



Upper beam: mechanogram of the stretch of the left inferior oblique muscle. Lower beam: unitary discharge recorded from a cell in the medial-dorsolateral part of the left semilunar ganglion of a lamb. The unit responded only to the stretching of the left inferior oblique muscle.

the stretch of the extraocular muscles. The units studied were spontaneously active in many cases and exhibited a discharge rate of 10–20/sec. The units showed a negative polarity with an amplitude of 100–200  $\mu$ V. A given unit was exclusively and consistently modified by stretching of a determined extraocular muscle; only when the record was a multifibre one, was the unitary discharge affected by stretching 2 or more extrinsic ocular muscles.

The response of such gasserian cells to the stretch of the extraocular muscles was characterized by a sudden increase in the discharge rate (up to 300/sec). The discharge of the units then settled down at a lower frequency (150–90/sec). The firing of the units decreased immediately or stopped for a few msec when the stretch was released and subsequently recommenced at the original resting rate (Figure). It is to be noted that such responses exhibited a very brief latency: 1–3 msec. All the units studied showed a slight adaptation.

The firing of the units during a stretch of a given extraocular muscle was inhibited by the electrically induced contraction of that muscle; thus the units could be identified as muscle spindle afferents<sup>17</sup>.

The gasserian units responsive to the stretch of the extraocular muscles were unaffected by movements of jaw or by stimulation of other ipsilateral trigeminal receptors. The responses were not abolished by Nembutal anaesthesia, whilst they disappeared totally after cutting the ipsilateral ophthalmic branch of the fifth nerve.

Summing up, stretching of the extraocular muscles provoked short latency-sustained responses of a limited group of cells contained in the semilunar ganglion of lambs. Such responses were of the type induced by muscle spindles. The conclusion was reached that proprioceptive fibres from extraocular muscles have their cell bodies in the semilunar ganglion.

*Riassunto.* È stato isolato nel ganglio semilunare dell'agnello un limitato gruppo di cellule la cui scarica unitaria è tipicamente ed esclusivamente modificata dallo stiramento di singoli muscoli oculari. Tali risposte sono del tipo di quelle indotte dai fusi neuromuscolari. Si trae la conclusione che nel ganglio di Gasser dell'agnello si trovano i pirenofori di fibre che provvedono alla sensibilità propriocettiva dei muscoli estrinseci dell'occhio.

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<sup>17</sup> B. H. C. MATTHEWS, *J. Physiol.* 78, 1 (1933).

## Responses of Crustacean Larval Chromatophores to Light and Endocrines

Evidence for the presence of functional amounts of chromatophorotropins from very early stages of development of crustaceans has been reported<sup>1,2</sup>. However, the times at which the chromatophoral systems in developing crustaceans first take on functional activity have been determined in few forms<sup>3–7</sup>. Zoeae of *Hippolyte* and *Palaemon*<sup>3</sup>, *Nephrops*<sup>4</sup>, *Crangon*<sup>5</sup> and *Carcinus*<sup>6</sup> failed to exhibit chromatic adaptation. By contrast, zoeae of *Palaemonetes* exhibited background responses<sup>7</sup>. PAUTSCH<sup>5,6</sup> reported that the chromatophores of zoeae of *Crangon* and *Carcinus* were unaffected by chromatophorotropins, while BROCH<sup>7</sup> reported that chromatophores of *Palaemonetes* zoea responded to adult chromatophorotropins. To determine the effect of chromatophorotropins on zoeae, PAUTSCH<sup>5,6</sup> has plunged the zoeae into an aqueous suspension of the nervous tissue ex-

tracts, while BROCH<sup>7</sup> has tested the efficacy of chromactive extracts on isolated pieces of carapace of zoea. Since such a fundamental difference exists between their techniques, it was felt worthwhile to repeat them, using another test species to facilitate proper interpretation of the available data.

During the present study, berried female *Ocypode macrocera* collected from the Visakhapatnam beach were

<sup>1</sup> J. D. COSTLOW JR., *Nature* 192, 183 (1961).

<sup>2</sup> J. D. COSTLOW JR. and M. I. SANDEEN, *Am. Zool.* 7, 443 (1961).

<sup>3</sup> F. W. KEEBLE and F. W. GAMBLE, *Phil. Trans. R. Soc. B* 196, 295 (1904).

<sup>4</sup> M. CARSTAM, *Colloques int. Cent. natn. Rech. scient.* 4, 139 (1947).

<sup>5</sup> F. PAUTSCH, *Bull. Acad. Sci., Cracovie, B II*, 511 (1951).

<sup>6</sup> F. PAUTSCH, *Acta Biol. med., Gdansk* 5, 105 (1961).

<sup>7</sup> E. S. BROCH, *Biol. Bull. mar. biol. Lab., Woods Hole* 119, 305 (1960).

brought to the laboratory and maintained in enamel basins containing sea water. Under such conditions the zoeae were liberated. Soon after hatching, the zoeae were isolated in finger bowls containing filtered sea water and this collection represented the stock group.

The chromatophoral system of *Ocypode* zoea is composed of monochromatic white and black chromatophores and dichromatic chromatophores containing black and white pigments. Changes of pigment migration in the chromatophores of zoeal carapace were staged following the scheme of HOGBEN and SLOME<sup>8</sup>.

10 zoeae were placed in each of 3 white-painted stender dishes and 3 black-painted stender dishes. One white and one black dish were placed in one of the following illuminations: 10, 100 and 1700 ft c. The zoeae were exposed to these light intensities for 30 min, at the end of which the chromatophore stages were recorded. Later the stender dishes at 10 ft c light intensity were placed at 100 ft c and those at 100 ft c changed to the 1700 ft c position. Zoeae at 1700 ft c were placed at 10 ft c light intensity. At 30 min after this change the chromatophores were staged. By repeating the above shift system and by exposing the larvae for 30 min to the changed light intensities, followed by staging the chromatophores, it was possible to determine the effect of the 3 different light intensities on each zoea. The experiment was repeated once using an identical number of zoeae. The results were averaged and are given in Table I.

From Table I it is evident that with increasing light intensity the black and white chromatophores of zoeae tend to disperse. The differences in the degree of dispersion of black pigment in zoeae adapted to black and white backgrounds at 10 and 100 ft c light intensity indicate

that the black chromatophores show also a secondary response to light.

To determine the responses of larval chromatophores to adult chromatophorotropins, the following 2 experiments were conducted at 10 ft c light intensity. In the first experiment 20 zoeae were used. They were divided into 2 lots of 10 each and placed in white stender dishes containing 2 ml filtered sea water. After 1 h the chromatophores were staged. Into one of the dishes was added 0.5 ml of an extract of eyestalk ganglia (10 eyestalks) of adult *O. macrocera*. Into the second dish 0.5 ml sea water was added and the zoeae therein served as controls. The chromatophores were staged at 5 and 30 min following the initiation of the experiment. The experiment was repeated once using an identical number of zoeae.

In the second experiment 20 zoeae adapted to a white background for 1 h were used. From each zoea a piece of the carapace containing the chromatophores was excised and placed in a cavity slide containing 0.05 ml sea water. Soon after dissection of the desired number of carapaces, they were divided into 2 groups of 10 each. For the experimental group 0.05 ml of an extract of eyestalk ganglia of *O. macrocera* (1 eyestalk/0.05 ml) was added to the medium of each piece of carapace, while those of the control group each received 0.05 ml of sea water. The chromatophores were staged at 5 and 30 min after the initiation of the experiment. The experiment was repeated once. The averaged results are given in Table II.

It is evident that the zoeal chromatophores are affected by chromatophorotropins of the crab. However, the effect was apparent only when isolated pieces of carapace were used for assay purposes. Experiments designed to determine the effect of extract of eyestalk ganglia of *Ocypode* on the chromatophores in the isolated carapace of zoeae of *Uca*, *Sesarma* and *Clibanarius* also yielded positive results. The method of using isolated pieces of carapace for assay purposes ensures the availability of the hormones to act on the chromatophores. A similar provision is most unlikely when entire zoeae are plunged into the extract. The present results suggest that the differences in the reported results on *Crangon*<sup>5</sup>, *Carcinus*<sup>6</sup> and *Palaemonetes*<sup>7</sup> are correlated to the differences between the methods employed<sup>9</sup>.

Table I. Relationship between the chromatophores of *Ocypode* zoea and light intensity

Zoeae	Light intensity (ft c)					
	10		100		1700	
	B*	W	B	W	B	W
White background	1.3	2.2	2.7	4.2	4.2	4.7
Black background	4.3	2.3	4.6	4.0	4.8	4.7

\* Stage of dispersion of black (B) and white (W) chromatophores.

Table II. Responses of chromatophores of *Ocypode* zoea to chromatophorotropins

Experimental material	Group	Chromatophore stages					
		Initial stage		At 5 min		At 30 min	
		W*	B	W	B	W	B
Entire zoeae	Experimental	2.3	1.3	2.3	1.4	2.2	1.4
	Control	2.3	1.4	2.2	1.4	2.3	1.4
Isolated pieces of carapace of zoeae	Experimental	2.2	1.4	3.6	3.2	4.8	4.6
	Control	2.3	1.5	2.2	1.4	2.2	1.4

\* W = white; B = black.

*Zusammenfassung.* Es wird eine deutliche Reaktion auf totale Beleuchtungs-(Primär)-Zeit in den schwarzen und weissen Pigmenten der Chromatophoren der *Ocypode macrocera* zoea beobachtet. Eine Albedo- (sekundäre oder Hintergrunds-) Reaktion zeigt sich in den schwarzen Pigmenten der *Ocypode* zoea. Extrakte aus Augenstielen der *Ocypode* bewirken Expansion der Chromatophoren der *Ocypode* zoea.

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<sup>8</sup> L. T. HOGBEN and D. SLOME, Proc. R. Soc. B. 108, 10 (1931).

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