**HISTOCHEMISTRY OF THE MUCOSUBSTANCES IN THE
GIANT NEUROSECRETORY CELLS IN THE THORACIC
GANGLION OF THE ADULT CRAB, *POTAMON*
MAGNUM MAGNUM (PRETZMAN)**

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The neurosecretory material of the giant cells, with and without axons, in the thoracic ganglion of the fresh-water crab *Potamon magnum magnum*, was studied. The results showed that the mucosubstances present in the secretory material in both cell types are acidic sulphated mucins. Further, the alcian blue-PAS test showed that the secretion is made up of periodate-reactive and alcianophilic components. The different alcian blue techniques and benzidine reaction used supported the presence of strongly acidic sulphated mucins.

A large number of morphological and histophysiological studies have been made on the neurosecretory system of decapod Crustacea (Enami, 1951; Bliss, Durand and Welsh, 1955; Scharrer, 1955; Knowles and Carlisle, 1956; Miyawaki, 1956a, 1960b, c; Matsumoto, 1958; Carlisle and Knowles, 1959; Szudarsky, 1963; Baid *et al.* 1966, 1968; Kato and Kamemoto, 1967, 1969; Kamemoto and Tullis, 1972; Tullis and Kamemoto, 1974 and Gorgees and Rashan, 1976).

In contrast, comparatively few investigations have been carried out to determine the chemical nature of the neurosecretory material in Crustacea. These studies include the work of Gabe, 1952a, b; Miyawaki, 1956a, b, 1960a, b; Rehm, 1959; Lake, 1969, 1970; Rashan and Gorgees, 1976 and Rashan *et al.* 1976.

The morphology of the neurosecretory cells in the thoracic ganglion of the fresh-water crab, *Potamon magnum magnum*, has been described by Baid *et al.* (1966, 1968). However, the detailed histochemical nature of the neurosecretory material has not yet been investigated. The present study was thus conducted in an effort to reveal the chemical nature of the mucosubstances in the giant neurosecretory cells, with and without axons, in the thoracic ganglion of this decapod.

MATERIALS AND METHODS

Adult specimens from both sexes of the fresh-water crab, *Potamon magnum magnum* (Pretzman), were collected from the suburbs of Mosul, a city in northern Iraq. The thoracic ganglia were dissected out and fixed in Bouin's fluid, Zenker's fluid, Carnoy's fluid and formalin. Paraffin horizontal sections of 8 microns thickness were obtained. All the histochemical tests were performed according to Pearse's method (1968).

The following histochemical techniques were carried out:

1. Alcian blue (8GX) technique, after Steedman (1950).
2. Alcian blue (pH 1.0), after Lev and Spicer (1964).
3. Alcian blue (pH 2.5), after Mowry (1963).
4. Alcian blue (CEC procedures), after Scott and Dorling (1965).
5. Alcian blue-periodic acid-Schiff technique, after Mowry (1963).
6. Acriflavine test, after Hollander (1964).
7. Benzidine reaction, after Bracco and Curti (1953).
8. Dialysed iron method, after Hale (1946).

RESULTS

The results of the various histochemical techniques are shown in Table 1.

The neurosecretory material in both the giant neurosecretory cell types, with and without axon, was stained clear blue-green with alcian blue and blue with dialysed iron method, probably indicating the presence of acid mucosubstances. The secretory material accumulated in the perinuclear zone and in the perikaryon periphery. The alcianophilic secretory material was further tested. Alcian blue (pH 1.0) showed very weak blue tinge in both cell types, indicating the presence of strongly acidic sulphated mucins. Alcian blue at pH 2.5 also indicated the presence of acidic mucosubstances in the neurosecretory material of both cell types (Figs. 1 and 2). The secretory material was stained weak blue in both giant secretory neurones. After treatment with alcian blue 8GX in 0.05 M sodium acetate buffer at pH 5.7 using different molar concentrations of magnesium chloride (0.1 M, 0.2 M, 0.5 M), it appears that the strongest positivity was obtained with 0.2 M, $MgCl_2$, indicating that the nature of the neurosecretory material is related to acidic sulpho-

TABLE 1. *Histochemical reactions of the giant neurosecretory cells in the thoracic ganglion of the adult crab, Potamon magnum magnum (Pretzman)*

Tests	Reaction		Results
	Giant cell with axon	Giant cell without axon	
Alcian blue	+ to ++	+ to ++	Acidic mucosubstances
Alcian blue (pH 2.5)	+	+	Acidic mucosubstances
Alcian blue (pH. 1)	+	+	Acidic sulphated mucins
Alcian blue (CEC Procedure)			
0.1 M. $MgCl_2$	+	+	Acidic sulphomucins
0.2 M. $MgCl_2$	##	##	Acidic sulphomucins
0.5 M. $MgCl_2$	++	++	Acidic sulphated mucins
Alcian blue-PAS	##	##	Periodate-reactive alcianophilic components
Acriflavine	##	++	Sulphomucosubstances
Benzidine reaction	++	##	Sulphated mucosubstances
Dialysed iron method	##	##	Strongly acidic mucosubstances

Strength of reactions are indicated as follows: +, weakly positive; ++, positive; ##, moderately positive.

FIG. 1. Horizontal section of the thoracic ganglion of adult crab, *Potamon magnum magnum*, showing giant neurosecretory cell without axon. Arrows indicate acidic sulphated mucins. Alcian blue (pH. 2.5). $\times 710$

FIG. 2. Horizontal section of the thoracic ganglion of adult crab, *Potamon magnum magnum*, showing giant neurosecretory cell with axon. Arrows indicate acidic sulphated mucins. Alcian blue (pH. 2.5). $\times 710$

FIG. 3. Horizontal section of the thoracic ganglion of adult crab, *Potamon magnum magnum*, showing giant neurosecretory cell without axon. Arrows indicate acriflavine-positive material. Acriflavine. $\times 650$

FIG. 4. Horizontal section of the thoracic ganglion of adult crab, *Potamon magnum magnum*, showing giant neurosecretory cell, with axon. Arrows indicate acriflavine-positive material. Acriflavine. $\times 650$

FIG. 5. Horizontal section of the thoracic ganglion of adult crab, *Potamon magnum magnum*, showing giant neurosecretory cell without axon. Arrows indicate periodate reactive and alcianophilic neurosecretory material. Alcian blue-PAS. $\times 650$

FIG. 6. Horizontal section of the thoracic ganglion of adult crab, *Potamon magnum magnum*, showing giant neurosecretory cell with axon. Arrows indicate periodate reactive and alcianophilic neurosecretory material. Alcian blue-PAS. $\times 650$

mucin. Combined alcian blue-PAS test showed that the neurosecretory material in these cells is made up of periodate reactive and alcianophilic components (Figs. 3 and 4). The secretory material is stained bluish-purple. With benzidine reaction, the secretory material was stained dark blue, suggesting the presence of sulphated mucosubstances. Its presence was further tested by acriflavine test, in which the secretory material was stained reddish-brown (Figs. 5 and 6).

DISCUSSION

The results of the histochemical tests clearly indicate that the mucosubstances found in the giant neurosecretory cells studied are acidic sulphated mucosubstances. With use of the alcian blue and dialysed iron staining methods, acidic mucosubstances were observed in both giant neurosecretory cell types in the thoracic ganglion of the crab, *Potamon magnum magnum*. Both the cell types showed moderate positivity, indicating the presence of medium amounts of acidic mucosubstances. In addition, acidic sulphated mucins were observed in the giant neurosecretory cell types after treatment with alcian blue (pH 1 and pH 2.5), alcian blue (CEC procedures), acriflavine and benzidine reactions.

Miyawaki (1956c), has reported the presence of neutral mucopolysaccharide or mucoprotein in the cytoplasmic vacuoles of the neurosecretory cells of the crab, *Telmessus cheiragonus*. Rehm (1959), has reported the presence of a alcianophilic-positive material in the crab, *Carcinus maenas*. Takeuchi (1965) and Schreiner (1966) have reported the absence of alcian blue-positive material in the neurosecretory cells in the earthworm, *Pheretima communissima* and in the milkweed bug, *Oncopeltus fasciatus*, respectively. Teichmann *et al.* (1966) have reported the absence of alcian blue-positive material in the neurosecretory cells of the earthworms, *Lumbricus herculeus* and *Eisenia foetida*.

The nature of the neurosecretory material was further histochemically tested. Combined alcian blue-PAS test showed that the secretory material in the giant cells is periodate-reactive and alcianophilic in nature.

The presence of PAS-positive material in the neurosecretory cells has been reported by several workers (Gabe, 1952a, b; Miyawaki, 1958, 1960a, b; Rehm, 1959; Lake, 1970 and Rashan *et al.* 1976). The AF- and CHP-positive neurosecretory cells of the thoracic ganglion contain considerable amount of carbohydrates with glycolipids and glycoproteins but no glycogen (Rashan *et al.* 1976).

It appears that the detailed nature of carbohydrates in the neurosecretory cells of decapod Crustaceans has not been studied thoroughly enough and deserves more attention. With this view, the present study was carried out in order to study the nature of the mucosubstances in the giant cells of the thoracic ganglion of *Potamon magnum magnum* (Pretzman).

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