egg but prior to cell membrane formation, over these "blastema" nuclei (Fig. 3). Synthetic activity in the future germ cells (pole cells) is either lower than that of other cells, or is asynchronous with other synthetic activities.

In contrast, leucine and phenylalanine are incorporated into all basophilic regions of the egg, including the cortex, from early cleavage onward—that is, 8 to 24 hours before the arrival of the nuclei in the cortex (Fig. 4). Amino acid incorporation increases two- to three-fold after the nuclei reach the cortex (Fig. 5). No local differentiation of this activity has as yet been detected.

Results of administration of antimetabolites, followed by histological comparison of the injected eggs to uninjected eggs from the same clutch, have confirmed these findings, in that puromycin (7 ng/mg live weight of the egg) stops development within minutes of being injected. Neither actinomycin D (0.02 to 0.2 ng/mg) nor tetracycline (8.4 ng/mg) appear to interfere with development prior to the late blastema stage. Thus, the evidence suggests that the Coleopteran egg early in development is capable of synthesizing protein in the absence of concomitant release of new information in the form of newly synthesized RNA. New information does not appear to be released until the migrating nuclei establish functional and physical contact with the highly differentiated regions of the peripheral cytoplasm. The techniques now available permit analysis of the interactions which occur at this time.

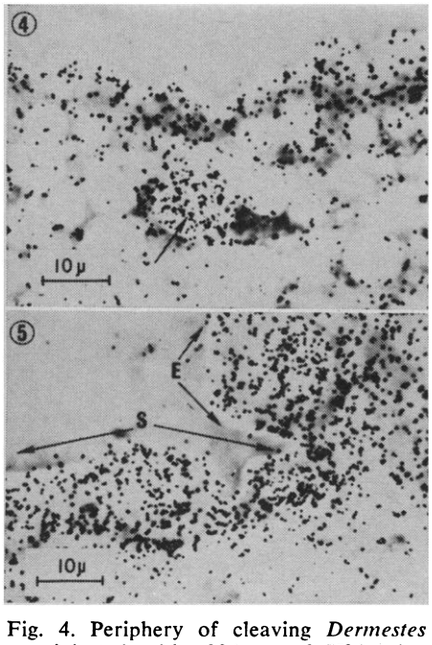

Fig. 4. Periphery of cleaving *Dermestes* egg injected with .004 μc of H^3-leucine and fixed 2 hours later. All basophilic regions of the cytoplasm incorporate the amino acid, even though nuclei (arrow) have not reached the periphery. Incorporation may also be seen during very early cleavage stages. Exposure time, 20 days. Fig. 5. Differentiating embryonic (*E*) and serosal (*S*) regions of *Dermestes* egg treated as described in Fig. 4. No spatial differentiation in the pattern of incorporation is distinguishable.

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References and Notes

1. F. Seidel, E. Bock, G. Krause, *Naturwissenschaften* **28**, 433 (1940); R. C. von Borstel, in *The Beginnings of Embryonic Development*, A. Tyler, R. C. von Borstel, C. B. Metz, Eds. (AAAS, Washington, D.C., 1957), p. 175; G. Krause, *Ergeb. Biol.* **20**, 159 (1958); S. J. Counce, *Ann. Rev. Entomol.* **6**, 295 (1961); I. Agrell, in *The Physiology of the Insecta*, M. Rockstein, Ed. (Academic Press, New York, 1964), p. 91.
2. A. Ewest, *Arch. Entwicklungsmech. Organ.* **135**, 689 (1937); A. Kanellis, *ibid.* **145**, 417 (1952).
3. A. Haget, *Bull. Biol. France Belgique* **87**, 123 (1953).
4. E. Mahr, *Arch. Entwicklungsmech. Organ.* **152**, 662 (1961); G. Krause and K. Sander, in *Advances in Morphogenesis*, M. Abercrombie and J. Brachet, Eds. (Academic Press, New York, 1962), vol. 2, p. 259; W. Schnetter, *Arch. Entwicklungsmech. Organ.* **155**, 637 (1965).
5. D. L. Hill, *Drosophila Inform. Serv.* **19**, 62 (1945).

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Rate of Movement and Redistribution of Stainable Neurosecretory Granules in Hypothalamic Neurons

Abstract. *Electrical stimulation of the olfactory tract of goldfish for one minute can deplete completely the stainable neurosecretory granules from cells of the preoptic nucleus as well as from their axons. Thus, in stimulated neurons secretory granules appear to move toward the neurohemal point of discharge at a rate of about 2 millimeters per minute. Reaccumulation of neurosecretory granules in depleted neurons to approximately normal numbers requires about 1 to 1.5 hours. Histological evidence indicates that, during the period of reaccumulation, granules move out of the perikaryon until normal granulation in the axons is achieved; finally, granulation of the perikaryon is restored.*

Neurosecretory cells, defined as a class, are neurons which end at a vascular space rather than at a synapse or upon an effector, as is more usual. Their secretory substance (or its "carrier") is stainable and usually appears to be in granular or droplet form (*1*); it is thought to be transported by axoplasmic flow toward the axonal terminals (*2*).

It is important to know whether the intracellular rate of movement of the neurosecretory substance is consistent with the known rates characteristic of physiological neuroendocrine responses, yet such information is difficult to obtain. Calculations based on indirect evidence (*3*) are tentative; observations of granular movements in living neurons (*4*) remain open to question since only limited areas of the axons can be studied and there is no certainty that what is being observed is, in fact, the neurosecretory substance or even the neurosecretory axon.

An opportunity to estimate rates of movement of neurosecretory granules developed from experiments in which the olfactory tract of goldfish was stimulated electrically. Sixty seconds of such stimulation evoked a complete "emptying" of neurosecretory granules from cells in the preoptic nucleus and from their axons. Because of the brevity and precision of the event it offered a means for relatively close approximation of rates of passage of these granules. Furthermore, with a planned delay between effective stimulation of the olfactory tract and death of the fish, the rate and pattern of reaccumulation could apparently be studied.

Thirty-eight goldfish immobilized with gallamine triethiodide (4 mg/kg) were wrapped in wet cloths and placed in a specimen-shaped holder of aluminum after dorsal exposure of their brains. Dechlorinated water was kept continuously flowing through their mouths and over their gills. Electrical stimuli (Grass S4 stimulator, 2 volt, 3 msec, 10 cy/sec) were delivered to the

exposed olfactory tract for total periods varying between 15 seconds and 10 minutes, with a single steel microelectrode on the tract and the grounded electrode in the saline solution bathing the brain. Tissues were fixed in Bouin's fluid, either immediately after stimulation, or after delays of up to 90 minutes, which permitted various degrees of recovery. Halmi's modification of Gomori's paraldehydefuchsin technique was used to stain hypothalamic neurosecretory material.

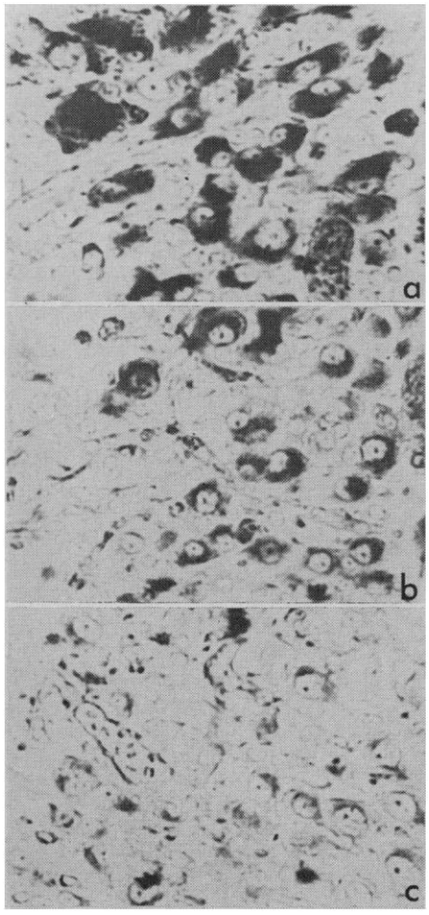

Fig. 1. Preoptic nuclei of three goldfish: (a) an untreated control; (b) and (c) were stimulated electrically through an isolation unit and electrode applied to one olfactory tract. Stimulus characteristics: 3 volt, 4-msec duration, repeated 10 cy/sec for 60 seconds. Tissues were stained by Halmi modification of Gomori's paraldehydefuchsin technique. In each photograph juxtasomal axons are at the left. In (b) some perikarya are almost completely degranulated, while others contain a reduced number of stainable neurosecretory granules. Compared to (a), it is clear that fewer axons contain stainable neurosecretions. In (c) most perikarya contain no stainable neurosecretion, but a few contain a very reduced number of granules near the nucleus. In (c) no granules are visible in the axons.

Stimulation for 15 seconds produced incomplete degranulation of the neurons of the preoptic nucleus; stimulation for 60 seconds evoked variable, but advanced, degrees of degranulation (Fig. 1, a–c), and in about one-third of the goldfish so treated, there was complete degranulation of cell bodies and axonal tracts, but not of the terminal axonal tips. After having been stimulated for 10 minutes, perikarya and axons generally contained no stainable granules, but there was still a recognizably reduced amount of stainable neurosecretion in the axonal terminals in the neurohypophysis.

Delays after stimulation, and before killing of the animal and fixing of its brain, permitted restoration of stainable neurosecretion. Delays as brief as 15 minutes were associated with visibly increased amounts of granulation. Delays of 60 minutes or more most often permitted reattainment of patterns of granular distribution approaching those of the untreated control, or "sham-operated" animal.

Despite the variety of durations of stimulation and periods of delay before fixation, the patterns of distribution of neurosecretory granules observed almost always could be represented by the alternatives diagrammed in Fig. 2.

Figure 2a indicates the condition in control goldfish, or in inadequately stimulated fish, or in specimens permitted a sufficiently long period of recovery. In these cases, perikarya, axons near the perikarya, axons in the hypothalamus, and hypophysial tracts all were well granulated. Figure 2b represents a minimal response consisting mostly of a visible depletion in the cell body, and to some extent in the juxtasomal axon. More complete degranulation of the soma, represented in Fig. 2c, was associated with agranular juxtasomal axons and depletion of axons in the tract. When the soma was completely degranulated (Fig. 2d), the entire axon was generally agranular, except for the tip. Less commonly, in such extreme instances, a slight degree of granulation remained in the tract region (Fig. 2d).

These patterns, observed under the various conditions specified, together with the fact that patterns of both depletion and recovery are similar, permit the following conclusions.

1) Stimulation for 60 seconds can deplete all visible neurosecretory granules from the soma and all parts of the

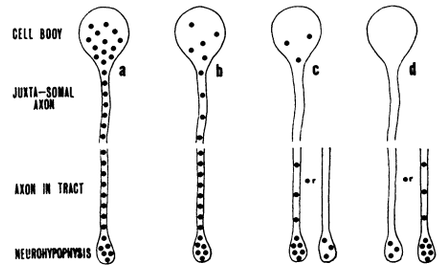

Fig. 2.

axon except the endings in the neurohypophysis. After stimulation for as long as 10 minutes, there is still much neurosecretory material in the endings, though it is reduced. Accordingly, perikaryal depletion and passage of granules through the entire length of the axons (about 2 mm) are relatively rapid processes that may be completed in a minute or less. Discharge of neurosecretory material from the axonal termination may or may not be just as rapid, but the store of material in the endings is sufficient so that 10 minutes of continuous stimulation only partly depletes it.

2) Delay in fixation after stimulation permits reaccumulation of neurosecretory material in the secretory neurons. As this occurs, over a 60- to 90-minute period, patterns of granular distribution are found that are the inverse, with time, of those found immediately after stimulation. That is, granules first appear to reaccumulate in the distal (tract) portions of the axons, then in the juxtasomal region, and finally in the perikaryon. This finding suggests that during the period of recovery after stimulation, granules produced in the perikaryon (*5*) leave as rapidly as they are formed and are transported toward the neurohypophysis. After the "storage" region in the neurohypophysis is restocked with a characteristic number of granules, then reaccumulating granules gradually fill the axon in a direction toward the soma. This suggests also that the synthetic process is not continuous and that it is halted when a particular level of reaccumulation in the soma is attained. Because of the rapidity of granule movement toward the axonal terminals, it is clear that the entire neuron—not just the terminal—can be used for "storage" of neurosecretory material. The somal and axonal stores are only the most visibly labile, and variation in amount of cytoplasmic accumulation may initiate or arrest the

process of new synthesis of neurosecretory granules.

3) This method of emptying the neurosecretory axons within a very short, experimentally controlled period of time offers many advantages for the study of the dynamic aspects of the neurosecretory process.

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References and Notes

1. H. A. Bern and I. R. Hagadorn, *Structure and Function in the Nervous Systems of Invertebrates*, T. H. Bullock and G. A. Horridge, Eds. (Freeman, San Francisco, 1965).
2. B. Hanstrom, *Forhandl. K. Fysiograf. Sallsk. Lund* **25**, 89 (1955).
3. J. D. Green and D. S. Maxwell, *Comparative Endocrinology*, A. Gorbman, Ed. (Wiley, New York, 1959).
4. W. Hild, *Z. Zellforsch.* **40**, 257 (1954); D. B. Carlisle, *International Symposium on Neurosecretion, 2nd*, W. Bargmann, Ed. (Springer-Verlag, Berlin, 1958).
5. It is assumed here that neurosecretory granules are formed in the perikaryon. However, these experiments offer no direct evidence concerning this question.
6. This work was supported by NIH grant NB-04887.
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Neurosecretory Cell: Capable of Conducting Impulse in Rats

Abstract. *Electric stimulation of the rat neurohypophysis in situ evoked the unit action potentials in the supraoptic nucleus. Compound and unit action potentials were recorded from the isolated neurohypophysis after electric stimulation. The application of solutions high in potassium or devoid of sodium made the neurohypophysis inexcitable. Mammalian neurosecretory cells have the neuronal properties of generation and conduction of action potentials.*

As discussed by Bern and Yagi (*1*), the question of whether mammalian neurosecretory cells have the ability to generate and conduct action potentials has not yet been solved. The mammalian neurohypophysis contains a very small number of nerve fibers; these range from sympathetic nerve fibers running with the posterior median hypophysial artery into the neurohypophysis (*2*), to the nerve fibers running within the hypothalamo-neurohypophysial tract originating in hypothalamic nuclei other than the supraoptic and paraventricular nuclei which send the neurosecretory fibers into the neurohypophysis. Moreover, not all neurons in the supraoptic and paraventricular nuclei are neurosecretory ones (*3*). Because of this anatomical situation, the possibility that the recorded impulses from supraoptic or paraventricular nuclei (*4*) and from the stalk of the neural lobe (*5*) might be derived from non-neurosecretory cells has not been excluded. In the present study we aim to indicate the capability of impulse conduction of rat neurosecretory cells by recording the antidromically conducted impulses from the supraoptic nucleus of the hypothalamus after electric stimulation of the neural lobe. The experiment on the isolated neurohypophysis was also conducted to examine the excitable nature of the membrane of neurosecretory cells.

For the in vivo experiments, the areas of the hypothalamus and hypophysis of five female rats anesthetized by intraperitoneal injection of urethane (1.5 g per kilogram body weight) were exposed from the ventral side with minimum bleeding. The rat was placed on its back, and artificial respiration was employed after an intramuscular injection of about 0.6 mg of tubocurarine chloride. With this procedure, locations of recording and stimulating electrodes can be identified under the dissecting microscope (*6*). For the in vitro experiments, 11 albino rats (body weight, 160 to 280 g) of both sexes were anesthetized and decapitated for removal of the neural lobe. The isolated neural lobe and a small piece of the attached stalk were placed in a vessel containing about 10 ml of Locke's solution. The solution was kept at 37°C and aerated continuously.

A stimulating current of spike shape (duration, 0.07 msec) was applied by an Aika E-1 stimulator attached to a pair of bipolar electrodes of silver wire, insulated by a glass capillary of about 0.3 mm OD. Bipolar electrodes of the same type were used for recording compound action potentials in vitro. Unipolar recording of unit action potentials was made with a silver microelectrode, similar to that used by Yagi and Bern (*7*), which was connected to an amplifier through a cathode follower. The action potentials were amplified by an EB-10 amplifier and displayed on a BO-207 cathode ray oscilloscope.

Single-unit action potentials could be induced in the supraoptic nucleus by stimulating the neural lobe in vivo (Fig. 1B). Some units, however, did not respond to the stimulation. The duration of unit spikes was 1 to 2 msec. A rough estimation of the velocity of antidromic conduction along the hypothalamo-neurohypophysial tract was 1 m/sec. This figure is comparable to the values of 0.5 m/sec in fish hypothalamic neurosecretory fibers (*8*), 1 m/sec in fish caudal neurosecretory fibers (*9*), and 0.6 to 1.4 m/sec in the cat hypothalamo-neurohypophysial tract (*5*). Our estimate includes errors arising from regarding the latent time between the stimulus and the spike potential as conduction time and from assuming the surface distance to be the length of the fiber. Changes in spontaneous firing frequencies were observed in these units after an infusion of hypertonic or hypotonic solution. The presence of antidromic responses indicates that the observed unit action

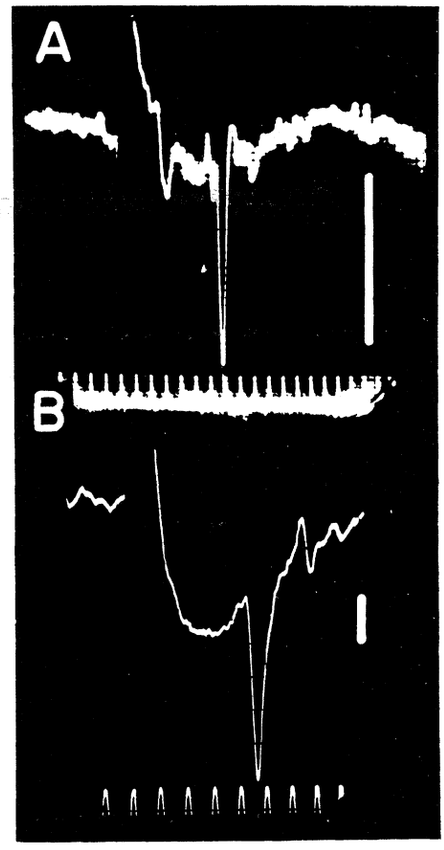

Fig. 1. Unit action potentials recorded from the isolated neural lobe (A) and from the supraoptic nucleus (B). (A) An electric shock of 55 volts was applied to the stalk region of the neurohypophysis in vitro. Vertical calibration indicates 50 μv. (B) An example of impulses conducted antidromically along the hypothalamo-neurohypophysial tract. Stimulation of 15 volts was given to the neural lobe in situ. Vertical line represents 100 μv. Time marks are equal to 1 msec.