Alterations in the Structure of the *Octopus vulgaris* Chromatophore by Cytochalasin B

The Cephalopod chromatophore is a highly specialized single cell, bearing pigment granules within an intracellular container (CLONEY and FLOREY[1]; MIROW[2]; FROESCH[3]). Expansion of the chromatophore is achieved by radially attached muscle fibres, and return from the dispersed condition is probably promoted by the pigment container itself, which is supposed to have elastic function (CLONEY and FLOREY[1]).

At any degree of expansion of the chromatophore, the pigment granules are dispersed homogeneously within the container. To account for this, there must be a co-ordinating mechanism for the pigment dispersion, although a respective structure has not been described so far. The present study is an experimental approach to the question of 'granule link', applying a combination of electrical stimulation and cytochalasin B (CB). CB interferes with certain cytoplasmic filaments (WESSELS et al.[4]); however, the mechanism is not understood (CARTER[5]).

Methods. The effect of CB on function and structure of the chromatophore was examined as follows: 10 pieces of skin were removed from 4 decapitated specimens of *Octopus vulgaris* and put together into an oxygenated bath containing 5 µg/ml CB in seawater at 10 °C. (1 mg CB was initially dissolved in 1 ml dimethyl sulphoxide (DMSO) and then made up with 200 ml seawater). After every 15 min the skin slices were electrically stimulated by square wave pulses (4 V, 100 Hz, 0.3 ms)

[1] A. R. CLONEY and E. FLOREY, Z. Zellforsch. *89*, 250 (1968).
[2] S. MIROW, Z. Zellforsch. *125*, 143 (1972).
[3] D. FROESCH, Z. Zellforsch. *145*, 119 (1973).
[4] N. K. WESSELS, B. S. SPOONER, M. O. BRADLEY, N. A. LUDUENA, E. L. TAYLOR, J. T. WRENN and N. K. YAMADA, Science *171*, 135 (1971).
[5] S. B. CARTER, Endeavour *113*, 77 (1972).

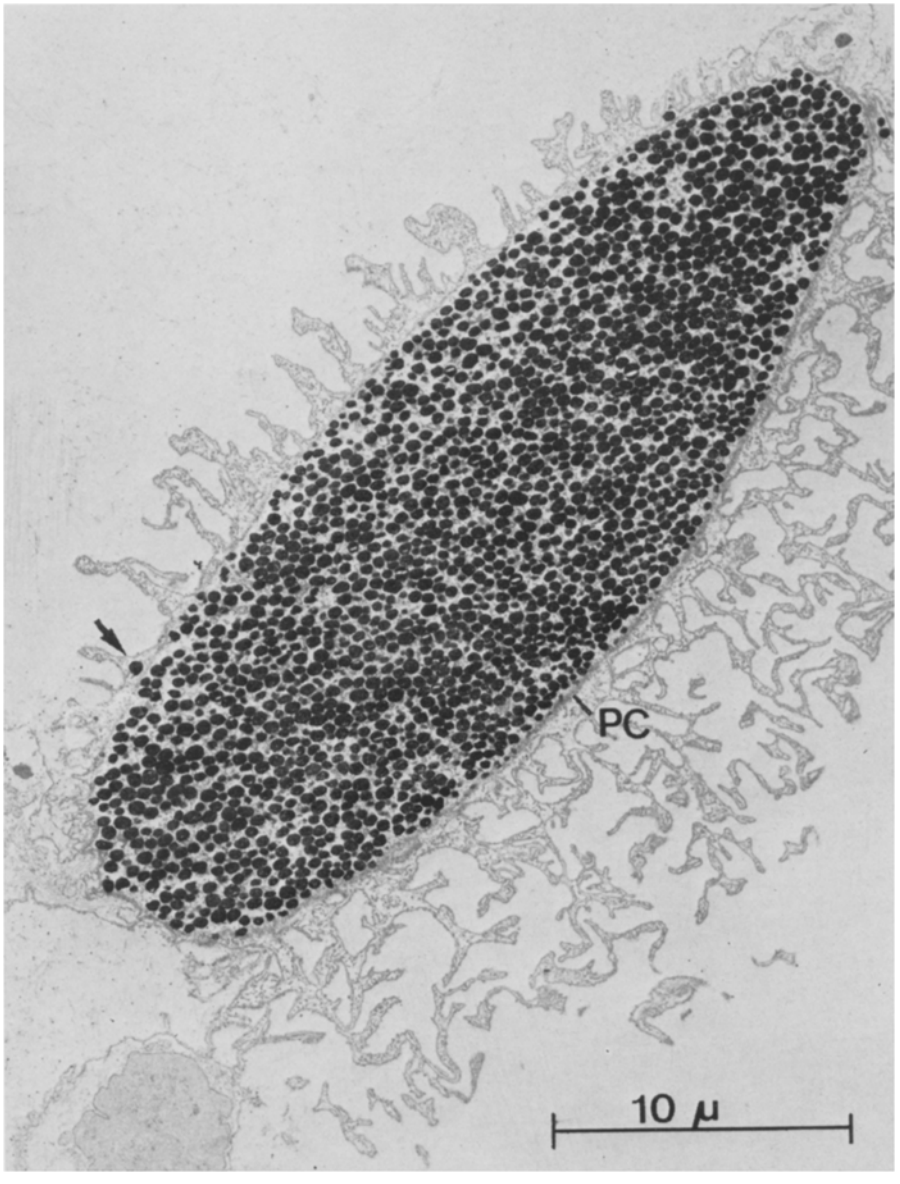

Fig. 1. Contracted *Octopus vulgaris* chromatophore cell of control specimen. The plasma membrane is extensively folded. The pigment granules are retained by an intracellular pigment container (PC). The arrow points to a pigment granule laying outside the container.

for 10 sec, in order to expand the chromatophores. After 30, 90 and 150 min, 1 slice was cut away from each specimen, fixed with osmic acid (2% in isosmotic solution, buffered with sodium cacodylate) and processed for EM observation as previously described (FROESCH[3]). Controls were bathed and stimulated at the same time in seawater containing 0.5% DMSO. At 90 and 150 min, samples were removed from the CB-bath and washed in oxygenated seawater (10 °C) for 30 min and then fixed. This was done to test the supposed reversibility of the effect of the drug.

Results. During the experiment, the chromatophores were observed under a binocular microscope. Stimulation always caused expansion of all the chromatophores and was followed by spontaneous contraction a few seconds after the last stimulus. In addition, numerous spontaneous expansions of the chromatophores, 'blinking', occurred in the periods between stimulation. This pattern remained constant throughout the experiment, implying that the function of the pigment container was unaffected by CB.

An effect of the CB was later recognized in the EM. In the untreated chromatophore all but avery few pigment granules are retained within the container (Figure 1). There is a marked increase in the number of granules outside the container in the CB-treated chromatophore, the pigment container appearing to leak these escaped granules (Figure 2). For each sample 50 sections of contracted chromatophores were examined with the EM, i.e. 500 alltogether. The escaped granules per unit circumference were counted. The proportion of escaped granules per 100 μm circumference rises from 2 in the control specimen to 5 (30 min), 7 (90 min) up to 8.5

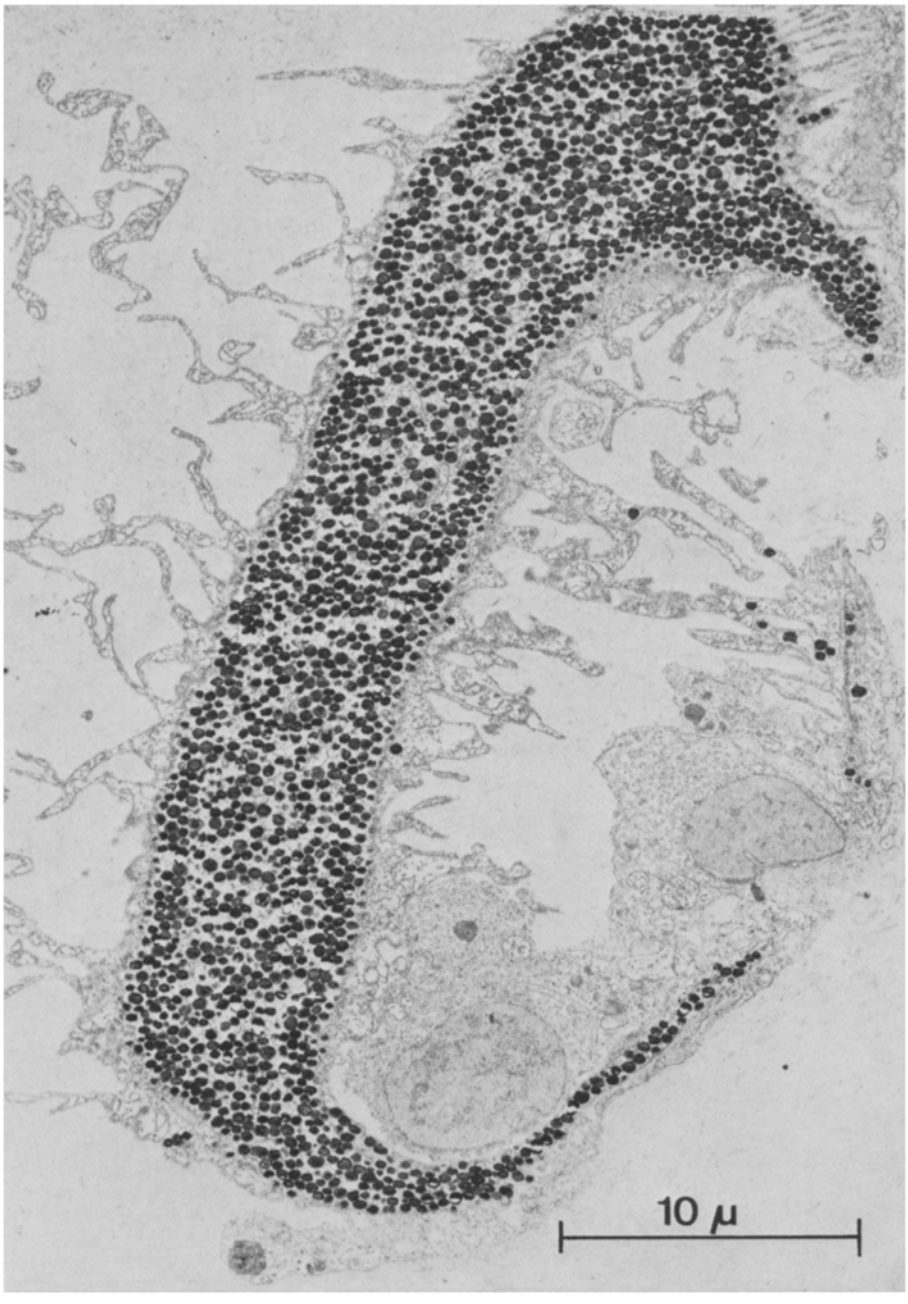

Fig. 2. Contracted *Octopus vulgaris* chromatophore, 150 min incubated with cytochalasin B at 5 μg/ml. Numerous pigment granules are situated outside the pigment container. The granules inside tend to assemble towards the periphery of the compartment.

(150 min), the overall increase being highly significant ($P < 0.01$).

Washing the specimens did not change the situation; rather, the 90-min CB-incubated chromatophores showed after washing exactly the same proportion of escaped granules as the 150-min specimens (Figure 3), showing that the escape effect is not reversible. In fact, it is highly improbable that once a granule has left the pigment container it would return to it ($P = [N \text{ outside}/N \text{ inside}]^2$). In this respect the irreversibility of the escape effect is secondary.

There is an additional phenomenon to be taken into consideration: in the control chromatophores the distribution of the pigment granules is very homogeneous, regardless of the state of contraction or expansion, (Figure 1). In the CB-incubated chromatophore, the pigment dispersion suffers considerable disorganization (Figure 2), the pigment granules tending to assemble towards the wall of the container. Rinsing in seawater for 30 min did not restore the normal organization.

Discussion. Though its function is essentially maintained, the chromatophore undergoes some structural change causing pigment granules to leave the container. To some extent they do so in the untreated chromatophore as well. Apparently the pigment container gradually fails to retain the granules the more their 'link' is damaged.

Ultrastructural studies of the pigment container have not shown its organization satisfactorily (CLONEY and FLOREY[1]; MIROW[2]; FROESCH[3]). In the squid it consists of 2 types of filaments, 250 Å and 50–70 Å respectively, whereas in *Octopus vulgaris* only filaments of less than 100 Å seem to be present. The squid pigment container is discontinuous in the plane of myochromatophoral junction. It is in these areas that the granules, which are found outside the container, may have escaped (MIROW[2]). In *Octopus* no such discontinuities have been found, suggesting that granules must penetrate the container in order to leave it.

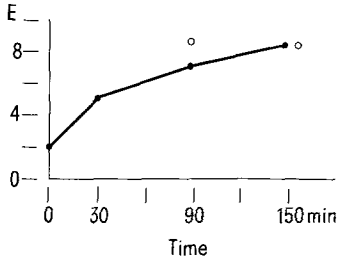

Fig. 3. ●, number of pigment granules (E)/100 µm circumference escaped from pigment container, at different times of cytochalasin B-incubation. ○, E of samples washed for 30 min after incubation with cytochalasin B.

However, the maintenance of elasticity of the pigment container throughout the experiment and the occurrence of escaped granules in the controls strongly indicate that the escape effect is not due to the influence of CB on the filaments of the pigment container.

In conclusion, I postulate the existence of a mechanism connecting the pigment granules and co-ordinating their dispersal in the chromatophore, the 'granule link'. Incubation with CB affects this mechanism and is followed by an increase of granules leaving the container. The number of granules entering into the statistics is small but sufficient to prove the significance of the result.

On the other hand CB does not interfere with the overall function of the pigment container. The sensitivity of the chromatophore to CB suggests the presence of filaments between the pigment granules. However, for direct proof evidence must be produced at the ultrastructural level, demonstrating 1. the filaments, 2. their insertion at the granules, and 3. their insertion at the pigment container or the plasma membrane.

Zusammenfassung. Nachweis, dass die Chromatophoren von *Octopus vulgaris* bei Inkubation mit Cytochalasin B strukturelle Veränderungen zeigen. Eine signifikante Zunahme von Pigmentkörnern ausserhalb des Pigmentbehälters ist festzustellen. Es wird angenommen, dass Cytochalasin B einen Mechanismus stört, der normalerweise Zusammenhalt und Verteilung der Pigmentgranula koordiniert.

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Some Structural Evidence in Support of Functional Integration within the Cephalic Neuroendocrine Complex of *Periplaneta americana* L.

The cephalic neuroendocrine system of insects, comprizing the median neurosecretory cells, their axonal pathways, corpora cardiaca (CC), corpora allata, and the various nerves associated with the retrocerebral complex, appears to act as one functional control unit[1]. A multicomponential control system can be effective only if its different components are functionally integrated. Functional integration in the cephalic neuroendocrine apparatus seems to be effected through neural and neurosecretory channels and conceivably also through feed-back humoral relations. In the course of our studies on the functional morphology of the cephalic neuroendocrine system of *P. americana* by in situ staining with performic acid Victoria blue[2], said to be specific for cystine/cysteine rich A-type neurosecretion, we came across an aberrant neurosecretory apparatus in an adult male. This is described and compared with the normal neurosecretory systems, inasmuch as together they throw some interesting light on the structural basis of functional integration in the cephalic neuroendocrine complex.

Neurosecretory cells in the pars intercerebralis medialis of the abnormal neuroendocrine apparatus are normal in configuration with the usual dendritic arborizations on their initial processes[3] (Figure 1); axonal collaterals

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