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A STUDY OF PIGMENTS IN SOME DEVELOPMENTAL STAGES OF *AURELIA AURITA LAM.*

by

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INTRODUCTION

Aurelia aurita Lam. is a cosmopolitan species of Scyphozoan jellyfish. Its life cycle is now well established and includes an asexually and a sexually reproducing phase. The male medusas shed their sperm into the sea and may fertilize the eggs released by the females. The zygotes are protected for a certain period by tissue folds surrounding the mouth of the female. The young are released as small, oval-shaped ciliated swimming larvae. These are called planulae. In a reproductive cycle which does not undergo an alternation of generations the planula is directly differentiated into the ephyra (Kakinuma, 1975). In the other case the planulae become attached to a substrate and differentiate into polyps with at first four, later eight and finally sixteen tentacles (scyphistomes).

Normally the scyphistomes start a period of asexual reproduction. Four different types have been observed (Kakinuma, 1975) :

1. The base of a well grown polyp gives rise to a stolon, which swells at its tip, and so produces a new polyp. There are also cases where a polyp buds from the middle part of a long stolon.
2. A young polyp grows out directly from the base of the body of a mother polyp. This outgrowth cuts off to become an independant unit which attaches to the substratum at a position immediately next to the mother.

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3. The polyp expands sidewise while increasing the number of tentacles and then splits longitudinally into two new polyps.
4. When a polyp moves from one location to another it may leave some of its tissues behind and a new polyp may arise from these remaining tissues.

In certain environmental conditions the sessile scyphistomes metamorphose into the free-swimming medusa. This process is called strobilation and it consists of three phases (Olmon and Webb, 1974) :

1. Formation of a constriction of the polyp body below the bases of the tentacles. This process is called neck formation.
2. Formation of many horizontal divisions. The polyp is now called a strobila.
3. Sequential development of ephyrae from the segments, each of which swims away and develops into an adult, sexually reproducing medusa.

Several publications treat the study of the environmental factors that may induce strobilation. It has now been clearly demonstrated that sufficient quantities of iodine and a low temperature pre-conditioning are of primary importance for this process (Spangenberg, 1967; Silverstone et al., 1977).

This paper is a first approach towards the identification of the pigments that can be found in some developmental stages of *Aurelia aurita Lam.* Planulae differentiate into young milkywhite polyps, which soon acquire a light pinkish colour when fed with *Artemia* nauplii. During strobilation a dark brown-red colour develops in the differentiating segments and the resulting ephyras show the same coloration. As the ephyra grows the colour disappears and is completely absent in medusas with a size of about 5 mm.

MATERIAL AND METHODS

The scyphopolyps used for these experiments originate from the Ueno Aquarium at Tokyo. A batch of about 300 polyps were transferred to the Aquarium of the Antwerp Zoo where they are kept in 60 liter aquaria, filled with natural seawater of a density of 1.024 and at a temperature of 22° to 26°C. The polyps are fed daily with brine-shrimp nauplii.

Under these conditions the scyphopolyps reproduce asexually by budding and their number increases rapidly.

Treatment for strobilation

In order to induce strobilation the methods described by Abe and Hisada (1969) are used.

The polyps needed for the experiments are separated from the holding tanks and transferred into small glass jars of 1 liter. Each jar is filled with natural seawater at 15°C and filled with 1000 polyps. The polyps are no longer fed and the jars are kept in a refrigerator at a temperature of 15°C. A permanent overhead lighting with 30 Watt fluorescent tube is present so that the polyps are kept constantly under 60 lux. After 1 to 3 weeks the first strobilas appear and the ephyras detach spontaneously. The free swimming ephyras are removed daily and collected in a cylindrical jar containing 400 liter of natural seawater at 20°C. There is constant aeration and the ephyras are fed daily with large quantities of brine-shrimp nauplii. After 3 to 4 weeks the ephyras grow into small medusae of 1 cm diameter. At this stage the small medusae are transferred into plastic aquaria from which the bottom and the sidewalls are perforated with 3 mm holes. Each aquarium has an aeration (large bubbles) and several of these aquaria are hanging together in a large tank with sandfiltration device. The natural seawater is kept at 20°C. The young medusae are fed every day with newly hatched brine-shrimp nauplii. After 4 to 6 weeks they reach 3 to 5 cm in diameter and at this moment several medusae are transferred into 60 liter aquaria with sandfiltration and with a slow water current. They are fed individually with brine-shrimp nauplii, chopped mussels, chopped krill and fishmeat. Finally they grow to medusae of approximately 8 cm in diameter.

Biochemical Methods

Extracts were prepared by mixing polyps, strobilas or ephyras in 0.05 M Tris-HCL, pH 8.7 with a Virtis homogeniser, followed by ultracentrifugation at 145.000 g and 4°C for 1 h. in a Beckman refrigerated centrifuge. Pigment spectra were measured with a Cary 118 spectrophotometer. Reduction of the pigments with $NaBH_4$ was done according to Krinsky and Goldschmidt (1960). Thin layer chromatography was carried out on silicagel layers (HPTLC Fertigplatten, Kieselgel 60, 10 × 10 cm, Merck) in petroleum benzine/aceton (1:1) and aceton/ methanol/ water (20:76:4) (Stahl, 1969). Gelfiltration was performed on Sephadex G-75 superfine (Pharmacia) in 0.01 M Tris-HCL, pH 8.0. electrophoresis on polycrylamide gels was done according to Davis (1964) and Ornstein (1964). Electrophoresis in SDS was based on Weber and Osborn (1969).

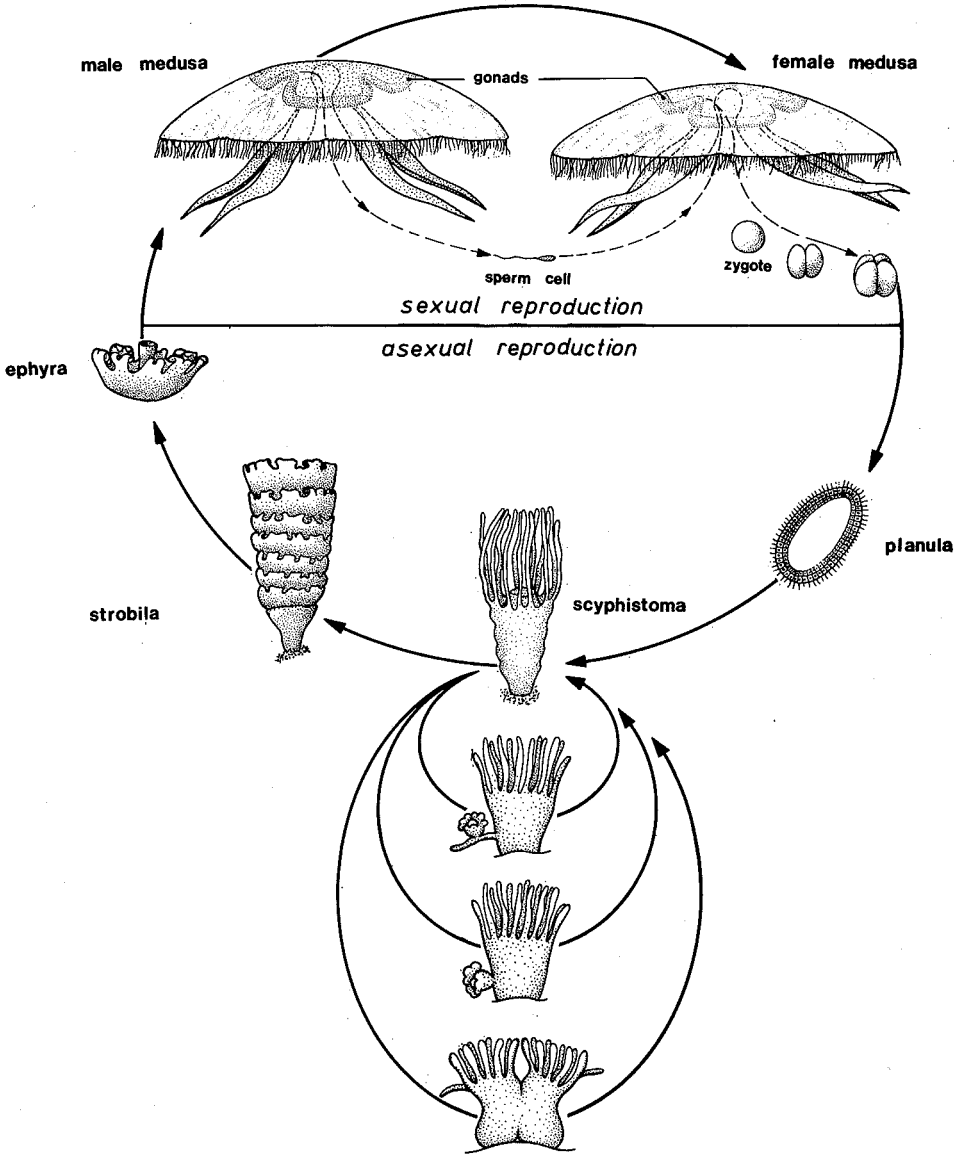

Fig. 1 – Different developmental stages in the life cycle of *Aurelia aurita*.
A. Polyp; B. Strobila; C. Ephyra; D. Medusa.

RESULTS

We have studied the pigmentation of polyps, strobilas and ephyras of *Aurelia aurita Lam.* These developmental stages are shown in figure 1.

Newly formed or unfed *Aurelia* polyps are white. Fed on *Artemia* nauplii the polyps become pinkish. *Artemia* nauplii are known to contain the carotenoids canthaxanthin and echinenone in a proportion 19:1 (Krinsky, 1965). We have

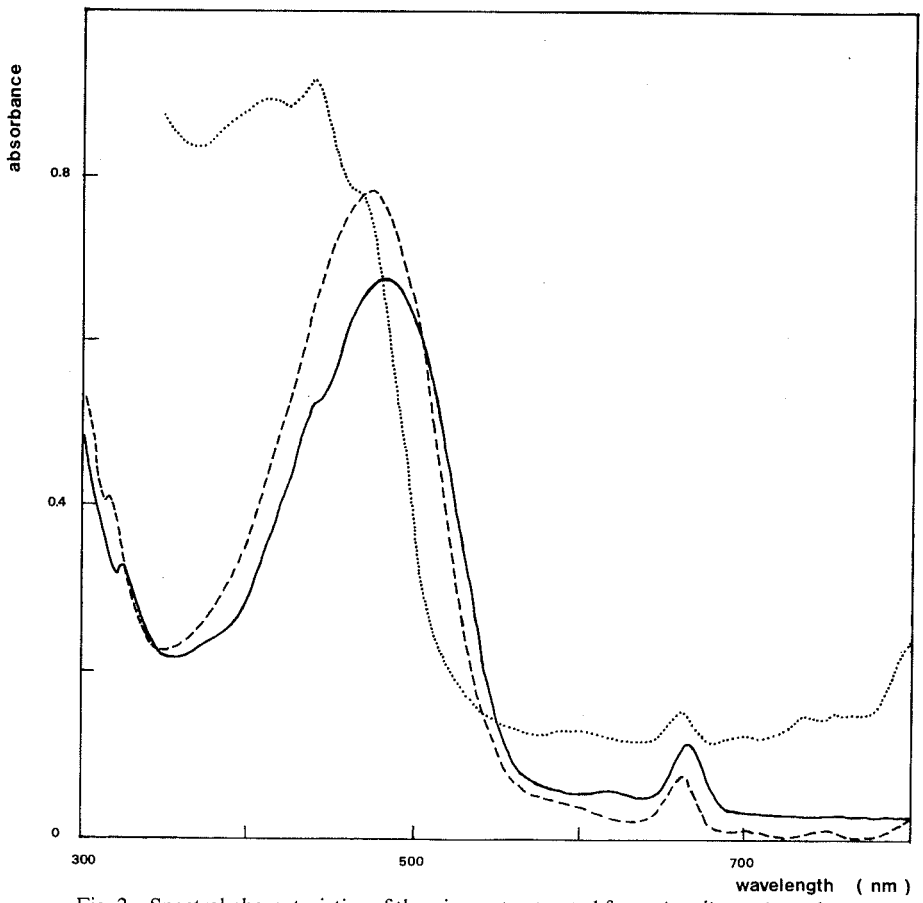

Fig. 2 – Spectral characteristics of the pigment extracted from *Aurelia aurita* polyps.
———— chloroform
— — — — — 95 % ethanol
. . . . . 95 % ethanol, after 90 minutes of reduction by $NaBH_4$.

prepared extracts of *Artemia* nauplii and polyps of both colours. The pellets formed during extraction were dissolved in chloroform or in 95 % ethanol. In extracts from both the nauplii and the pinkish polyps the spectrum showed a maximum at 482 nm in choroform and at 475 nm in ethanol. After reduction by $NaBH_4$ a typical spectrum with three maxima at 413, 440 and 467 nm appeared. This is shown in figure 2. These results correspond with the values found for canthaxanthin (Goodwin, 1955; Hager and Stransky, 1970; Krinsky, 1965). In white polyps no canthaxanthin was found.

Thin layer chromatography of the extracts confirmed the presence of canthaxanthin in pinkish polyps (figure 3). Furthermore in both the pinkish and the white polyps there was a weak indication of a yellow-green pigment of unknown structure and a second pigment of carotenoid nature. After extraction of the pinkish polyps the aqueous supernatant always was slightly pink. This pigment could be extracted from the water phase by shaking with petroleum benzine. In this solvent the spectrum showed the same maximum as canthaxanthin (462 nm in petroleum benzine – Liaaen-Jensen, 1965). So probably part of the canthaxanthin in the pink polyps of *Aurelia* is bound to a protein.

The beginning of strobilation in *Aurelia* is easily detected by the appearance of a brown-red colouration in those parts of the polyps, where segments are formed. Because we have always induced strobilation by a low temperature preincubation combined with starvation the appearance of coloured strobilas was very striking amidst the milky white polyps. A few days later dark-red ephyras are liberated.

When an extract of the ephyras and the strobilas was prepared, a dark-brown pellet and a brown supernatant are formed.

The pigment in the pellet could not be dissolved in the common aqueous or organic solvents. It was however dissolved in 20 % KOH. The spectrum between 350 and 800 nm showed no maxima. These results point to the presence of melanin. The definite identification of a pigment as melanin is very difficult. Therefore we have searched for additional information by using histochemical techniques.

Ephyras are remarkably fit for histochemical investigations. They are very thin structures in which we found large well-limited pigment grains distributed along the radial channel walls. Silverimpregnation and investigation of tyrosinase-activity support the idea of melanin being the main pigment in the ephyras. An electron microscope study of the strobilas showed two subcellular structures that possibly represent the pigment grains : one was a membrane-less

inclusion with an amorphous content, not coloured by OsO_4 and not contrasted by uranylacetate; the other was a granule with a membrane and a granulated content, coloured by OsO_4. These structural aspects of our study will be the subject of a separate publication.

The brown supernatant found in extracts of ephyras and strobilas showed a spectrum with a maximum at 455 nm. The pigment in this extracts was rather unstable, precipitated easily and could not be dissolved again in the common

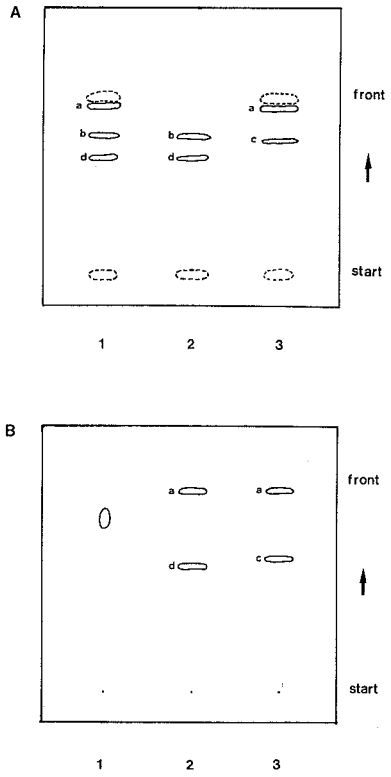

Fig. 3 – Comparative thin layer chromatography of the pigments from *Aurelia aurita* polyps and from *Artemia salina* nauplii on silicagel layers.
A. Solvent : petroleum benzine/aceton (1:1)
1. Pink polyps, 2. White polyps, 3. *Artemia salina*
B. Solvent : aceton/methanol/water (20:76:4)
1. Pink polyps, (supernatant), 2. Pink polyps (pellet), 3. *Artemia salina*
a. canthaxanthin; b. a yellow-green pigment; c. echinenone; d. a carotenoid pigment of unknown structure. The full spots are visible after chromatography; the dotted spots are only visible after spraying the layer with H_2SO_4.

solvents. Gelfiltration of the extracts on Sephadex G-75 superfine indicated a molecular weight of more than 75000 for the pigment fraction. SDS-gelelectrophoresis on the other hand showed a molecular weight of 14500, thus demonstrating that the pigment, probably bound to a protein, is composed of several subunits.

Disc-gelelectrophoresis on polyacrylamide gels showed two coloured bands. So the subunits probably aggregate to at least two polymeres of different size.

DISCUSSION

We have shown that the pink colour of polyps of *Aurelia aurita Lam.* is due to the presence of canthaxanthin. This carotenoid is derived from the food (nauplii of *Artemia salina L.*) where it occurs in the same form. Next to this, polyps contain a yellow-green pigment and a second carotenoid which occur in so low concentrations that chemical identification is very difficult.

The brown-red colour of strobilas and ephyras is formed in the animal itself and has been shown to be melanin accompanied by a smaller fraction of a protein bound brown pigment. These pigments appear as soon as strobilation is started and disappear some time after the liberation of the ephyras from the strobila.

These results suggest that the brown colour has some metabolic link with the strobila metabolism. We think that this link might very well be tyrosine. This amino acid may indeed be at the origin of both melanin biosynthesis and different iodinated compounds.

For the moment scyphozoans are the only invertebrates in which a function of iodide has been demonstrated and just like in amphibians it seems to play an important role in metamorphosis. At the onset of strobilation iodine is taken up by the polyps and subsequently organified by the biosynthesis of mainly mono-and diïodotyrosine (MIT and DIT). Furthermore iodotyrosines and especially DIT are secreted from the polyps into the surrounding medium (Silverstone et al., 1978). This explains why polyps may be seen strobilating synchronously or in waves radiating from a central area as Loeb (1973) has described for the sea nettle, *Chrysaora quinquecirrha.* The active principle has been called the neck-inducing factor. In the light of these findings we put forward the working hypothesis that the striking colouration which accompanies strobilation is due to melanin which forms as a by-product of iodium- and tyrosin metabolism which characterizes the onset of strobilation.

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SUMMARY

Unfed polyps of *Aurelia aurita* are white. Fed with *Artemia salina* nauplii they turn to pinkish because of the canthaxanthin present in the food. The deep brown-red colour of strobila and ephyra is due to melanin.

SAMENVATTING

Niet-gevoede poliepen van *Aurelia aurita* zijn wit. Gevoed met *Artemia salina* nauplii worden zij roze, door het canthaxanthine in het voedsel aanwezig. De donkere bruin-rode kleur van strobila en ephyra wordt veroorzaakt door melanine.

RÉSUMÉ

Quand ils ne sont pas nourris, les polypes d'*Aurelia aurita* sont blancs. Nourris avec des nauplii d'*Artemia salina* ils deviennent roses, à cause de la canthaxanthine dans les nauplii. La couleur brun-rouge fonçé du strobile et de l'éphyra est de nature mélanique.

ZUSAMMENFASSUNG

Nicht gefütterte Polypen von *Aurelia aurita* sind weiß. Gefüttert mit *Artemia salina* nauplii erhalten sie eine rosa Farbe wegen des Kanthaxanthins in der Nahrung. Die dunkel braun-rote Farbe von Strobila und Ephyra wird durch das Melanin verursacht.

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