Changes in the Histochemical Nature of Neurosecretory Materials During Axonal Transport in the Crab *Paragrapsus gaimardii*

Histochemical evidence of modification or transformation of neurosecretory material during its transport from the cell perikarya to axonal endings in the neurohaemal organs is available for frogs[1] and insects[2]. The technique used in these studies was PEUTE's[3] modification of RAVETTO's[4] alcian yellow/alcian blue procedure. This technique distinguishes between the weakly acidic glycol groups and the strongly acidic S–S and SH groups present in the neurosecretory material. In frogs[1] and insects[2], a yellow colour was observed in the neurosecretory material at or near the cell perikarya, indicating the presence of strong acidic groups, while at the axonal endings, the colour was blue-green, indicating the presence of weak acidic glycol groups.

From work on the eyestalks of decapod crustaceans, there is evidence that the chemical nature of the neurosecretory material changes after leaving the perikarya, but before its release into the haemolymph[5–10].

Materials and methods. Mature crabs of the species *Paragrapsus gaimardii*, of both sexes, at inter-moult were used. The eyestalks of the crabs, without the exoskeleton, were fixed in Masson's bouin[11] and embedded in paraplast. Serial sections, 7–8 μ thick, were cut and stained with either the paraldehyde-fuchsin technique[12] or with the alcian yellow/alcian blue technique[3]. The latter technique was also applied to sections of the pericardial organs and thoracic ganglia of *Paragrapsus gaimardii* and also to sections of the brain, corpora cardiaca and corpora allata of the insects *Periplaneta americana* and *Enithares woodwardi*.

Results and discussion. In the eyestalk, neurosecretory material from the medulla terminalis X-organ is transported to the sinus gland by the distinct sinus gland tract. An increase in the basophilia of the secretory material during its axonal transport was observed, using the paraldehyde-fuchsin technique.

With the alcian yellow/alcian blue technique, the secretory material in many of the cells of the medulla terminalis X-organ was stained blue. Axonal neurosecretory material, notably in the sinus gland tract, was stained yellow-green and green. In the sinus gland, 3 types of secretory materials were present. Almost all of the secretory material consisted of large green globules (8–10 μ in diameter) and medium-sized, yellow-green globules (4–5.5 μ in diameter). A few small light-blue globules were also observed near the basement membrane of the sinus gland. The actual site of the major part of the modification of the neurosecretory material appears to be in the sinus gland tract and within the sinus gland itself.

These results suggest that the neurosecretory material in the perikarya of the neurosecretory cells contains weak acidic glycol groups and that of the axonal endings contains strong acidic S–S or SH groups. This is further confirmed by other histochemical tests: diastase-removable, PAS-positive material is clearly present in the cell perikarya of the medulla terminalis X-organ but not to any great extent in the sinus gland. The performic acid–alcian blue test[13] reveals a mild reactivity for S–S groups in the cell perikarya and a strong reaction in the sinus gland.

The pericardial organs of the Decapoda have been shown to release materials which have a powerful excitatory effect on the heart[14,15]. These materials may be indole amines[15,16] but polypeptides are also present[15,17]. The neurosecretory material is synthesized in the 'B' and 'C' cells of the thoracic ganglion and is transported from them via the first thoracic segmental nerves, to the pericardial organs[18].

With the alcian yellow/alcian blue technique, the secretory granules in the 'C' cells stained an intense blue, and the finer granules of the 'B' cells stained a light blue. Axonal secretory material in the thoracic ganglion and the pericardial nerves stained greenish-blue. Neurosecretory material in the axonal terminals of the pericardial organs consisted of fine granules which stained green to yellowish-green. Thus the modification of the neurosecretory material in the pericardial organ system is similar to that occurring in the medulla terminalis X-organ sinus gland system though it is not as marked.

As a control measure, insect material was stained with the alcian yellow/alcian blue technique. In both insects, the neurosecretory material became less acidic as it was transported axonally. These results agree with those of GABE[2]. Thus there appear to be fundamental differences in the process of axonal modification or transformation of neurosecretory material, as revealed by histochemical techniques, between the crab *Paragrapsus gaimardii* and both insects and frogs. The direction of the process of modification of the neurosecretory material in the eyestalk and the pericardial neurosecretory systems in *Paragrapsus gaimardii* appears to be the reverse of that occurring in insects and frogs[19].

Résumé. Dans le système neurosécréteur du pédoncule oculaire et les organes péricardiques du crabe *Paragrapsus gaimardii*, la direction du processus de modification du produit de neurosécrétion durant le cheminement axonal semble être de sens opposé à celle qu'on observe chez les insectes et les grenouilles.

P. S. LAKE

Department of Zoology, University of Tasmania, Hobart (Australia), 9 July 1969.

[1] J. PEUTE and J. C. VAN DE KAMER, Z. Zellforsch. mikrosk. Anat. *83*, 441 (1967).
[2] M. GABE, C. r. hebd. Seanc. Acad. Sci., Paris *264*, 943 (1967).
[3] J. C. VAN DE KAMER, in *IV Internat. Symp. Neurosekretion* (Ed. F. STUTINSKY; Springer-Verlag, Berlin 1967), p. 95.
[4] C. RAVETTO, J. Histochem. Cytochem. *12*, 44 (1964).
[5] F. G. W. KNOWLES and D. B. CARLISLE, Biol. Rev. *31*, 396 (1956).
[6] D. B. CARLISLE, Nature *182*, 33 (1958).
[7] D. B. CARLISLE, J. mar. biol. Ass. U.K. *38*, 381 (1959).
[8] M. REHM, Acta Histochem. *7*, 88 (1959).
[9] F. G. W. KNOWLES, Endeavour *14*, 95 (1955).
[10] C. HUMBERT, Trav. Inst. scient. chérif., Sér. Zool. *32*, 1 (1965).
[11] N. C. FOOT, Stain Technol. *8*, 101 (1933).
[12] A. B. EWEN, Trans. Am. microsc. Soc. *81*, 94 (1962).
[13] C. W. M. ADAMS and J. C. SLOPER, J. Endocrin. *13*, 221 (1956).
[14] J. S. ALEXANDROWICZ and D. B. CARLISLE, J. mar. biol. Ass. U.K. *32*, 175 (1953).
[15] D. M. MAYNARD and J. H. WELSH, J. Physiol. *149*, 215 (1959).
[16] D. B. CARLISLE, Biochem. J. *63*, 32P (1956).
[17] F. A. BELAMARICH, Biol. Bull. Woods Hole. *124*, 9 (1963).
[18] D. M. MAYNARD, Gen. comp. Endocrin. *1*, 237 (1961).
[19] This study was financed by a University of Tasmania research grant. I wish to thank Mrs. L. LOWREY and Mr. B. DIXON for technical assistance, Prof. B. JOHNSON for discussion and criticism of the manuscript and Dr. I. LANSBURY of the Hope Department of Entomology, University Museum, Oxford, England, for identifying the notonectid.