The extent and duration of amantadine and amphetamine effects are confirmed by the observation of significant signs of hyperactivity in noradrenaline producing medullary cells. The Golgi apparatus shows a clear hypertrophy and it is surrounded by a great number of new formation vesicles, characterized by their low optical density and by the concentric disposition of the granule contents respect to the membrane. We have not found an increase of exocytosis with any of the two drugs employed in the study. On the contrary, we have observed an obvious increase in the number and size of cytoplasmatic microtubules during the releasing phase.

*Comments.* Morphological observation proves to be a reliable rule for investigating the pharmacological effect of some drugs. The peripheric action of amantadine is better clarified by the fact that the drug has a definite and specific activity at medullary level via a release of noradrenaline.

The morphological data in our hands seems to support that the mechanism of release of catecholamines from the adrenal cells is due to a process of hemiocytosis. This is in contrast with the observations realized by SCHNEIDER[21], SMITH and VAN ORDEN[25] and TRIFARO and coworkers[26]; but, on the other hand, our data confirm the results obtained by other authors[27–29] who think that the microtubular system is involved in the noradrenaline and adrenaline release from adrenal medullary cells.

The actions of amantadine seems therefore to be the overall results of an effect on the CNS and of the activity at peripheric level.

[25] D. J. SMITH and L. S. VAN ORDEN, Neuropharmacology *12*, 875 (1973).
[26] J. M. TRIFARO, B. COLLIER and A. LASTOWECKA, Mol. Pharmac. *8*, 264 (1972).
[27] A. M. POISNER and J. BERNSTEIN, J. Pharmac. exp. Ther. *177*, 102 (1971).
[28] R. G. RAHWAN, J. L. BOROWITZ and E. J. HINSMAN, Arch. int. Pharmacodyn. *206*, 345 (1973).
[29] D. A. REDBURN, A. M. POISNER and E. F. SAMSON, Jr., Brain Res. *44*, 615 (1972).

## An indirect demonstration of the substructure of the lamina basalis in the branchial heart of Sepia officinalis L. by means of cholinesterase reaction[1]

R. Schipp

*I. Zoologisches Institut, Universität, D–63 Giessen (Federal Republic of Germany, BRD), 13 September 1976*

*Summary.* Using a cytochemical cholinesterase reaction, a filamentous substructure of the lamina basalis of the peripheral epithelium in the branchial heart of Sepia officinalis L. can be demonstrated. The reaction product can be identified between the probable collagenous microfilaments, so that a form of negative staining is given.

The basement lamina has been identified as a fundamental structure of many epithelia with transporting functions. In excretory and blood vessel systems, this lamina is a structure which, as a 'semipermeable' barrier, separates the blood, hemolymph, of excretory fluid from the fluid which washes around the basal plasmalemma of the epithelial cells. Biochemical and cytochemical findings[2–4] have produced some indications of the chemical composition of the basement lamina in vertebrates; but its substructure is not well clarified[5].

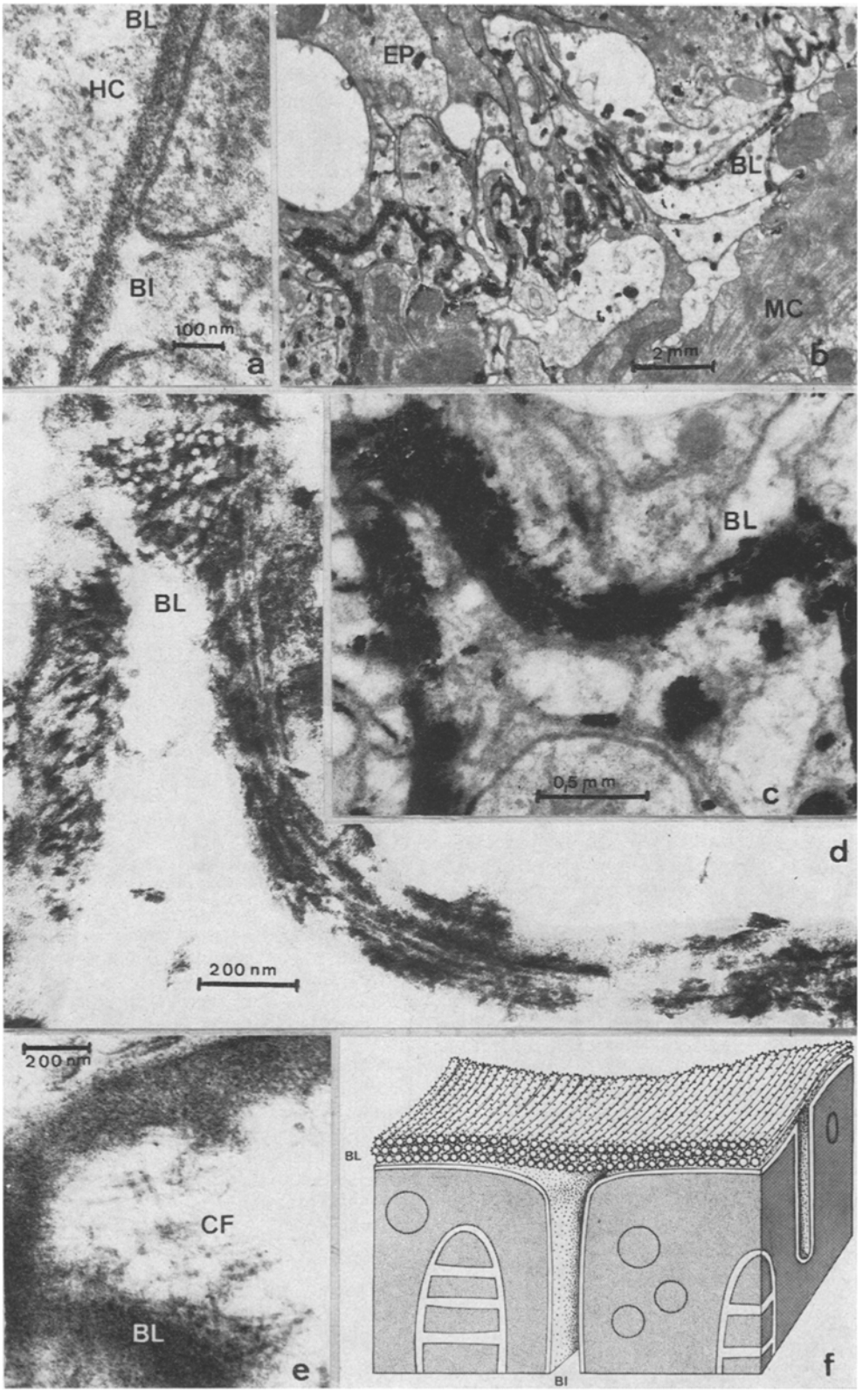
In our cytochemical and electron microscopical investigations concerning the circulatory and excretory system of dibranchiate Cepahlopods, we were able to see that the epithelia and endothelia of these organs (the branchial and systemic heart, the blood vessels, the renal and pancreatic appendages, the gills, etc.) always have a very large and distinct basement lamina of 200 nm thickness which very often builds up the only closed border of the blood sinus in the periphery of the organs[6,7]. With normal electron microscopical fixations and staining with uranyl acetate and lead citrate, the lamina shows a granular, amorphous, or partially a grid-like substructure (figure, a) in higher magnifications.

Cytochemical identification of the non-specific cholinesterase in the peripheral epithelium of the branchial heart of Sepia officinalis will provide new aspects of the fine structure of this extracellular layer in cephalopods.

*Material and methods.* The tissue was taken from the branchial heart of juvenile Sepia officinalis (L.) anaesthetized with 0.5–1% Ethanol Seawater. Fixation of specimens (⌀ 0.5–1 mm): 4% glutaraldehyde in phosphate buffer (Sørensen), pH 7.4; 1050 mOsm; 2 h; 6 °C; rinsing: in 10% sucrose for 30 min at 20 °C; incubation[8]: for 30 min in acetylthiocholineiodide 70 mg, 0,1 M sodium acetate 22 ml, 0,1 N acetic acid 3 ml, 3.75% glycine 1 ml, 0.1 M $CuSO_4$ 1 ml, 0.5% $PbNO_3$ 1 ml; the reaction can be inhibited with $10^{-6}$ M Mipafox; rinsing: seawater 5 min, $H_2S$ saturated sea water 5–6 min, seawater 5 min; postfixation: 1.5% $OsO_4$ in phosphate buffer; embedding: Durcupan® ACM (araldit); thin sections were cut on a LKB ultramicrotome and viewed – without staining with uranyl acetate or lead citrate – in a Zeiss EM 9A or a Philips EM 300. – In addition some samples were stained with alcian blue and the PAS-reaction for light microscopic analysis.

*Results.* The branchial heart sections primarily show reaction products on the surface of the ovoid cells[9] and in the lamina basalis of the peripheral brush border epithelium bordering the organ to the pericardial coeloma (figure, b). Some precipitations can be seen in the alcian blue and PAS-positive basic region of the epithelial cells and also in the microvilli of the brush border.

1 This work was supported by the Deutsche Forschungsgemeingemeinschaft.
2 R. P. Misra and L. B. Berman, Proc. Soc. exp. Biol. Med. *122*, 705 (1966).
3 W. Ferwerda, J. F. M. Meijer, D. H. Van den Eijnden and W. Van Dijk, Hoppe-Seylers Z. Physiol. Chem. *355*, 976 (1974).
4 W. Ferwerda and W. Van Dijk, Hoppe-Seylers Z. Physiol. Chem. 356, 1671 (1975).
5 D. B. Moffat, 000 Cambridge Univ. Press 1975.
6 R. Schipp, P. Höhn and A. Schäfer, Z. Zellforsch. *117*, 252 (1971).
7 R. Schipp, S. von Boletzky and G. Doell, Z. Morph. Tiere *279*, 304 (1975).
8 P. Kasa and B. Csillik, J. Neurochem. *13*, 1345 (1966).
9 R. Schipp and A. Schäfer, Z. Zellforsch. *101*, 367 (1969).




Basal lamina (BL) of Sepia officinalis: *a* of the pancreatic epithelium after a normal fixation and staining with uranyl acetate and lead citrate, *b–c* of the branchial heart epithelium after the cholinesterase reaction (unstained), showing a 'microtubular' substructure, *e* of the vena cephalica with corresponding collagenous fibrils (Cf), *f* in a schematic reconstruction after the figures *b–c*; hemocyanin (HC); epithelium (EP); muscle cell (MC); basal infoldings of plasmalemma (BI). (Further explanations in the text.)

Higher magnifications of the dark contrasted lamina basalis in longitudinal sections show a tubular substructure; this means that the reaction product can be found in the area between long unstained 'microtubuli'-like structures (figure, c). The diameters of their roughly circular cross sections are constant 210–215 Å. They lack membranes and are demonstrated indirectly in a kind of 'negative staining' by their dark stained surroundings. As we can see in the slightly developed micrograph (figure, d), the 'microtubuli'-like structures are always situated parallel to each other and build up the lamina basalis with 3–5 layers; there is no evidence for a grid-like orientation or periodical substructure of them.

*Discussion.* Using a cytochemical cholinesterase[8], we can demonstrate a 'microtubular'-like substructure in the lamina basalis of the peripheral epithelium in the branchial heart of Sepia officinalis L. which can be compared with the substructure of the lamina basalis in the midgut of Aedes[10]. The membrane-less 'microtubuli' are not stained themselves by using this method, but their surrounding matrix contains the reacting enzyme and binds the cytochemical reaction product. In normal fixed and stained thin sections, these tubular structures cannot be seen distinctly; the lamina basalis has an amorphous or sometimes a grid-like substructure there (figure, a), and the more or less parallel located 'microtubuli' are covered by the uranyl acetate or lead citrate precipitates or they are invisible because they do not possess any distinct and homogenous selfcontrast. The diameter of these structures (210–215 Å) (figure, f) is the same as that of the non-striated collagenous fibres surrounding the lamina basalis. This correspondence, as well as the fact that such non-striated collagenous fibres are very often in close contact to the lamina basalis, sometimes the collagenous fibres seem to originate in the amorphous lamina (figure e) indicates that the 'microtubular' – or better filamentous – structure is built up of collagenous material. This hypothesis is supported by the findings concerning the chemical nature of the lamina basalis in vertebrates i.e. of the glomerular basement membrane[2,3]. The analysis of this shows that the material of which it is composed is a member of the collagenous family[5]. – The chemical composition of the lamina basalis in Cephalopods is not yet known. Thus it is not yet possible to characterize the nature of the site-to-site bindings of the microfilaments or to determine the cause of the amorphous or sometimes grid-like structure resulting from staining by uranyl acetate and lead citrate.

10 J. A. Terzakis, J. cell. Biol. *35*, 273 (1967).

## Morphometric study of the aortic body type I cell[1]

J. T. HANSEN

*Department of Anatomy, The University of Texas Health Science Center, San Antonio (Texas 78284, USA),*
*29 June 1976*

*Summary.* The subclavian glomus (aortic body) of New Zealand white rabbits was examined ultrastructurally using stereological morphometric analysis. The Type I cells of the glomus possess numerous electron-opaque vesicles which occupy approximately 12% of the cytoplasmic volume of the cells. The amine-containing vesicles comprise a heterogeneous population of vesicles with a mean caliper diameter of 113.5 nm. Differences in vesicle diameters may indicate the storage of different biogenic amines, different secretion or maturation states between glomera and/or additional physiological functions for the glomera.

The subclavian glomus, located at the root of the subclavian artery, is one of a group of chemoreceptive organs known as the aortic bodies[2]. The cells of the subclavian glomera are of 2 types: Type I (chief) cells possess large round or oval nuclei, the normal complement of cytoplasmic organelles and numerous electron-opaque cytoplasmic vesicles (Figure). The Type I cells appear similar to the carotid body chief cells[3]. Type II (sustentacular) cells possess angular nuclei, attenuated cytoplasmic processes, which partially or completely envelop the Type I cells, and juxtanuclear mitochondria, Golgi complexes and granular endoplasmic reticulum[4]. The Type II cells are avesicular.

The electron-opaque cytoplasmic vesicles observed in the Type I cells of the subclavian glomus are known to contain biogenic amines. These organs yield an intense yellow-green fluorescence when treated with formaldehyde gas at 80 °C for 1 h[4], indicating that the cells contain primary catecholamines and possibly serotonin[5]. It appears likely that the electron-opaque vesicles of the Type I cells are the storage sites for these amines. It is well documented that both the carotid and aortic bodies are chemoreceptors[6,7] however, the significance of the conspicuous biogenic amine-containing Type I cells in the

Morphometric data on Type I cells

| | Volume densities (%) | | | Mean |
|---|---|---|---|---|
| | Nucleus[a] | Mitochondria[b] | Vesicles[b] | vesicle diameters (nm) |
| Mean | 35.77 ± 2.43 | 11.65 ± 0.81 | 12.07 ± 0.57 | 113.5 ± 0.7 |
| Range | 32.58–38.97 | 9.64–13.51 | 10.74–13.58 | |
| No. | 6 | 4 | 4 | 624 |

[a] Whole cell; [b] cytoplasm. Means ± S.E.M.

[1] Supported by NIH Grant 5-TO1-GM793 and The University of Texas.

[2] A. HOWE, J. Physiol. *134*, 311 (1956).
[3] J. T. HANSEN, I-LI CHEN and R. D. YATES, Texas Rep. Biol. Med. *32*, 854 (1974).
[4] J. T. HANSEN and R. D. YATES, Am. J. Anat. *144*, 477 (1975).
[5] B. FALCK and C. OWMAN, Acta Univ. Lund. Sec. II *7*, 1 (1965).
[6] J. F. HEYMANS and C. HEYMANS, Arch. int. Pharmacodyn. *33*, 273 (1927).
[7] J. H. COMROE, jr., in *Handbook of Physiology and Respiration* (Am. Physiol. Soc., Washington, D.C. 1964), p. 557.