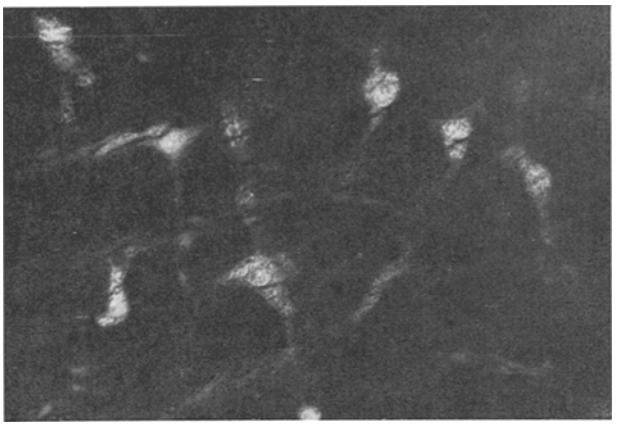

Fig. 3. Raphé bulbaire, IMAO-I DC 50 mg/kg-Dopa: persiste encore une légère fluorescence de l'endothélium capillaire; intense fluorescence verte des neurones contenant normalement de la sérotonine; prolongements neuronaux au contact des capillaires.

Appréciation de la barrière enzymatique pour la Dopa, par la dose d'IDC nécessaire pour abolir la fluorescence endothéliale

	Dose d'IDC nécessaire pour abolir la fluorescence endothéliale des capillaires du cerveau provoquée par la Dopa
Cortex cérébral	10 mg/kg
Cortex cérébelleux	15 mg/kg
Striatum	8 mg/kg
Locus niger et groupe A10 de FALCK	4 mg/kg
Noyaux latéraux pontobulbaires à catécholamines intraneuronales	10 mg/kg
Noyaux tronculaires sans monoamines intraneuronales et substance blanche	15 mg/kg
Noyaux raphé	50 mg/kg

Dopa, «des fenêtres» dans la barrière enzymatique capillaire. Cette hypothèse est suggérée par l'existence au niveau du locus niger de contacts étroits entre capillaires et neurones et surtout par le comportement des neurones du raphé médian. A ce niveau, la barrière enzymatique capillaire pour la Dopa est très intense. La fluorescence capillaire ne disparaît pas complètement avec 50 mg d'IDC. Pourtant les neurones normalement chargés de sérotonine accumulent des catécholamines aprés injection de Dopa. Or, ils présentent de curieux prolongements qui viennent au contact des capillaires.

L'accumulation de catécholamines par des neurones normalement chargés de sérotonine remet en cause la spécifité des neurones à monoamines et pose le problème de l'ambiguité de certains d'entre eux.

Summary. An enzymatic barrier for Dopa exists at the level of the cerebral capillaries which changes Dopa into Dopamine by means of endothelial decarboxylase. It is abolished by a decarboxylase inhibitor. Its intensity is weaker in structures containing Dopamine (Striatum, Locus Niger). Neurones of the Raphé containing serotonin can be charged with catecholamines. There are close contacts between neurones and capillaries.

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Action of Biogenic Amines on Crustacean Chromatophores: I. Differential Effect of Certain Indolealkylamines on the Melanophores of the Crabs *Uca pugilator* and *Carcinus maenas*

Although it is generally accepted that color changes in crustaceans are regulated by blood-borne substances[1,2], some recent studies[3–6] indicate that in addition to the well-established peptide neurosecretory products some indolealkylamines may also be involved. However, because the latter agents were tested in vivo only, the question arises whether they act directly on the chromatophores or indirectly by stimulating the release of neurohormones (peptides) from the neurohemal organs. A solution to this problem would be to simultaneously assay these amines by in vitro as well as in vivo methods on the chromatophores.

Shore crabs, *Carcinus maenas*, supplied by the Marine Biological Laboratory, Woods Hole, Massachusetts, and fiddler crabs, *Uca pugilator*, supplied by the Gulf Specimen Company, Panacea, Florida, were used in this investigation. The crabs were destalked at least 18 h prior to use in the experiments. All experiments were conducted on eyestalkless crabs at a temperature of 22–24 °C and under a light intensity of 377 meter-candles. Under these conditions the melanophoric pigment in the eyestalkless *Carcinus* and *Uca* was maximally concentrated. Each of the following substances, serotonin creatinine sulfate, bufotenin bioxalate, tryptamine, 5-hydroxytryptophan, and creatinine sulfate, was tested in dosages ranging between 0.01 and 100 µg per crab. They were dissolved in crustacean saline[7] and injected in doses of 0.05 ml per crab into *Uca* and 0.1 ml per crab into *Carcinus*. The controls received corresponding doses of crustacean saline alone. For comparison melanin-dispersing hormone in extracts of the eyestalks from *Carcinus* was injected into eyestalkless *Carcinus*. Eyestalks were freshly dissected, extracted in saline, boiled briefly, centrifuged, and the supernate

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was diluted with sufficient saline to provide the desired concentrations of the hormone. The melanophores were staged according to the scheme of HOGBEN and SLOME[8].

Injection of each of the substances to be tested in dosages of 0.01, 0.1, 1, 10, 50 and 100 µg per crab had no effect on the melanophores of *Uca pugilator*. However, injection of serotonin and bufotenin into eyestalkless *Carcinus* resulted in dispersion of the melanophoric pigment (Figure 1). These results confirm the previous findings of BAUCHAU and MENGEOT[6] that serotonin disperses the pigment in melanophores of *Carcinus*. It is seen here, for the first time, that the melanophores in *Carcinus* respond to bufotenin also, although to a lesser degree than to serotonin. The other substances, 5-hydroxytryptophan, tryptamine, and creatinine sulfate, were as ineffective on the melanophores of *Carcinus* as they had been on the melanophores of *Uca*. The present study clearly demonstrates a difference between the responses of melanophores of *Uca* and *Carcinus* to serotonin and bufotenin. Differences had previously been found between their responses to photic stimuli; the pigment in the melanophores of eyestalkless *Uca* dispersed in response to bright light[9] while the pigment in the melanophores of *Carcinus* failed to do so[10].

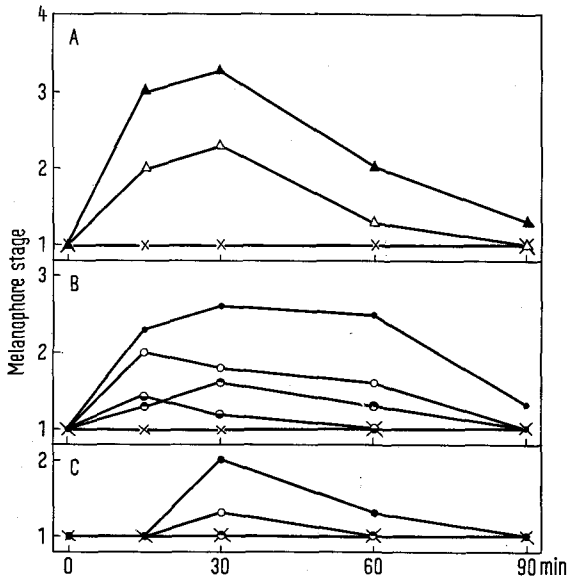

Fig. 1. Responses of the malanophores in eyestalkless *Carcinus maenas* to injection of extracts of the eyestalks (A), varying concentrations of serotonin creatinine sulfate (B) and bufotenin bioxalate (C) in a dose of 0.1 ml per crab. The concentrations of the eyestalk extracts were 0.05 (empty triangles) and 0.1 eyestalk equivalent (solid triangles) per dose. The concentrations of the drugs were 100 (solid circles), 50 (circles), 10 (circles half-filled on the top) and 1 µg (circles half-filled on the bottom) per dose. The controls (lines joined by ×) received injections of 0.1 ml saline per crab. Each curve represents the averaged data from 8–12 crabs.

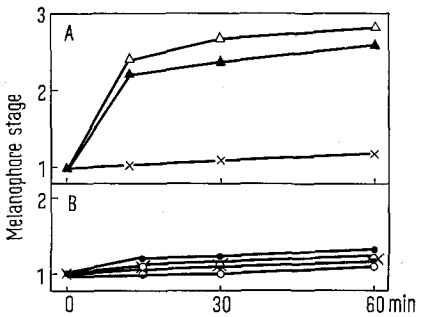

Fig. 2. Responses of the melanophores in legs isolated from eyestalkless *Carcinus maenas* and perfused with extracts of eyestalks from *Carcinus* (A) and varied concentrations of serotonin creatinine sulfate (B) in a dose of 0.1 ml per leg. The concentrations of the eyestalk extracts were 0.01 (empty triangles) and 0.005 eyestalk equivalent (solid triangles) per 0.1 ml. The concentrations of serotonin were 10 (solid circles), 1 (circles) and 0.1 µg (half-filled circles) per 0.1 ml. The controls (lines joined by ×) received 0.1 ml saline per leg. Each curve is based on the averaged data from 12–18 legs.

The integrated melanin-dispersing response evoked by 100 µg serotonin was approximately the same as that evoked by an extract of eyestalks in a dosage of 0.1 eyestalk equivalent per crab (Figure 1). The aim of the next experiment then was to determine whether qualitatively and quantitatively similar responses would be evoked in vitro by melanin-dispersing hormone and serotonin. Both substances were tested on the melanophores in legs isolated from eyestalkless *Carcinus*, utilizing the technique of FINGERMAN, MIYAWAKI, and OGURO[11] with the modification that each leg was perfused with 0.1 ml of the test solution. The eyestalk extracts and serotonin were then tested on isolated legs in dosages up to $^1/_{10}$th of the maximum injected into the eyestalkless crabs. The results (Figure 2) clearly show that under the given experimental conditions the eyestalk extracts elicited pigment dispersion in the melanophores, whereas serotonin in dosages of 0.1–10 µg per leg had no significant melanin-dispersing effect. The fact that eyestalk extracts evoke pigment dispersion in the melanophores in vivo and in vitro suggests that the well-known melanin-dispersing hormone acts directly on the melanophores. Furthermore, the observation that serotonin evokes melanin dispersion in vivo but not in vitro reveals that the mechanism of the drug-induced melanin dispersion is different from that of the hormonally-induced melanin dispersion. Whether serotonin stimulated the release of the melanin-dispersing hormone known to be present in the central nervous organs of *Carcinus*[12] remains to be investigated[13].

Résumé. Des extraits des pédoncules oculaires de *Carcinus* ont stimulé la dispersion de la mélanine in vivo et in vitro. Il paraît, pourtant, que le mécanisme par lequel la sérotonine provoque la dispersion de mélanine est différent de celui que déclenchent les extraits des pédoncules oculaires.

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[13] This investigation was supported by Grant No. GB-7595X from the National Science Foundation.