(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.

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.

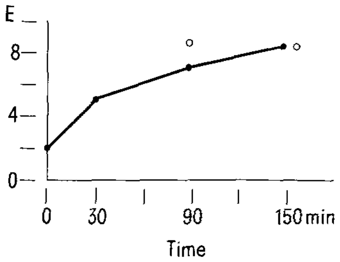

Fig. 3. ●, number of pigment granules (E)/100 μm circumference escaped from pigment container, at different times of cytochalasin B-incubation. ○, E of samples washed for 30 min after incubation with cytochalasin B.

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

[1] K. G. Adiyodi, Vijn. Kair. *1*, 57 (1969).
[2] G. S. Dogra and B. K. Tandon, Q. Jl microsc. Sci. *105*, 455 (1964).
[3] K. G. Adiyodi and H. A. Bern, Gen. comp. Endocr. *11*, 88 (1968).

containing performic acid Victoria blue-positive material could also be distinguished. It is well known that in pterygote insects, including *P. americana*[4,5], the axons of the 2 median groups of neurosecretory neurons run rostrally, medially and ventrally, decussate, turn posteriorly and leave the cerebral ganglion ventrally as the nervi corporis cardiaci interni (NCC I)[6]. The intracerebral portions of NCC I do not decussate in the abnormal neuroendocrine apparatus, though they seem to exchange some fibres at different levels (Figure 1). Our observations show that the chiasma is not complete in normal *P. americana*: processes of a small number of median neurosecretory cells do not decussate with the result that axons of each median group of neurosecretory neurons innervate both ipsilateral and contralateral CC. Some exchange of fibres between the 2 axonal pathways appear to occur normally also in *Locusta migratoria migratorioides*[7]. In the abnormal neuroendocrine apparatus axonal tracts from the 2 groups of median neurosecretory cells enter jointly the right CC, completely bypassing the left one (Figure 2). The left NCC I is nevertheless distinguishable extracerebrally, but is bleak in the wholemount, being devoid of viable outgoing neurosecretory processes within; possibly, the nerve in this case contained only non-neurosecretory ('ordinary') fibres and or stromal sheath. Neither the lateral groups of neurosecretory cells, nor the intracerebral or extracerebral portions of their axonal pathways (the nervi corporis cardiaci externi; NCC II) stain with performic acid Victoria blue in *P. americana*, apparently due to deficiency of cystine/cysteine residues in their secretory products. A few fine neurosecretory processes carrying granules and derived from the bunch of viable fibres in the left CC (see below) could be seen meandering from below into NCC I and NCC II. Such ascending processes are observed in the CC of normal animals also. Neurosecretory processes carrying stainable blobs and granules are far fewer in the left CC than in normal CC (Figures 2 and 3). The neurosecretory fibres present ramify over a large part of the left CC (except anterodorsally adjoining the anterior cardiac commissure described by Willey[4]) and in the proximal portions of the nervus corporis allati I (NCA I) (Figure 2). Neurosecretory stores can be seen to be relatively more abundant in regions proximal to the left nervus cardiostomatogastricus (NCS). The neurosecretory processes in the left half of the retrocerebral complex are clearly derived from the cardiostomatogastric commissural fibres from the main axonal bundle in the right CC (Figures 2 and 4). Ramifications of cardiostomatogastric commissural neurosecretory fibres with secretions can be seen in the right and left NCS (Figure 4) and also spreading over an area posterior to the cardiostomatogastric junction in the nervus oesophageus (Figure 2). A solitary neurosecretory process with granules could be traced from the right NCS going up the hypocerebral ganglion-nervus recurrens complex (Figure 4). Such ascending neurosecretory processes into the stomatogastric system are of common occurrence in wholemount preparations of *P. americana*.

As could be expected, the right NCC I, NCC II, NCS, CC and NCA I of the abnormal neuroendocrine apparatus are rich in stores of neurosecretion; secretory granules were abundant also in extensions of the NCA I, enveloping the right corpus allatum, whereas at the opposite side, regions of NCA I adjoining the corpus allatum had only a few secretory granules. Neurosecretory material was scarce or absent in the nervi corpori allati II and the postallatal nerves.

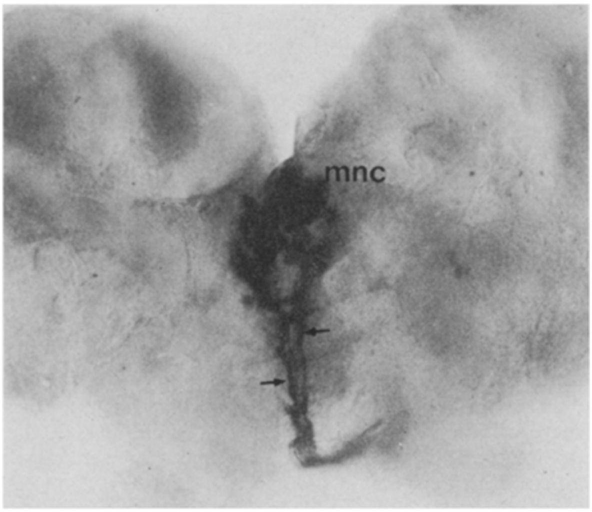

Fig. 1. Brain of the abnormal adult male stained in situ with performic acid Victoria blue to show median neurosecretory cells (mnc) and their non-decussating axonal pathways (arrows). The latter can be seen to leave the brain jointly. ×80.

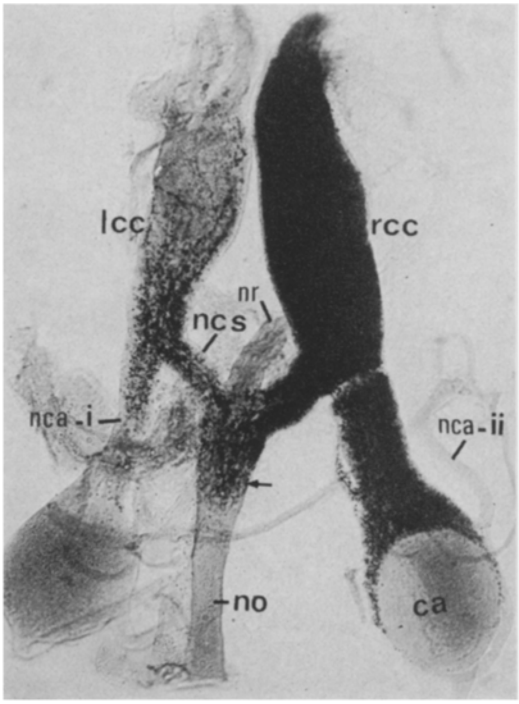

Fig. 2. Retrocerebral complex of the same animal stained in situ as in Figure 1. Note the nature of neurosecretory innervation of the left corpus cardiacum (lcc) by fibres from the right corpus cardiacum (rcc) through the nervi cardiostomatogastrici (ncs). Arrow points to the area on nervus oesophageus (no) rich in neurosecretion. ca, Corpus allatum; nca-i, nervus corporis allati I; nca-ii, nervus corporis allati II; nr, nervus recurrens. ×80.

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[4] R. B. Willey, J. Morph. *108*, 219 (1961).

[5] G. S. Dogra, Acta anat. *70*, 288 (1968).

[6] M. Gabe, *Neurosecretion* (Pergamon Press, Oxford 1966), p. 322.

[7] K. C. Highnam and M. W. West, Gen. comp. Endocr. *16*, 574 (1971).

Morphology of the cephalic neuroendocrine apparatus varies within fairly wide limits among individuals of *P. americana*, asymmetry of paired components being common. We have also come across a small number of individuals with uneven amounts of stored secretion in the 2 halves of the retrocerebral complex, though the difference was never as pronounced as described herein. Some structural and functional compensation in the right half of the retrocerebral complex in the abnormal neuroendocrine apparatus may only be anticipated, as this half has to cope with the storage, translocation and outlet of secretion by almost the double complement of median neurosecretory axons. The number of neurosecretory processes from the right CC crossing over to the left is, however, comparable to that in normal males. Other neurosecretory fibres being absent in the left CC, the picture presented here may, therefore, be taken as illustrative of normal or near normal neurosecretory innervation of a CC from the contralateral CC through the NCS for functional coordination (Figure 4). BRONSKILL and HANNAY[8] and DOGRA[5] had briefly indicated the presence of a cardiostomatogastric commissure in *P. americana*, though its extent could not be appreciated by them.

Fig. 3. Retrocerebral complex of a normal adult male *P. americana*, stained in situ as above. Note the distribution of neurosecretory fibres in various components of the retrocerebral complex, and compare with Figure 2. Arrow points to the area on nervus oesophageus rich in neurosecretion. ×80.

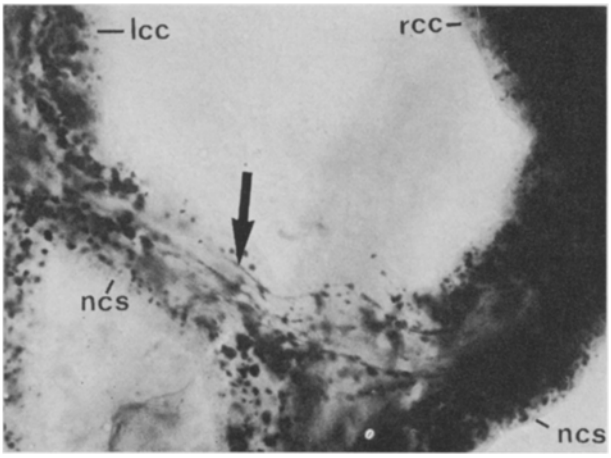

Fig. 4. A portion of the abnormal neuroendocrine apparatus given in Figure 2 enlarged to show the course of neurosecretory fibres (arrow) from the right corpus cardiacum (rcc) through the cardiostomatogastric nerves (ncs) into the left corpus cardiacum (lcc). Note the neurosecretory process with stainable granules ascending from the right ncs into the hypocerebral ganglion-nervus recurrens complex. ×300.

Our observations on normal and abnormal cockroach neurosecretory systems suggest that functional integration by neurosecretion between the 2 halves of the cephalic neuroendocrine apparatus may be brought about by 1. exchange of neurosecretory fibres intracerebrally at different levels between the median axonal pathways, 2. neurosecretory innervation of ipsilateral and contralateral CC by each median group of fibres and 3. exchange of neurosecretory fibres between the CC mainly through the NCS with possibly some linking with the stomatogastric system. Functions, if any, of neurosecretory processes ascending NCC I, NCC II and the nervus recurrens are unknown. The area adjoining the cardiostomatogastric junction in the nervus oesophageus which is rich in performic acid Victoria blue-positive granules (Figures 2 and 3) may serve as one of the sites of release of neurosecretion. Variations in the content of stainable material have been observed in this area in different individuals, according to different physiological states. Axonal translocation of neurosecretion from this area down the nervus oesophageus alone (Figure 3) can hardly account for such variations.

Zusammenfassung. Die funktionelle Integration der beiden Hälften des cephalen neurosekretorischen Systems kann mittels Neurosekret auf dreierlei Weise zustande kommen: 1. durch Austausch neurosekretorischer Fasern zwischen den beiden medianen Axonbündeln in verschiedenen Bereichen innerhalb des Gehirns, 2. über die neurosekretorische Innervierung der ipsi- und contralateralen Corpora cardiaca (CC) durch jede der beiden medianen Fasergruppen und 3. durch Austausch neurosekretorischer Fasern zwischen den CC über die Nervi cardiostomatogastrici.

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[8] J. F. BRONSKILL and C. L. HANNAY, Proc. XII Int. Congr. Ent., London 1964 (1965), p. 137.

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