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# A STUDY OF THE CHROMATOPHORE PIGMENTS IN THE SKIN OF THE CEPHALOPOD *SEPIA OFFICINALIS* L.

by

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ABSTRACT. — In the chromatophores of the dorsal skin of the cuttlefish *Sepia officinalis* L. at least three different pigments can be found. They belong to the ommochromes and they differ from each other by their color, solubility and redox behavior.

## INTRODUCTION

Cephalopods possess a unique system of chromatophore organs and iridophores which allow very rapid physiological color changes. The iridophores form a layer of immobile reflector cells which is only exposed when the chromatophore cells are absent or fully contracted (HOLMES, 1940 and MIROW, 1972, a).

The morphology and structure of the chromatophore organ has been studied in detail (MIROW, 1972, b - SERENI, 1930 - BOYCOTT, 1948). It is composed of a central pigment containing cell, several radially arranged obliquely striated muscle fibers, nerve cells, Schwann cells and sheath cells. The cell bodies of the fibres innervating the chromatophore muscles are concentrated in anterior and posterior chromatophore lobes, which were classified as lower motor centres (BOYCOTT and YOUNG, 1950). A possible endocrine regulation of chromatophores has been proposed by KAHR (1958 and 1959).

The different color changes and color patterns of cephalopods and the corresponding complex behavioral patterns have been the subject of several studies (WELLS, 1962 - KUHN, 1950 - HOLMES, 1940 - SERENI, 1930).

The cuttlefish, *Sepia officinalis* L. shows extreme rapid color changes as compared to most other cephalopods. HILL and SOLANDT (1935) show that the change from complete contraction to complete expansion in a chromato-

phore can take place in two-thirds of a second. Moreover the chromatophore system of *Sepia officinalis* and *Octopus vulgaris* is organized in such a way that a much wider variety of complicated color patterns can be produced than in any other cephalopod (BOYCOTT, 1948). According to WELLS (1962) one out of every three hundred cells in the brain of these animals is involved in color displays.

The chromatophores in *Sepia officinalis* lie in three layers parallel with the outer surface of the animal. Those of the outer layer contain a bright yellow pigment, in the middle layer the pigment is orange red and in the basal layer the pigment is brown violet (HOLMES, 1940). The chromatophore pigments were studied by SCHWINCK (1953 and 1955). She demonstrated that the pigments in the chromatophores and in the eyes of *Sepia officinalis*, *Octopus vulgaris* and *Eledone moschata* are ommochromes.

This paper describes our first efforts to isolate and characterize the different chromatophore pigments from the skin of *Sepia officinalis*.

## MATERIAL AND METHODS

We used adult specimens of *Sepia officinalis* L. They were obtained either from the culture of RICHARD (1976) or from the "Expedition Cephalomanche". For our experiments only the dorsal skin of the cuttlefish was taken. After washing with tap water it was extracted with consecutively 0,05 M TRIS-HCl buffer pH = 7,2, methanol-HCl 0,1 N and formic acid (99-100%). Each extraction was repeated until no more pigment was released. The extracts were centrifuged at 30.000 g for 20 minutes at 4°C. The resultant supernatants were used for spectrophotometric analyses or freeze-dried. The dry powders were partitioned into different fractions for solubility tests and further study.

Absorption spectra were obtained using a Cary 118 spectrophotometer over the range 650-300 nm. Redox reactions were tested by adding $H_2O_2$ (3% final) or excess ascorbic acid or sodium thiosulphate to the measuring vessel. Formation of a purple halochrome was tested by addition of concentrated sulpuric acid (BECKER, 1941).

## RESULTS

With our extraction procedure we succeeded in isolating three differently colored pigments. We call them pigment A (buffer extraction), pigment B (acid methanol extraction) and pigment C (formic acid extraction). They all show a typical redox color and halochrome (Table 1).

Table 1

Redox color of cuttlefish skin pigments

| Pigment | Pigment color | | | |
|---|---|---|---|---|
| | In extraction fluid | Reduced (+ ascorbic acid) | Oxidized (+ $H_2O_2$) | Halochrome (+ $H_2SO_4$) |
| A | Yellow | Pink purple | Yellow | Ligth purple |
| B | Brown red | Red purple | Yellow | Brown purple |
| C | Violet purple | Violet purple | Yellow | Violet purple |

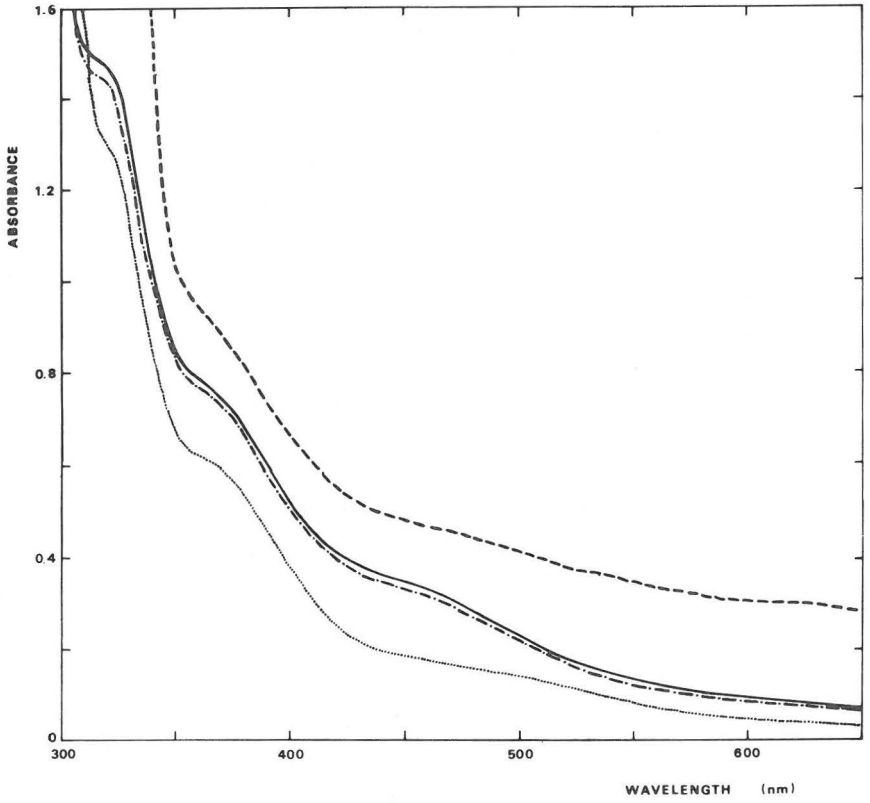


FIG. 1. — Spectrophotometric analysis of pigment A in 0,05 M TRIS-HCl buffer pH = 7,2.

———— Untreated pigment.
..... After addition of ascorbic acid.
—.—.—.—. After addition of sodium thiosulphate.
— — — — After oxidation with $H_2O_2$.

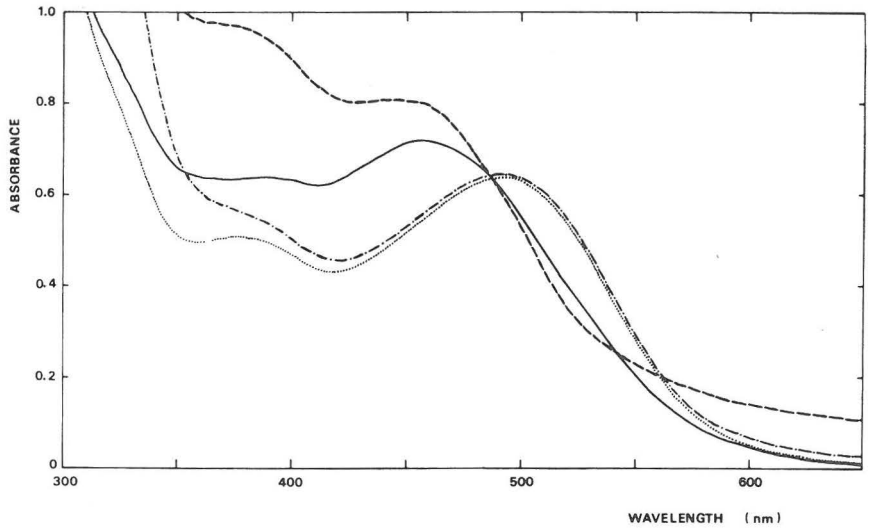


FIG. 2. — Spectrophotometric analysis of pigment B in methanol-HCl 0,1 N.

——— Untreated pigment.
..... After addition of ascorbic acid.
—.—.—.—. After addition of sodium thiosulphate.
— — — — After oxidation with $H_2O_2$.

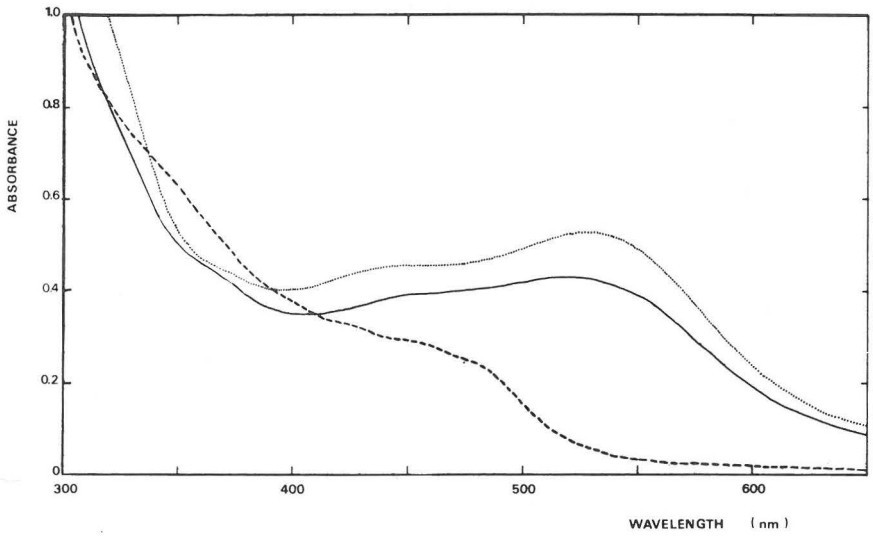


FIG. 3. — Spectrophotometric analysis of pigment C in formic acid.

——— Untreated pigment.
..... After addition of ascorbic acid.
— — — — After oxidation with $H_2O_2$.

The absorption spectra of the extracts without any treatment and after addition of oxidizing and reducing agents are shown in figure 1 (pigment A), figure 2 (pigment B) and figure 3 (pigment C).

The solubility of the three pigments was studied by freezedrying the original extracts and dissolving the dry powder in different solvents. The results of this are summarized in table 2.

Table 2

The solubility and colors of cuttlefish skin pigments

| Solvent | Pigments | | |
|---|---|---|---|
| | A | B | C |
| Aqua dest. | + + + + yellow | + + + brown | — |
| 0,1 M Na acetate buffer pH = 4,4 | + + + + yellowish | + + + yellow brown | — |
| 0,1 M Phosphate buffer pH = 6,3 | + + + + yellow | + + + pink brown | — |
| 0,05 M glycine/NaOH buffer pH = 9,8 | + + + + yellow | + + + yellow brown | + + + pink purple |
| 1 N HCl | + + + yellow | + + + + brown | — |
| 20% KOH | + + + + yellow | + + + + brown red | + + + + violet purple |
| Ethanol | — | — | — |
| Methanol | — | + brown | — |
| Aceton | — | — | — |
| Acetic acid | + + + yellowish | + brown red | + violet purple |
| Formic acid | + + + + yellow | + + + + brown red | + + + + violet purple |
| Methanol/HCl 0,1 N | + + + yellow | + + + + brown red | + + orange purple |
| Pyridine | — | + + + yellow brown | — |
| $CS_2$ | — | + + pinkish | — |

—: no solubility.

+, + +, + + +, and + + + + : very weak, weak, good or very good solubility according to visual appreciation.

## Discussion

In this preliminary study of the skin pigments in *Sepia officinalis* L. we have isolated three pigment fractions, which show a typical redox- and

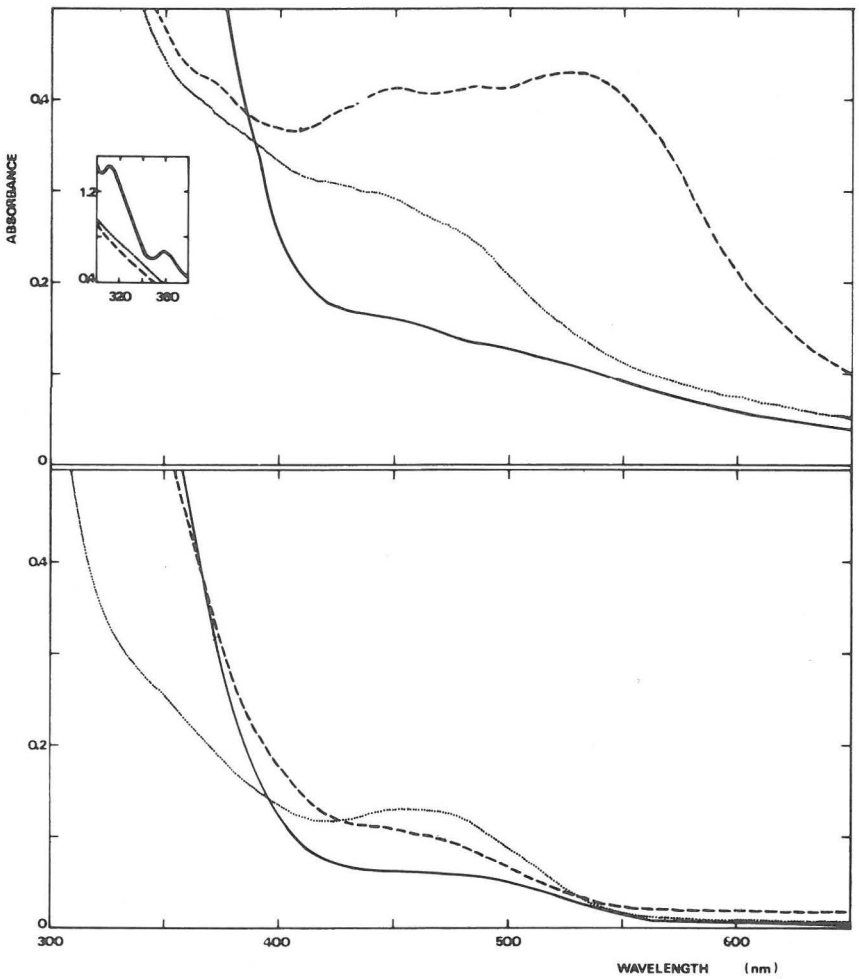


Fig. 4. — Comparative spectrophotometric analysis of the pigments A, B and C in formic acid.

The pigments reduced with ascorbic acid are presented in the top part of the figure, the pigments oxidized with $H_2O_2$ in the bottom part.

———— Pigment A.

..... Pigment B.

— — — — Pigment C.

halochrome reaction. They are not dissolved by neutral organic solvents but are soluble in alkali, acid methanol and formic acid. All these properties correspond with the definition of ommochromes as given by BECKER (1941) and LINZEN (1967). This confirms the publications of SCHWINCK (1953 and 1955) who found that the chromatophore pigments in the skin of cephalopods are ommochromes. Moreover we were able to demonstrate that at least three different pigments are present in the chromatophores of *Sepia officinalis*. They differ by their color (table 1), solubility (table 2) and redox-reaction. A comparison of the oxidized and reduced absorption curves of these pigments in the same solvent (formic acid) is presented in figure 4.

As our pigment fractions are respectively yellow (fraction A), brown red (fraction B) and violet purple (fraction C) it is plausible to put forward a correspondence of these pigments with the three chromatophore layers which were described as yellow, orange red and brown violet. However this is a subject for further research. We also plan to study the further purification and identification of the three pigments and the way in which they are bound to their substrates.


## SAMENVATTING

In de chromatophoren van de rughuid van de zeekat, *Sepia officinalis* L. komen ten minste drie verschillende pigmenten voor. Het zijn ommochromen en ze verschillen onderling in kleur, oplosbaarheid en redoxreaktie.

## RÉSUMÉ

Dans les chromatophores de la seiche, *Sepia officinalis* L. on peut démontrer la présence d'au moins trois pigments différents. Ce sont des ommochromes et ils se distinguent par leur couleur, leur solubilité et leurs propriétés d'oxydoréduction.



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