

## STUDIES ON THE SHRIMP *CARIDINA LAEVIS* HELLER

### VI. THE INTEGUMENT

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THIS paper deals with the structure of the integument in *Caridina laevis*. The various other systems of the animal have already been published (Pillai, 1960 a, b; 1961 a, b; 1962).

#### METHODS

The integument freed from body wall by maceration overnight in water was used in this study. The differential staining reaction based on the difference in the iso-electric points of the component parts of the cuticle was carried out (Loeb, 1922). The presence of chitin was determined by the chitosan-iodine colour test (Richards 1951). Thin microtome sections of the integument were stained in Safranin-Light Green and in Mallory's triple stain. Tegumental glands and the epidermis were studied by the help of sections stained in Heidenhain's haematoxylin, Heidenhain's Azan and Mallory's triple stain.

#### INTEGUMENT

The component parts of the crustacean and insectan integument have received different names. Wigglesworth (1933) has recognised two regions in the insectan integument which is applicable to the entire phylum, an outer layer without chitin (epicuticle) and an inner layer with chitin (procuticle of Richards (1951)).

The thickness of the cuticle varies in the different regions of the body. It measures about 4  $\mu$  in thickness in freshly moulted animals to 20  $\mu$  in some ovigerous females. The intertergal arthro-dial membrane is less than 0.5  $\mu$ . The cuticle inside the foregut viz., the stomach is about 3  $\mu$  thick.

#### *Epicuticle*

This layer has been shown to be distinct from the underlying layer by the help of staining reactions in Crustacea (Yonge 1932) and by X-ray diffraction studies in insects (Fraenkel and Rudall, 1947). The epicuticle in *Caridina*, where detectable is an extremely thin layer, about a fraction of a micron thick. It has not been possible to demonstrate the epicuticle throughout the integumental surface by either chemical or staining reactions. Where it is demonstrable are usually regions of comparatively heavy deposition. These are (i) molar surface of the mandible (12 to 14  $\mu$  thick) (ii) posterior surface of the head of the mandible (3  $\mu$  thick) (iii) outer side of the molar process (1.5  $\mu$  thick) and (iv) apex of the rostral spines (0.2 to 0.5  $\mu$ ).

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*Procuticle*

The procuticle has a laminar structure being composed of alternate light and dark bands having different refractive indices. The laminae are clearly seen when mounted in glycerine. It is composed of two sheets, an outer and an inner, both giving the Prussian blue and reddish brown colour by the method of Loeb (1922). It is not clear whether these correspond to the exocuticle and the endocuticle. The outer layer stains a shade deeper blue than the inner in Mallory's triple stain. This region of the cuticle is positive to chitosan-iodine colour test.

Melanin is not demonstrable in the cuticle by the Dublin application of the Bodian method (Glick, 1949). The presence of free tyrosine could not be established either, by the Millon reaction. This condition recalls closely that in the fairy shrimp (Dennel, 1947) where the process of tanning is restricted to the tip of the mandibles. The presence of calcium has been demonstrated inside the procuticle by the following way. Cuticle removed from the body wall was treated with 4 % sulphuric acid. Monoclinic crystals of calcium sulphate were detected under the microscope. Von Kossa silver test (Glick, 1949) also gave positive results for calcium in the outer layer of the procuticle.

*Pore canals*

Pore canals are minute vertical ducts inside the procuticle and are as long as the cuticle is thick. Because of their distinct stainability and refractibility, they are clearly seen inside the procuticle in cut material. Each consists of a series of discontinuous rods which take up nuclear dyes as has been observed in other arthropods (Braun, 1875 in *Astacus fluviatilis* and Wigglesworth, 1948 in *Tenebrio*). The contents of the pore canals are negative to tests for chitin. The density of distribution of the pore canals varies in the different parts of the body. From the cuticle of the coxa of the walking leg of a female specimen, an average of 34 pore canals have been counted in an area 5  $\mu$  square. They were also observed in the cuticle of the foregut and hindgut.

*Setae*

Two types of setae are distinguishable, mechanical and sensory. Mechanical setae are abundant inside the stomach. These are unjointed and nonplumose. Whether these are epicuticular or chitinous in nature could not be ascertained as some show definitely an epicuticular nature and a few show a chitinous nature of staining. Sensory setae are numerous and have a very wide distribution. A few types have been recognised as follows. Spines are abundant as those on the rostrum and elsewhere with an inner core of living tissue. The constricted setae found in between the rostral spines are long and filiform with a basal constriction. One microtrichium enters the core of this. A modification of this is the jointed plumose seta, very characteristic of the appendages. Here the proximal portion represents a node upto where only the epidermal cells extend. The ovigerous setae are neither jointed nor plumose ; they are much longer and have a longer core of living material.

## EPIDERMIS

The epidermis is unilaminar ranging from 4 to 14  $\mu$  in thickness. The cells are cuboid in section and hexagonal in surface view. Nuclei are spherical. Larger nuclei of the undifferentiated embryonic cells have been observed occasionally. In adults no mitosis has been noticed.

*Tegumental glands*

Tegumental glands are comparatively few and sunk deep in the dermis. The gland proper measures about  $43 \mu$  in length and  $14 \mu$  across. The duct is about  $20 \mu$  in length. The gland consists of about 35 to 40 cells which do not seem to be arranged in a layer as in the rosette glands of other crustaceans but form an ovoid mass so that an internal lumen is not clearly seen. The long duct appears to be composed of about 2 or 3 cells, judged from the number of nuclei arranged along its length.

## CHROMATOPHORES

The chromatophores are binucleate, the nuclei appearing as two refractile bodies under the phase contrast microscope. The pigments are of two types, dark and white. The dark ones constitute red and brown pigments. In many places, during their maximal dispersion they tend to look alike. The second type is the white or reflecting pigment which, strictly, is of a faint golden yellow colour in reflected light and slate grey and slightly opaque in transmitted light. Both monochromatic and polychromatic chromatophores are observed. The following are the types of chromatophoral pigment combinations :

1. Monochromatic red.
2. Monochromatic brown.
3. Monochromatic reflecting.
4. Dichromatic red and brown.
5. Dichromatic reflecting and 6. Trichromatic reflecting, red and brown.

The monochromatic and dichromatic chromatophores are more abundant although di- and trichromatic chromatophores are characteristic of Caridea (Knowles and Carlisle 1956).

Red chromatophores are most conspicuous and are distributed along the antennule, branchiostegite, posterior two-thirds of the abdominal terga and the tail fan. The brown chromatophores are less conspicuous and are interspersed among the red. The reflecting pigments are scarce and are distributed scantily on the rostrum, anterior aspect of the eye stalk, joints of pereopods, branchiostegite, median hind end of abdominal terga and the tail fan. Except for the white, a tendency has been noticed for like chromatophores to aggregate.

A blue pigment, noticed always in association with the red, is not lodged within the chromatophoral body and hence is not included under the chromatophoral pigments. That a blue pigment diffuses outwards from the red and that it is biochemically related to the red was shown by Keeble and Gamble (1904).

Specimens of *Caridina* adapted to darkness and those with extirpated eye stalks have been observed to be in the same state of chromatophoral dispersion. The red and brown chromatophores get completely dispersed and lichen-shaped. The dispersion of the brown is more thorough, the chromorhizae are longer and the intensity of colour lesser. These measure about  $250 \mu$  across. The red ones at their maximal dispersion are not more than  $120 \mu$ . In the dichromatic red and reflecting, the red pigment which is placed above the other is alone dispersed, their chromorhizae restricted to one or two sides. The reflecting pigment is concentrated to form a central golden yellow spot. The dispersion of the dark pigments in a light-adapted animal commences within 20 minutes after the extirpation of the eye stalk. The dispersion of the brown pigment is quicker than the red.

When a dark adapted animal is exposed to strong sun light, the dark pigments concentrate and the reflecting pigments disperse. The dispersion of the reflecting pigment is rather quick, it takes only 7 to 10 minutes. The dispersed reflecting pigment has a central disc from where narrow chromorhizae arise and end in a lanceolate fashion. These measure about 150  $\mu$  across.

## SUMMARY

The integument of *Caridina laevis* is very thin, an epicuticular layer is not demonstrable throughout the surface. Calcium salts are present but not free tyrosine or melanin. Tegumental glands are scarce and small. Six chromatophoral pigment combinations have been observed.

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