phases of the prothoracic glands and corpora allata to epidermal secretions during embryonic molts in *Oncopeltus*. These activity phases were marked by glandular volume expansion (secretion), followed by contraction (quiescence), phenomena typical of glandular activity in postembryonic development (*18*). Using chromatographic and radioimmunoassay methods, Lageux *et al.* (*19*) detected well-defined peaks of ecdysone titer in *Locusta migratoria* embryos that correlated with cuticular apolysis, an indication of hormonal regulation of embryonic molting. Because of the mutual interaction between prothoracic gland and corpora allata functions during insect larva development (*18*), JH may well assume a parallel indispensability during embryonic ecdysis.

Kaplanis *et al.* (*20*) found that *M. sexta* eggs 0 to 24 hours old were devoid of molting hormone, whereas older eggs yielded relatively large quantities of 26-hydroxy-α-ecdysone. These data and ours reveal an apparent correlation between the appearance of molting hormone and juvenile hormone in *M. sexta* embryos (*5*, *20*). The major embryonic molting hormone differs chemically from that found in larvae (*21*) and pupae (*22*); we see an analogous situation with JH, where the major constituents of embryonic JH's (0 and I) are distinct from those of larval (*5*) (JH II, lesser amount of JH I) and adult female (*5*) (JH II and JH III) JH in *M. sexta*. It is noteworthy that JH 0, biochemically the most unusual of the JH's, occurs in *M. sexta* only in the ontogenetically primitive stages.

It appears that embryonic development in insects requires a functioning endocrine system, similar to that demanded for postembryonic development. However, in embryos of *M. sexta*, structural alteration in the respective hormones could imply that biosynthetic enzyme systems of differing precursor specificities are present. Whether the structural differences are in fact obligatory during embryogenesis or simply reflect a fortuitous biosynthetic occurrence has yet to be resolved.

B. JOHN BERGOT
GENE C. JAMIESON
MILTON A. RATCLIFF
DAVID A. SCHOOLEY
Zoecon Corporation Research Laboratory, Palo Alto, California 94304

References and Notes

1. D. A. Schooley, in *Analytical Biochemistry of Insects*, R. B. Turner, Ed. (Elsevier, Amsterdam, 1977), p. 241.
2. K. H. Trautmann, M. Suchy, P. Masner, H.-K. Wipf, A. Schuler, in *The Juvenile Hormones*, L. I. Gilbert, Ed. (Plenum, New York, 1976), p. 118.
3. D. A. Schooley, K. J. Judy, B. J. Bergot, M. S. Hall, J. B. Siddall, *Proc. Natl. Acad. Sci. U.S.A.* **70**, 2921 (1973).
4. B. Lanzrein, M. Hashimoto, V. Parmakovich, K. Nakanishi, R. Wilhelm, M. Lüscher, *Life Sci.* **16**, 1271 (1975).
5. B. J. Bergot, A. A. Furrer, M. S. Hall, K. J. Judy, D. A. Schooley, unpublished data.
6. B. J. Bergot, M. A. Ratcliff, D. A. Schooley, *J. Chromatogr.*, in press.
7. R. J. Anderson, V. L. Corbin, G. Cotterrell, G. R. Cox, C. A. Henrick, F. Schaub, J. B. Siddall [*J. Am. Chem. Soc.* **97**, 1197 (1975)] synthesized the 2*E*,6*E*,10-*cis* and 2*E*,6*E*,10-*trans* isomers of JH 0, together with related substances, as analogs of JH I and JH II, using a stereoselective synthetic route that allowed assignment of geometrical configuration.
8. R. J. Liedtke and C. Djerassi, *J. Org. Chem.* **37**, 2111 (1972).
9. F. J. Ritter, I. E. M. Brüggemann-Rotgans, P. E. J. Verwiel, C. J. Persoons, E. Talman, *Tetrahedron Lett.* **1977**, 2617 (1977).
10. H. Röller, K. H. Dahm, C. C. Sweeley, B. M. Trost, *Angew. Chem. Int. Ed. Engl.* **6**, 179 (1967).
11. A. Pfiffner, in *Aspects of Terpenoid Chemistry and Biochemistry*, T. W. Goodwin, Ed. (Academic Press, New York, 1971), p. 94.
12. T. Koyama, K. Ogura, S. Seto, *Chem. Lett.* **1973**, 401 (1973).
13. E. D. Morgan, K. Parry, R. C. Tyler, *Insect Biochem.*, **9**, 117 (1979).
14. E. D. Morgan and L. J. Wadhams, *J. Insect Physiol.* **18**, 1125 (1972).
15. G. B. Staal, *Pontif. Accad. Sci., Scripta Varia* **41**, 333 (1977).
16. L. I. Gilbert and H. A. Schneiderman, *Gen. Comp. Endocrinol.* **1**, 453 (1961), using bioassay techniques, observed the presence of JH-like material in extracts of developing embryos whose progenitors were allatectomized females. No JH could be detected in corresponding unfertilized eggs; the authors then concluded that the embryos synthesize JH in an unspecified fashion.
17. A. Dorn, *Z. Morphol. Tiere* **71**, 52 (1972).
18. V. J. A. Novak, *Insect Hormones* [Wiley, New York, ed. 4 (2nd English edition), 1975], p. 219.
19. M. Laguex, C. Hetru, F. Goltzene, C. Kappler, J. A. Hoffmann, *J. Insect Physiol.* **25**, 709 (1979).
20. J. N. Kaplanis, W. E. Robbins, M. J. Thompson, S. R. Dutky, *Science* **180**, 307 (1973).
21. W. E. Bollenbacher, W. V. Vedeckis, L. I. Gilbert, J. D. O'Connor, *Dev. Biol.* **44**, 46 (1975).
22. M. J. Thompson, J. N. Kaplanis, W. E. Robbins, R. T. Yamamoto, *J. Chem. Soc. Chem. Commun.* **1967**, 650 (1967).
23. We thank M. Sharon Hall for collecting and synchronizing *M. sexta* eggs, and NSF for partial financial support (PCM 79-18307).

14 April 1980; revised 24 June 1980

Swelling of Nerve Fibers Associated with Action Potentials

Abstract. *Swelling of nerve fibers during the action potential was demonstrated by three different methods. Generation of a propagated nerve impulse in a crab nerve produced an outward movement of 50 to 100 angstroms of the nerve surface and a rise in swelling pressure on the order of 5 dynes per square centimeter. In squid giant axons, the amplitude of the observed outward movement of the surface was small.*

We have demonstrated a small movement of the nerve surface associated with the propagation of an action potential. In spite of reports by previous investigators (*1*) on this subject, to our knowledge the existence of such changes has never been substantiated. In particular, there has been no unequivocal demonstration whether it is a swelling or a shrinkage. Obviously, extremely small, transient movements of soft tissues (*2*) have escaped reliable measurements. Using three independent methods, two optical and one mechanoelectrical, however, we have succeeded in obtaining evidence for the occurrence of a rapid and transient swelling of the crab nerve accompanied by excitation. A preliminary experiment on squid giant axons indicated the existence of a similar mechanical change at the surface.

One of the methods used was to modulate the intensity of the light transmitted from a source to a photodetector by the movement of a small object placed on the nerve (Fig. 1A). The light source was a 100-W quartz-iodine lamp (Osram). The light from the lamp was led to the surface of a nerve by means of a bundle of four plastic fiber optics (each 0.12 μm in diameter). In most cases, claw nerves of the crab *Callinectes sapidus* were used. The chamber in which optical measurements were carried out was so designed that a small tension could be applied to the nerve. A small (about 3 mg)

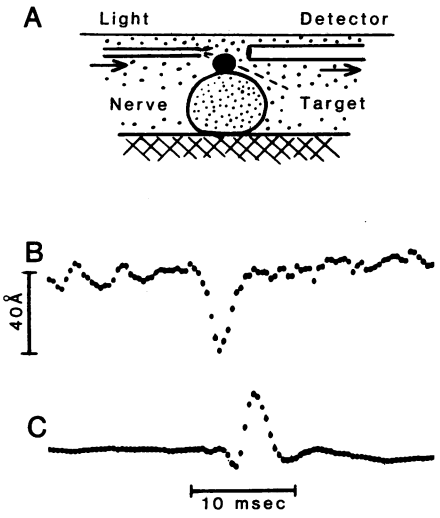

Fig. 1. (A) The method of detecting a small movement of the light-obstructing target on a crab nerve associated with production of action potentials. (B) Transient decrease in the light intensity (an upward movement of the target) produced by nerve stimulation. (C) Extracellularly recorded action potential. Brief stimulating pulses were delivered at the time marked by the beginning of the time marker. The temperature of the preparation was 21°C.

0036-8075/80/1017-0338$00.50/0

piece of platinum ("target") was placed in the middle of the de-sheathed portion of the nerve. The light beam, partially blocked by the target, was led to a photodetector (Pin-10, United Detector Corp.) with a bundle of fiber optics about 0.5 mm in diameter. The nerve, as well as the tips of the fiber optics, was kept beneath the surface of the seawater in the chamber. The output of the photodiode was amplified with a capacity-coupled amplifier, and a transient change in the light intensity was recorded with a signal averager (model SW71B, Nicolet Instrument). Stimulating current pulses about 0.1 msec long were delivered to the nerve near one end; the action potentials induced were recorded extracellularly near the other end.

The optical signal in Fig. 1, B and C, is an example of the records obtained after averaging 128 trials. A transient reduction in the light intensity was associated with the production of a propagated nerve impulse. The magnitude of the displacement was calculated by measuring the output (d-c) voltage of the photodiode as a function of the distance between the nerve surface and the fiber optics. (The two bundles of fiber optics were held together and moved with a heavy-duty micromanipulator.) The optical signals obtained were produced by a transient upward movement of the target, bringing about an increased obstruction of light. The peak value of the displacement was 40 to 80 Å. From the latency and duration of the optical signals, the displacement of the surface of the nerve was estimated to be roughly simultaneous with the action potential at the site of the target.

In one series of experiments, we used an optical sensor (Fotonic, model KD1104, Mechanical Technology, Inc.) in conjunction with an oscilloscope. This sensor consists of a bundle of fiber optics for transmitting light from the source to the light-reflecting target and another bundle for carrying light from the target to the photodiode; all the fibers are thoroughly mixed at the sensing end. Measured with this sensor, the intensity of the light detected by the photodiode changed with the distance between the target and the sensing tip. Again, the nerve under study was placed in a chamber filled with seawater in such a manner that the lower surface of the nerve was pressed against the bottom of the chamber. A small piece of platinum (approximately 3 mg) was placed on the curved top of the nerve and used as the light-reflecting target. The light source and the photodetector were the same as those described. When crab nerves were examined by this method, clear signs of swelling of the nerve fiber during excitation were observed. The upward movement of the nerve surface obtained by this method varied between 50 and 100 Å. This finding is consistent with that obtained with the first (light-modulation) method.

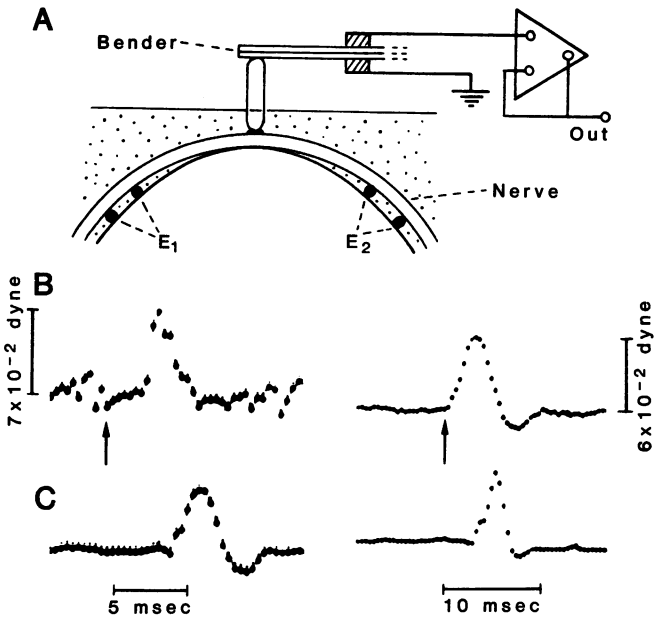

Fig. 2. (A) Measuring a small change in the swelling pressure of the nerve fibers during action potentials; E_1 and E_2 represent electrodes used for stimulating the nerve and for recording electric responses extracellularly. (B) Mechanical and (C) electrical responses of crab nerve fibers. The arrows indicate when brief stimulating shocks were delivered. The preparation was maintained at 21° to 22°C.

The appearance of an upward movement of the target on the nerve indicates that a positive pressure is developed inside the nerve fibers during excitation. An attempt was made to detect this pressure change by using a piezoelectric transducer, a bender element (model R050, Gulton) (Fig. 2A). Near the end of an about 8-mm-long movable portion of the bender, a loop of bristles was attached. A small disk (0.01 cm^2) was glued at the lower end of the loop. A heavy-duty micromanipulator was used to lower the loop slowly to the surface of the nerve. The chamber was essentially the same as that already described. The bender was connected first to a voltage-follower (model AD515, Analog Devices). The output of the follower was led to a capacity-coupled amplifier and, finally, to a signal averager.

Figure 2, B and C, shows two examples of the records obtained with a piezoelectric bender. The upward deflection of the upper trace represents the appearance of an upwardly directed force on the order of 2×10^{-2} to 8×10^{-2} dyne. Since the area of contact between the nerve and the loop of bristles was about 0.01 cm^2, we estimated the increment of the pressure observed at the peak of nerve excitation to be between 2 and 8 dyne/cm^2. From these observations, we concluded that the result obtained by the piezoelectric method is consistent with those acquired with the optical methods.

We are concerned primarily with the reliability of our optical and mechanoelectric techniques. All three methods we have used yielded consistent results, both in time course and in magnitude, which indicates the occurrence of swelling of nerve fibers associated with the development of an action potential. The results we obtained disagree with those described by previous investigators; the reason for this disagreement will be discussed elsewhere.

Further experimental studies are required in order to clarify the significance of the phenomenon of swelling described in this report, which might be compared to swelling of polyelectrolyte gels and of charged membranes studied by a number of physical chemists (*3*).

KUNIHIKO IWASA
ICHIJI TASAKI
ROBERT C. GIBBONS
Laboratory of Neurobiology, National Institute of Mental Health, Bethesda, Maryland 20205, and *Marine Biological Laboratory, Woods Hole, Massachusetts 02543*

References

1. L. P. Kayushin and P. G. Lyudkovskaya, *Dokl. Akad. Nauk SSSR* **95**, 253 (1954); *ibid.* **102**, 727 (1955); J. Lettvin, personal communication; paper presented in a seminar at the Marine Biological Laboratory, Woods Hole, Mass., August 1979; B. C. Hill, E. D. Schubert, M. A. Nokes, R. P. Michelson, *Science* **196**, 426 (1977).
2. S. M. Khanna, J. Tonndorf, W. W. Walcott, *J. Acoust. Soc. Am.* **44**, 1555 (1968).
3. P. H. Hermans, in *Colloid Science*, H. R. Kruyt, Ed. (Elsevier, New York, 1949), pp. 483-650; A. Katchalsky and I. Michaeli, *J. Polym. Sci.* **15**, 69 (1955); H. P. Gregor, *J. Am. Chem. Soc.* **73**, 642 (1951).

14 May 1980