of fractions separated by counter-current distribution. Solid lines represent theoretical extrapolation of actual values (dotted lines). The figure depicts the 100-tube equivalent of a 200-tube run.

WALTER J. BURDETTE

Laboratory of Clinical Biology and *Department of Surgery, University of Utah College of Medicine, Salt Lake City*

MILON W. BULLOCK

Chemical Research and Development Laboratories, Agricultural Division, American Cyanamid Company, Princeton, New Jersey

References and Notes

1. M. Kobayashi, J. Kirimura, M. Saito, *Mushi* **36**, 85 (1962); *Nature* **195**, 515 (1962).
2. V. P. Schmialek, Z. *Naturforsch.* **16b**, 461 (1961); V. B. Wigglesworth, *J. Insect Physiol.* **7**, 73 (1961).
3. A. Butenandt and P. Karlson, *Z. Naturforsch.* **9b**, 389 (1954).
4. P. Karlson, *Angew. Chemie*, in press (1963).
5. W. J. Burdette and R. L. Coda, *Proc. Soc. Exptl. Biol. Med.* **112**, 216 (1963).
6. G. T. Rudkin, *Genetics* **40**, 593 (1955).
7. T. S. Painter, *ibid.* **20**, 301 (1935).
8. H. J. Becker, *Chromosoma* **10**, 654 (1959).
9. M. E. Breuer and C. Pavan, *ibid.* **7**, 371 (1955). W. Beerman, *Cold Spring Harbor Symp. Quant. Biol.* **21**, 217 (1956).
10. W. J. Burdette and R. S. Anderson, unpublished.
11. U. Clever and P. Karlson, *Exptl. Cell Res.* **20**, 623 (1960); U. Clever, *Chromosoma* **12**, 607 (1961).

12. The technical assistance of L. Paul, K. Nomura, R. Berndt, and G. Henkel is gratefully acknowledged.

13. W. J. Burdette, *Science* **135**, 432 (1962).
14. ———, *ibid.* **138**, 987 (1962); ——— and R. C. Richards, *Nature* **189**, 666 (1961).

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