

Pigments Present in Arms and Pinnules of the Crinoid, *Antedon bifida* (Pennant)

IN *A. bifida* red pigment usually occurs in protein granules in superficial connective tissue and in the eggs. The skeleton is colourless. When freshly detached pinnules are placed on slides and covered with sodium hydrosulphite solution, microscopic examination reveals that the red colour is immediately lost, but is restored in the granules when this solution is replaced quickly by sea water, and more markedly if drops of hydrogen peroxide are added to the sea water. Cannan¹ applied this test to *Arbacia* and *Echinus* echinochrome with similar results. The pigment restored in colour passes gradually into the protein pyriform bodies in the sacculi in *Antedon*. In distilled water the red pigment also passes into these bodies, which are discharged. Ball² used acid alcohol to extract echinochrome from *Arbacia*, and this solvent proved to be effective in removing selectively the red pigment from *Antedon* from Plymouth. Extraction procedure and tests performed were as follows:

1. Living arms and pinnules of 10 animals were cut up into 50 ml. of acid alcohol (70 per cent methyl alcohol containing 0.75 per cent conc. hydrochloric acid). Pigment extracted was placed in a separating funnel.

2. 50 ml. of petroleum ether and later 60 ml. of distilled water were added to the extract, which was shaken so that carotenoids and lipids might pass into the epiphase.

3. The hypophase containing the red pigment was run into a further separating funnel. About 20 ml. of distilled water of pH 5 (from a metal still) and 50 ml. of diethyl ether were added. The red pigment passed into the epiphase. If 20 ml. of glass distilled water is used instead, the red pigment remains in the hypophase, but on addition of a little hydrochloric or acetic acid it rises into the ether. In this instability the pigment resembles the hydroxy-naphthaquinone extracted by Ball and Cooper³ from *Arbacia*, and by Millott^{4,5} from *Diadema antillarum*; furthermore it is orange in aqueous acid solution and deep red in alkaline. A green colour, indicative of a phenol, appears if ferric chloride solution is added to a sample of this pigment in distilled water acidified with hydrochloric acid.

4. Samples of the ethereal solution of pigment dried with anhydrous sodium sulphate were examined in a Beckman spectrophotometer.

Absorption peaks occur at 262 m μ , 337 m μ , 460 m μ and there is an inflexion at 510 m μ —all as in the naphthaquinone pigment from *Diadema*^{4,5} except that the maxima at 337 and 460 m μ are less well marked.

Yellow-brown lipid granules (which take up sudan dyes) are present in deposits in connective tissue and in coelomocytes. The brown colour is not removed by changes of acetone or ethylene chlorohydrin. In sections these granules blacken with alkaline silver methenamine. Since 0.13 per cent *l*-tyrosine is blackened slowly by ground arms and pinnules, some tyrosinase is possibly present in them, but use of the Hueck and Long Ziehl-Neelsen tests (see Pearse⁶) indicate that the yellow-brown material contains lipofuscin (oxidized lipid), not melanin, and bleaching tests bear this out, for very little brown colour is removed when sections 7 μ thick are placed in 30 vol. hydrogen peroxide or 1 per cent bromine water for 48 hr.

Carotenoid pigments are present in gut wall and gonad, but can be extracted from the region of the arm-tip, which contains practically no gonad. I am indebted to Dr. L. R. Fisher for identification (using the methods of Fisher, Kon and Thompson⁷) of β -carotene, esterified astaxanthin (peak at 465 m μ in *n*-hexane), astaxanthin and xanthophyll (peak at 445–447 m μ in *n*-hexane) in extracts of arms and pinnules of *A. bifida*. Echinenone and vitamin A appeared to be absent. In its arms *A. bifida* seems to have several carotenoid pigments which resemble those found in the integument of starfish such as *Marthasterias glacialis*⁸. The carotenoids, α -carotene, β -carotene, echinenone, esterified astaxanthin and xanthophyll have been found in echinoderms and the occurrence of some of these pigments in the crinoid *A. bifida* might be expected.

Certain pigments present may be of dietary origin. *A. bifida* is both a detritus and suspension feeder. Small copepods are ingested with detritus. Diatoms are digested.

Like the echinoid *Diadema*⁹, this crinoid responds to light. Animals so placed that aboral cirri rest on the bottoms of bowls will stay still in very dim light for half an hour, but rise up and swim when exposed to 160 ft. candles of light from an electric bulb for 4 sec. Preliminary work indicates that this response is also given when green, but not when blue, yellow or red filters are placed in front of the light; a light-sensitive pigment may be present in the nervous system.

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⁴ Millott, N., *Nature*, **178**, 1185 (1956).

⁵ Millott, N., *Proc. Zool. Soc. Lond.*, **129**, 263 (1957).

⁶ Pearse, A. G. E., "Histochemistry" (London, 1954).

⁷ Fisher, L. F., Kon, S. K., and Thompson, S. Y., *J. Mar. Biol. Assoc. U.K.*, **35**, 41 (1956).

⁸ Vevers, H. G., and Millott, N., *Proc. Zool. Soc. Lond.*, **129**, 75 (1957).

⁹ Millott, N., *Phil. Trans. Roy. Soc.*, B, **238**, 187 (1954).

The Finer Structure of the Galeal Lamella in Adult Mosquitoes

THE maxilla of the adult mosquito is a reduced structure in that the cardo and lacinia are almost lost, and the stipes is embedded below the alimentary canal. The functional maxilla is a lancet-like galea consisting of a chitinized rod with a trough-shaped membranous lamella on its outer edge. This lamella has been described in *Anopheles maculipennis* as possessing fine anastomosing striations and bearing at its distal extremity small, backwardly pointing teeth¹. The piercing action of the maxillary galea has been described elsewhere².

Studies being carried out upon the detailed structure of the trophi of adult Culicidae have revealed that in many species the lamella has a more complex structure than previously believed. In *Aedes argenteoventralis*, *Culex horridus*, *C. cinereus* and *C. fatigans*, a very fine non-striated lamella on the inner side of the galea has been observed. The structure of the outer lamella has particularly been studied in *Aedes aegypti*, *A. grahami*, *Culex nebulosus*, *C. cinerellus*, *C. fatigans*, *Uranotaenia bilineata* and *Hodgesia*