Zoomorphology (1988) 108:1–11

Zoomorphology

Cytomorphology and copper content of the basal cells in the midgut gland of *Nautilus* (Cephalopoda, Tetrabranchiata)

A contribution to the localization of hemocyanin synthesis

P. Ruth, R. Schipp, and B. Klüssendorf

Institut für Allgemeine und Spezielle Zoologie, Justus-Liebig-Universität, D-6300 Gießen, Federal Republic of Germany, and ORSTOM, Aquarium of Nouméa, New Caledonia

Summary. By means of light and electron microscopical studies on *Nautilus pompilius* and *Nautilus macromphalus*, special basal cells within the alveolary enlarged terminal parts of the tubules of the midgut gland were revealed. Their abundant rough endoplasmic reticulum (RER) with enlarged cisterns synthesizes ring-shaped macromolecules that resemble the hemocyanin molecules within the bloodspaces in size and form and seem to be secreted throughout the lamina basalis, locally discharged into the adjacent blood sinus. The hypothesis that this cell type represents a site of hemocyanin synthesis was confirmed by Zeeman AAS and ASTEM analyses of the content and distribution of copper within the glandular tissue, especially the different compartments of this cell type.

A. Introduction

Previous electron microscopical and histochemical studies of the branchial gland of different coleoid species have provided striking evidence of a hematopoetic (i.e. hemocyanin-synthesizing) function of this organ. Its glandular cells are characterized by an abundant rough endoplasmatic reticulum containing, within the enlarged cisternae, a large quantity of ring-shaped structures that correspond in size and shape to the hemocyanin molecules within the blood vessels and the lacunae (Dilly and Messenger 1972; Schipp et al. 1973; Muzii et al. 1974; Messenger et al. 1974).

The existence of a branchial gland in Nautiloidea gives rise to controversy. Some authors have claimed it does exist (Joubin 1980; Taki 1964); while others have reached the conclusion that there is no formation of such a gland in Tetrabranchiata (Naef 1913; Mangold-Wirz and Fioroni 1970; Saure et al. 1987). Recent investigations on the gill complex of nautiloids indicate that there are groups of glandular cells within the ligamentum branchiale that resemble the coleoid glandular cells mentioned above and could possibly be a site of hemocyanin synthesis (Saure et al. 1987).

As only few cells can be found there, it is still not known whether or not other tissues or organs may also be involved in hemocyanin synthesis in nautiloids. This paper is concerned with a similarly specialized type of cell that can be found within the alveolar enlarged terminal parts of the hepatopancreas or midgut gland (MGG) tubules of *Nautilus macromphalus* (Sowerby 1849) and *Nautilus pompilius* (L.) that might possibly play a part in the process of hemocyanin synthesis.

Offprint requests to: P. Ruth

B. Materials and methods

The investigation was carried out on organs of three adult *Nautilus macromphalus* (G.B. Sowerby, 1849) from the coral reefs in New Caledonia and those of one adult and one juvenile *Nautilus pompilius* (Linné, 1758) specimen from Philippine costal waters. The animals were anesthetized with 1%–2% ethanol/seawater. Tissue was pre-fixed with 4% glutaraldehyde in isotonic phosphate or cacodylate buffer (pH 7.3; 1000 mosmol) for 2 h at 4° C increasing to 20° C. The post-fixation was carried out in 1.5% OsO_4 in phosphate or cacodylate buffer for 2 h. The material was embedded in araldite (Durcupan and Vestopal) and cut on a Reichert OM/U2 ultramicrotome.

Light microscopical studies. Sections 1 µm thick were stained with methylene-blue-azure II and basic fuchsin (Humphrey and Pittman 1974). They were studied by phase-contrast microscopy using a Zeiss "Photomikroskop II".

Transmission electron microscopy. Ultra-thin sections were put on copper grids, stained with uranyle acetat and lead citrate and viewed in a Zeiss EM 9 A and a Philips EM 300 transmission electron microscope (TEM). Because of the high content of lipids in the tissue of the midgut gland, fixing and embedding this rather fluid material proved difficult.

Scanning electron microscopy. Tissue fixed in a formaldehyde-seawater solution was dehydrated in paramylester and

List of animals used:

Animal no.	Stage	Species	Total weight (g)	Shell weight (g)	Diameter of shell (cm)
1	Adult	*N. macr.*	587	189.5	16.0
2	Adult	*N. pomp.*	355	92.3	12.0
3	Adult	*N. macr.*	513	185.0	14.6
4	Juvenile	*N. pomp.*	95	30.0	7.8
5	Adult	*N. macr.*	423	158.5	13.6

dried first in the air and then in a vacuum, because the almost fluid consistency of this tissue ruled out the use of the critical-point method for drying the specimens. The scanning electron microscope (SEM) investigations were carried out on a Cambridge Stereoscan 4 microscope.

Analytical scanning transmission electron microscopy. Sections from the araldite material with a thickness of 1000 Å were placed on nickel or plastic grids. The spectra of elements were recorded with a Hitachi H 600 ASTEM (analytical scanning transmission electron microscope) based on energy-dispersive X-ray microanalysis. The duration of measurement at each point was 100 s at 25 kV.

Atomic absorption spectroscopy. To determine the copper concentration in the midgut gland only fresh tissue or fresh frozen material was used. Measurements were taken with a Zeeman atomic absorption spectrometer. The materials 1577a (bovine liver), NBS (National Bureau of Standards), and used for calibration were dried milk (F9).

C. Results

1. Light and scanning electron microscopical findings

The MGG of nautiloida is a paired organ connecting with the midgut via the ductus hepatopancreas, which is also paired, and the caecum. Morphologically it is composed of numerous acini that are in direct contact with the above-mentioned ductus by means of distally ramified ductuli. The single acinus is surrounded by a thin but multilayered septum representing the barrier between the acinary blood sinus and the coelom (Fig. 1).

The functional units of an acinus are ramified, blind-ending tubuli, which are morphologically as well as histochemically differentiated in a longer transitional part and an enlarged terminal alveolus (Fig. 2). The transitional part is characterized by a high columnar epithelium with basally localized nuclei and a distinct apical microvilli border. Further striking characteristics of these *storage cells* that absorb chyme particles are their basal infoldings and, in particular, the high content of lipid droplets (Fig. 2).

Three different types of cell can be distinguished within the terminal alveolus – even when viewed under the LM (Fig. 2).

Chief cells. These high columnar cells, found from the basal lamina up to the lumen, contain a great many lipid droplets, as well as other inclusions, and have a flatter, sometimes irregular microvilli border.

Vacuolized cells. They are principally located in the entrance area of the alveolus; they are lower in height than the chief cells and contain numerous vacuoles and only a few dense bodies within a transparent cytoplasm.

Basal cells. These triangularly shaped cells can be found only at the basal area of the terminal alveolus, i.e. within the triangular space between the basal part of the chief cells and the adjacent bordering blood sinus. Their most obvious characteristic is the extended endoplasmatic reticulum (ER), which, as can be seen even by LM, forms concentrically layered accumulations with a diameter of up to 13 µm. Another typical feature of the basal cells is the sharply outlined nucleolus within their centrally situated ovoid nuclei.

2. Electron microscopical findings

The purpose of this chapter is to focus on an ultrastructural description of the *basal cells,* which are of particular interest in the case of hemocyanin synthesis, which is dealt with here. The other types of cell within the tubulus and the alveolus will be described later in a more detailed comparative ultrastructural and histochemical study.

As mentioned above the abundant ER fills up nearly the whole volume of the basal cell surrounding the nucleus. It primarily consists of the granular or rough type (RER) and is very densely studded with ribosomes. However, the other cellular organelles (i.e. mitochondria, lipid droplets, dictyosomes and dense bodies) appear only sparsely between the RER, which is often concentrically layered, and are dispersed over the cell (Figs. 3, 4).

The different appearance and/or organization of the RER (i.e., changing density of ribosomes) and the differing sizes and diameters of the ER cisternae are essential to any further classification of this cell type.

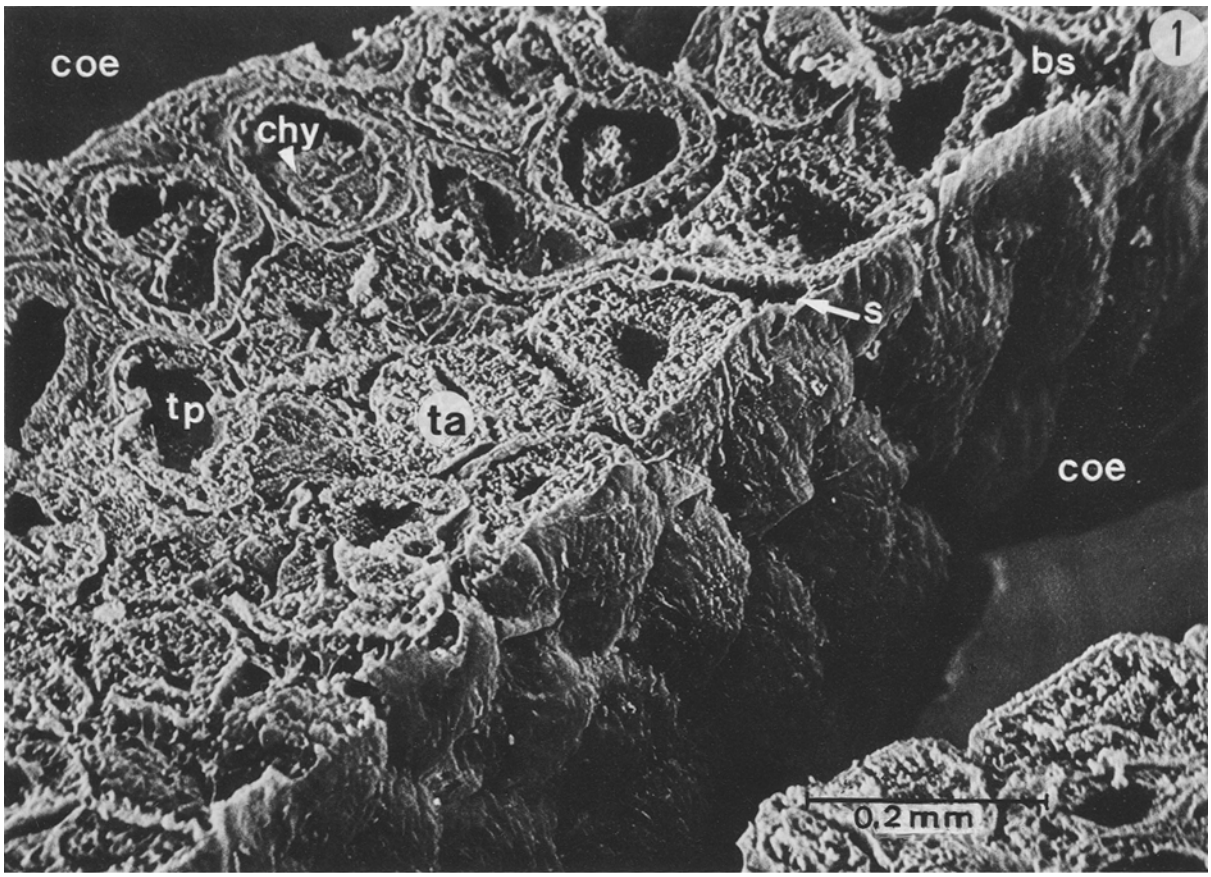
Basal cell I. Concentric agglomerations of the RER, sometimes with a high density of bound and free ribosomes, seem to be the main content of these cells. The cisternae are generally small in diameter and nearly empty (Figs. 3, 5). Lipid droplets can often be found.

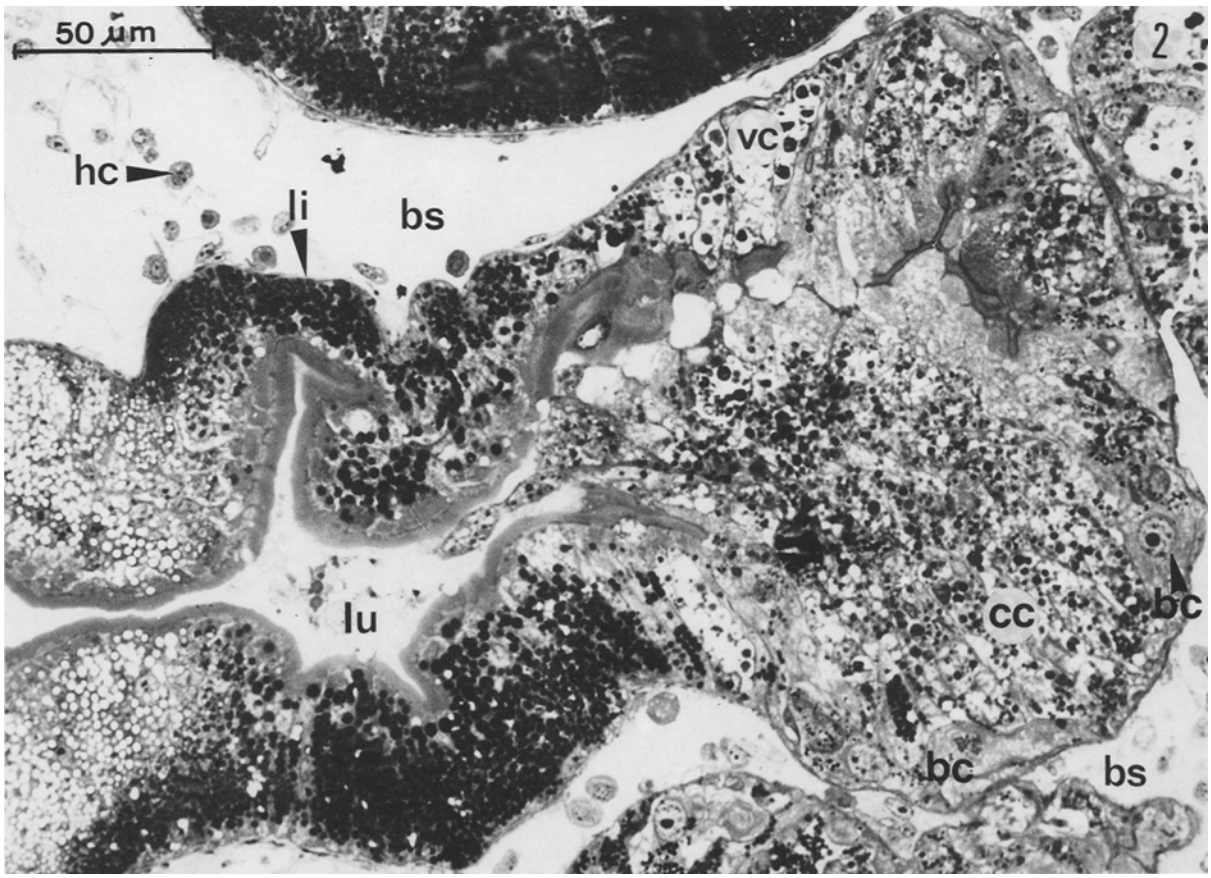
Basal cell II. The RER of these cells commonly has a higher density and partially enlarged cisternae containing ring-shaped particles with a diameter of 204 Å ± 15.1 (Figs. 4, 9). Furthermore, spirally layered myeline bodies (diameter: 7.2 µm) can frequently be found within their cisternae, which seem to be partially despiralized, forming transparent vacuoles (Fig. 9). The lamina basalis, bordering on the blood sinus, seems to become loose, revealing a spongy network of collagenous fibers (Fig. 4).

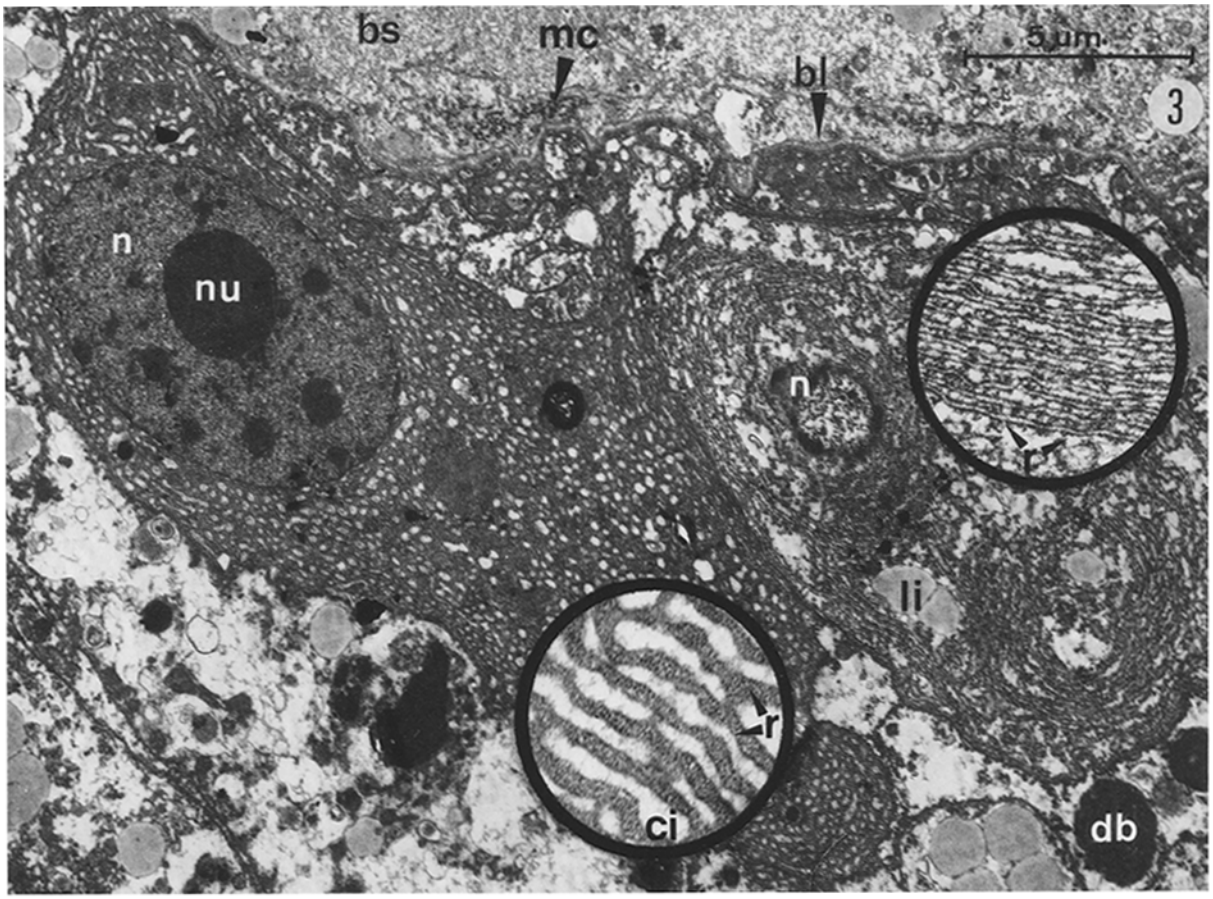
Basal cell III. The cisternae of these cells are greatly enlarged and contain high quantities of the ring-shaped molecules mentioned above (Figs. 3, 6, 7, 11). In correlation to the very large amount of ribosomes within the reduced areas of cytosol between the cisternae membranes, numerous sharply outlined nucleoli occur in the karyoplasm (Fig. 3), indicating a high rate of RNA synthesis. The dis-

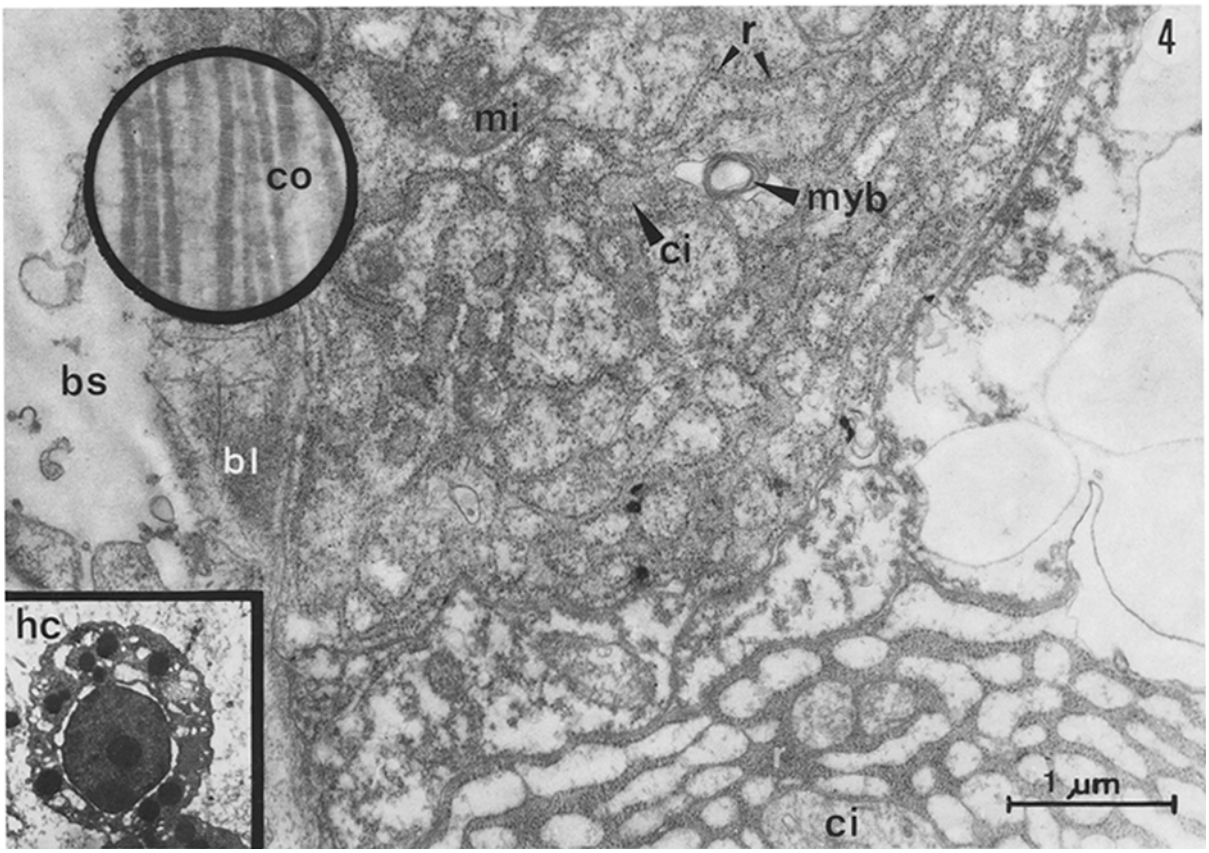
Fig. 1. Stereoscan electron micrograph of an acinus of the midgut gland of *Nautilus macromphalus*. The acinus is surrounded by a septum (*s*), which separates the blood space (*bs*) from the coelom (*coe*). The terminal alveoli (*ta*), characterized by a narrow lumen, can be distinguished from the transition parts (*tp*), characterized by a wide lumen. The transition parts are partially filled with chyme (*chy*)

Fig. 2. Longitudinal section of a blind-ending glandular tubulus of the midgut gland of *Nautilus pompilius* seen by phase-contrast microscopy. The transition part is marked by a great number of lipid inclusions (*li*) and a wide lumen (*lu*). Basal cells (*bc*), chief cells (*cc*) and vacuolized cells (*vc*) can be found in the terminal alveolus. *bs* Blood space; *hc* hemocyte


coe
chy
bs
1
s
tp
ta
coe
0,2 mm


50 µm
2
hc
li
bs
vc
lu
cc
bc
bc
bs


bs
mc
bl
5 µm
3
n
nu
n
r
li
r
ci
db


r
4
mi
co
myb
ci
bs
bl
hc
1 µm
ci

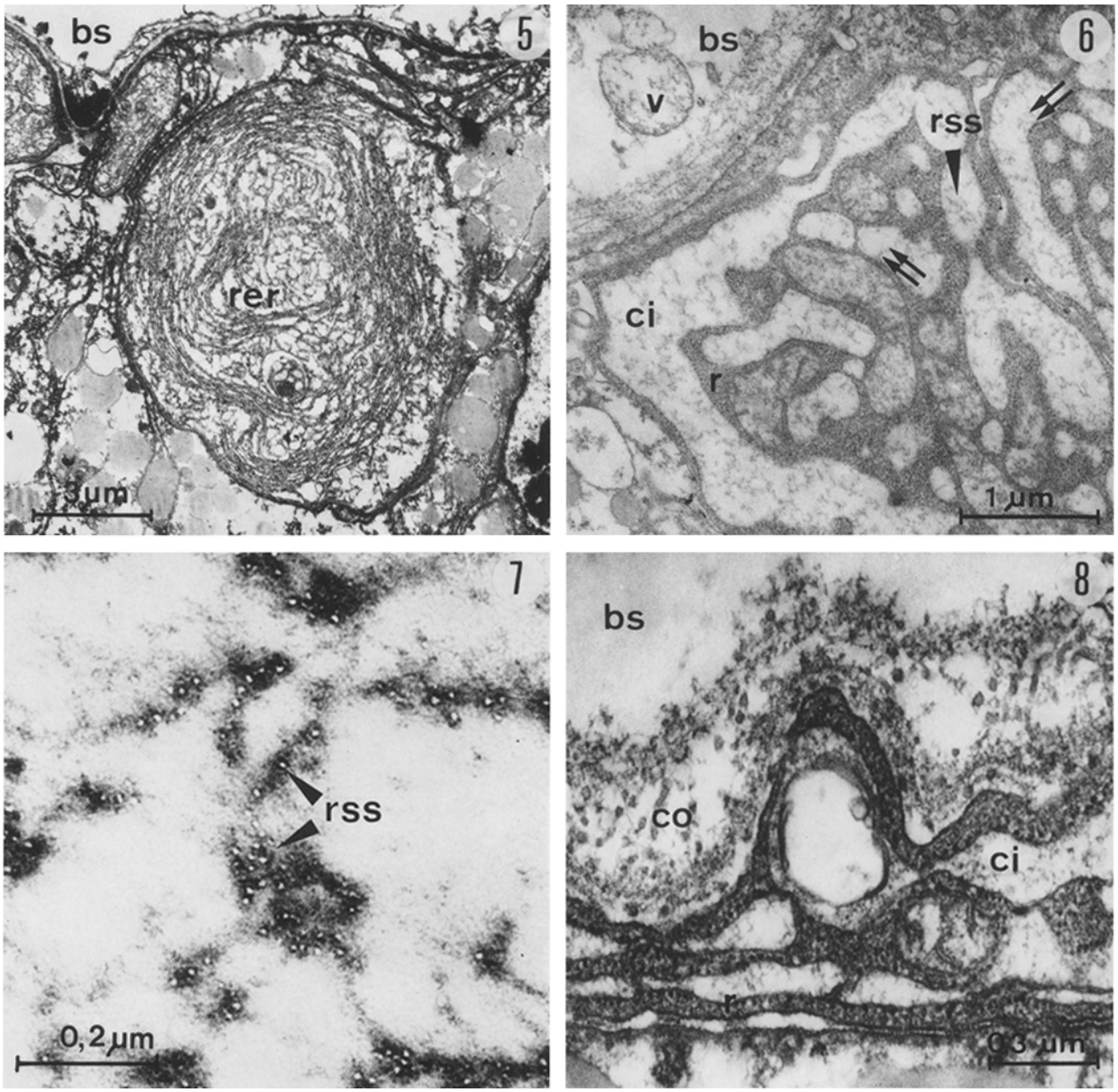


Fig. 5. *Nautilus pompilius*. Basal cell I with concentric piles of rough endoplasmatic reticulum (*rer*). *bs* Bloodspace

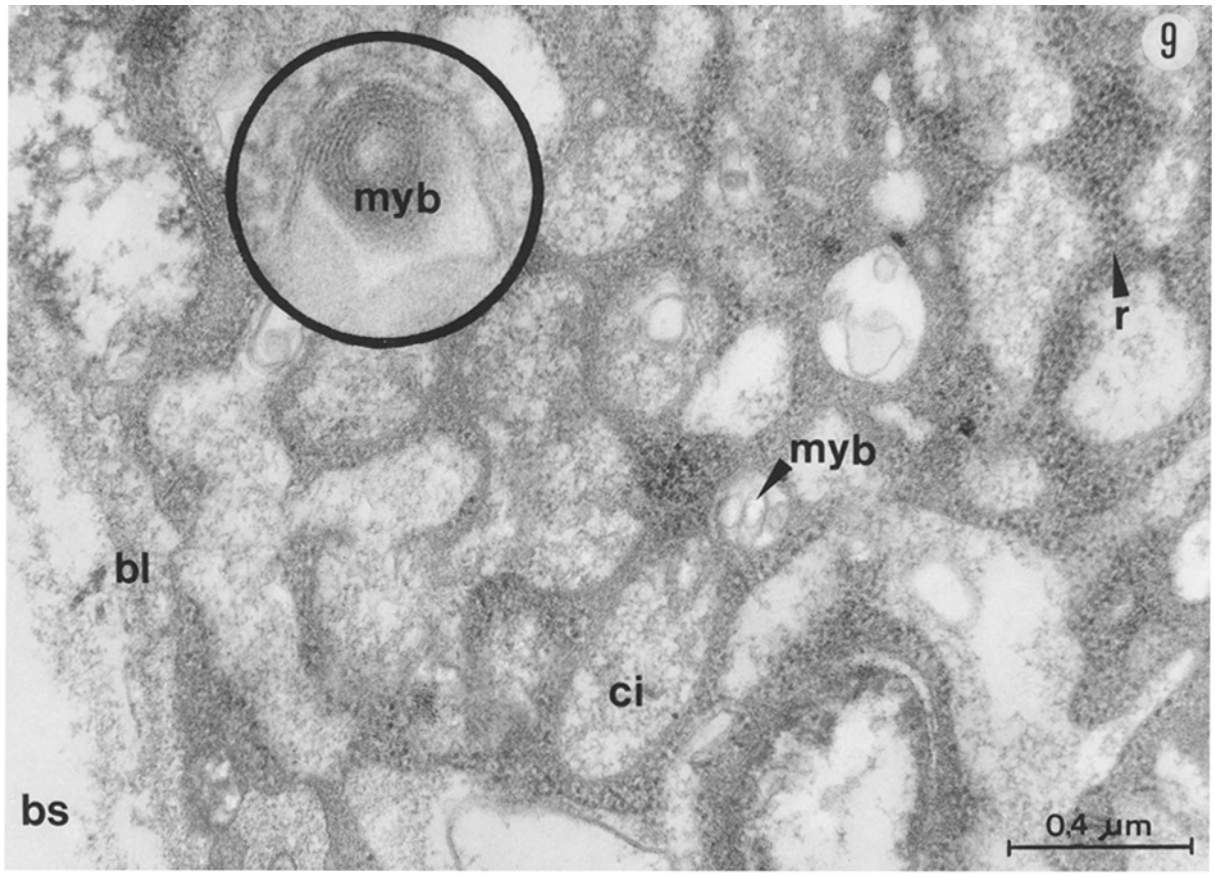
Fig. 6. *Nautilus pompilius*. In the cisternae (*ci*) of the RER of the basal cell III there are ring-shaped structures (*rss*) and products of synthesis (→) of the ribosomes (*r*). *bs* Blood space; *v* vesicle

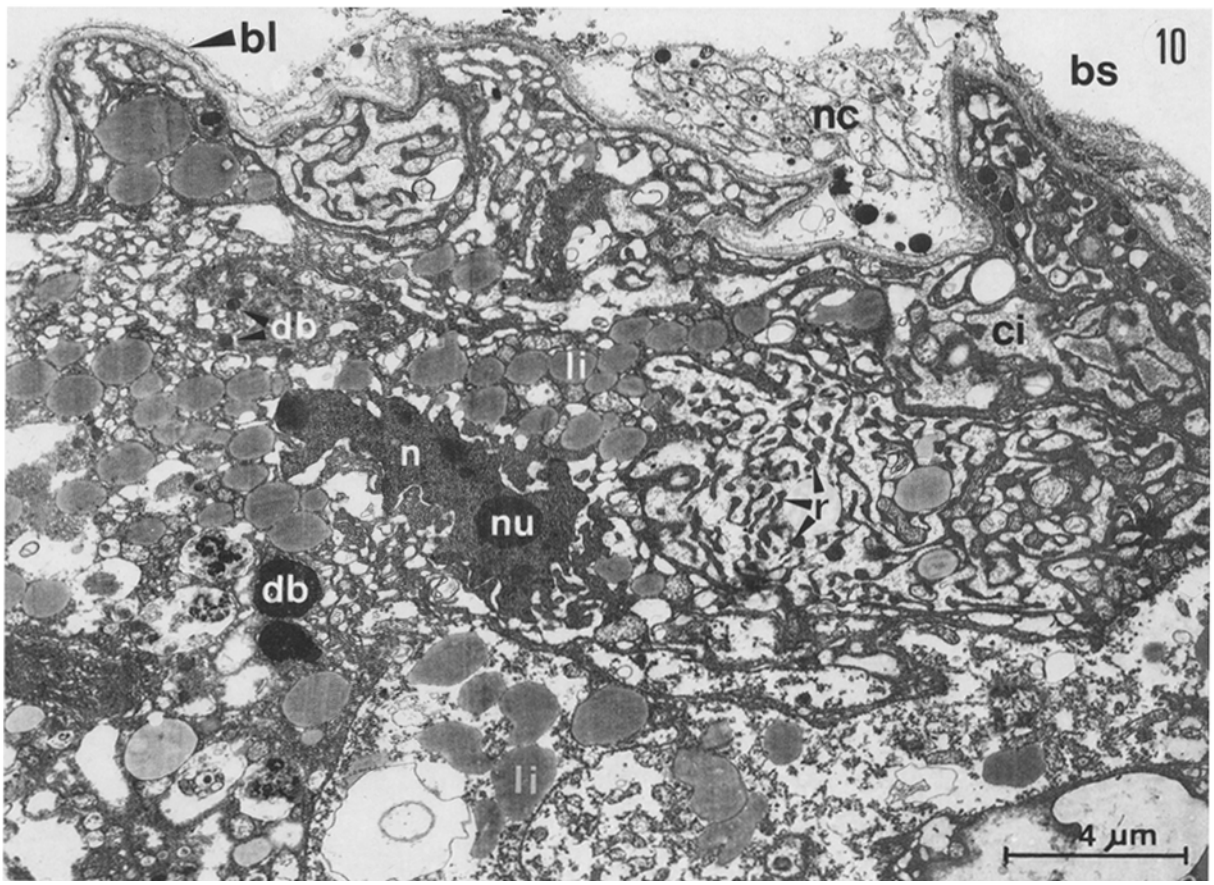
Fig. 7. *Nautilus macromphalus*. Ring-shaped structures (*rss*) with osmiophobe lumina of a cisterna of a basal cell III in a negatively stained section

Fig. 8. *Nautilus pompilius*. Basal part of a basal cell III. *bs* Blood space; *ci* cisternae of the RER; *co* collagen; *r* ribosomes

Fig. 3. A basal cell I of *Nautilus pompilius* with extended rough endoplasmatic reticulum (*inset*) is shown in the right part; nucleus (*n*). *Inset* magnification: ×17100. A basal cell III in the *left part*. Sharply outlined nucleoli (*nu*) in the nucleus (*n*). the cisternae (*ci*) of the RER are enlarged. *bl* Basal lamina; *bs* blood space; *db* dense body; *li* lipid; *mc* muscle cell; *r* ribosomes. *Inset* (*left*) magnification: ×27300

Fig. 4. The basal cell II of *Nautilus pompilius* with a loose basal lamina (*bl*) in the upper part. The cisternae (*ci*) of the RER are filled with ring-shaped synthesis products of the ribosomes (*r*) and myeline bodies (*myb*). A part of a basal cell III is shown below a basal cell II. *bs* Blood space; *co* collagen; *hc* hemocyte; *mi* mitochondrium. *Inset* magnifications: ×85760 (*above*); ×4700 (*below*)


9
myb
r
myb
bl
ci
bs
0,4 µm


bl
10
bs
nc
db
ci
li
n
nu
r
db
li
4 µm

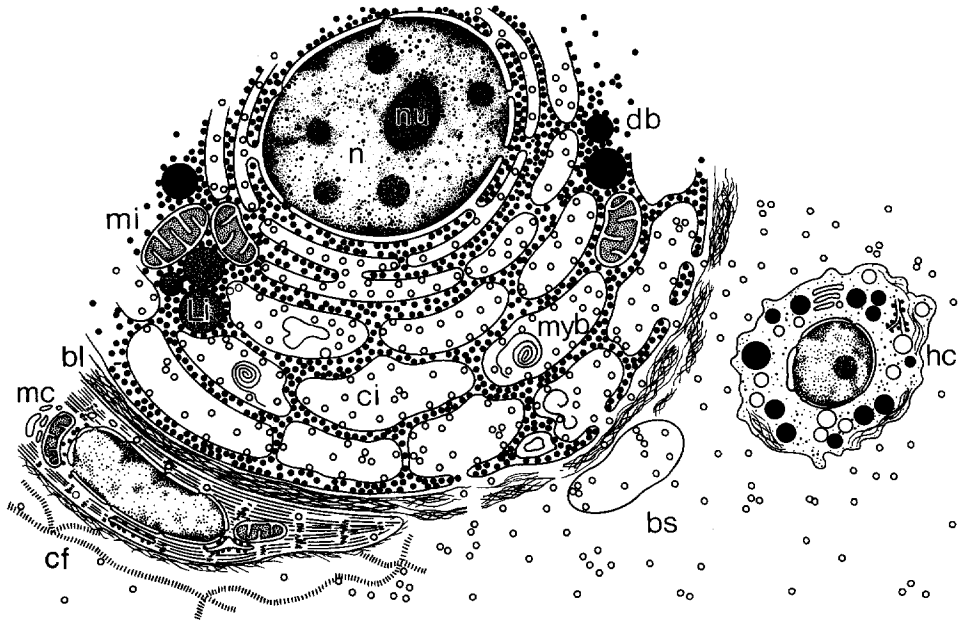


Fig. 11. Schematic diagram of a basal cell III. The cisternae (*ci*) of the RER are enlarged. *bs* Blood space; *cf* collagenous fibers; *db* dense body; *hc* hemocyte; *li* lipid; *mi* mitochondria; *mc* myocyte; *myb* myeline body. The ring-shaped molecules (*o*) may leave the cell through the loose basal lamina (*bl*)

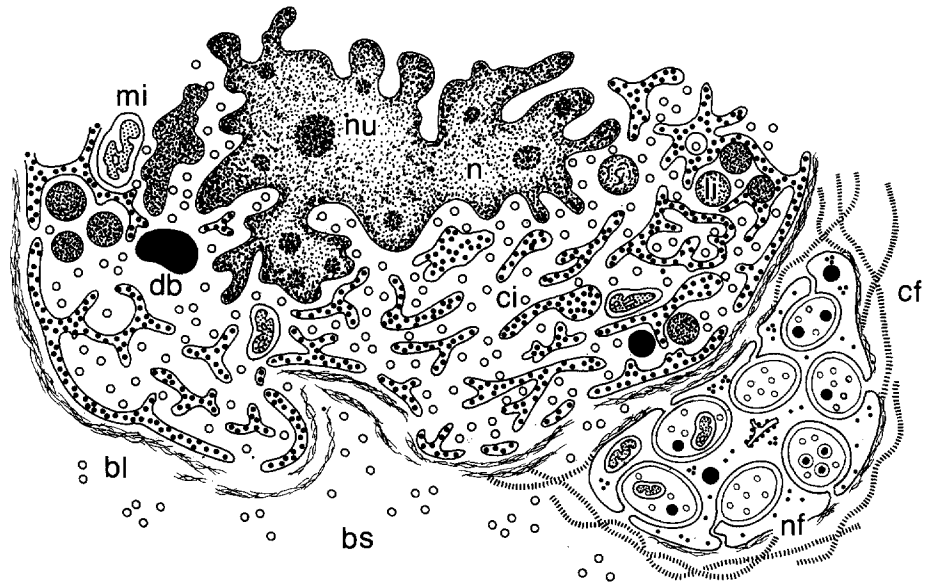


Fig. 12. Scheme of a basal cell IV. This type is characterized by the beginning of a general karyolysis. *bl* Basal lamina; *bs* blood space; *co* collagenous fibers; *db* dense body; *li* lipid; *mi* mitochondria; *nf* nerve fiber; *n* nucleus; *nu* nucleolus

solving tendency of the lamina basalis described for the basal cell II seems to be generally intensified (Fig. 8).

Basal cell IV. The nuclei of these cells (diameter: 7.2 µm) have an irregular lobed shape; their karyoplasm is of high density and contains numerous pyknotic dark – stained DNA particles. Peripherally the nuclear membrane seems to break up slightly in order to build dense irregular frag-

Table 1. Cu content of the midgut gland of *Nautilus macromphalus* (Cu µg/g fresh weight; .222.6 nm)

Animal	*n*	*x*	*s*
1	6	851	±196
3	5	320	± 25
5	6	249	± 18

Fig. 9. *Nautilus pompilius.* The cisternae (*ci*) of the basal cell III contains numerous myeline bodies (*myb*). *bl* Basal lamina; *bs* blood space; *r* ribosomes. *Inset* magnification: ×216800

Fig. 10. The basal cell IV of *Nautilus pompilius* is characterized by an autolytic nucleus (*n*) with sharply outlined nucleoli (*nu*). A non-myelinated polyaxonal nerve (*nc*) in the blood space (*bss*) is remarkable. *bl* Basal lamina; *ci* cisternae; *db* dense bodies with a different electron density; *li* lipid; *r* ribosomes

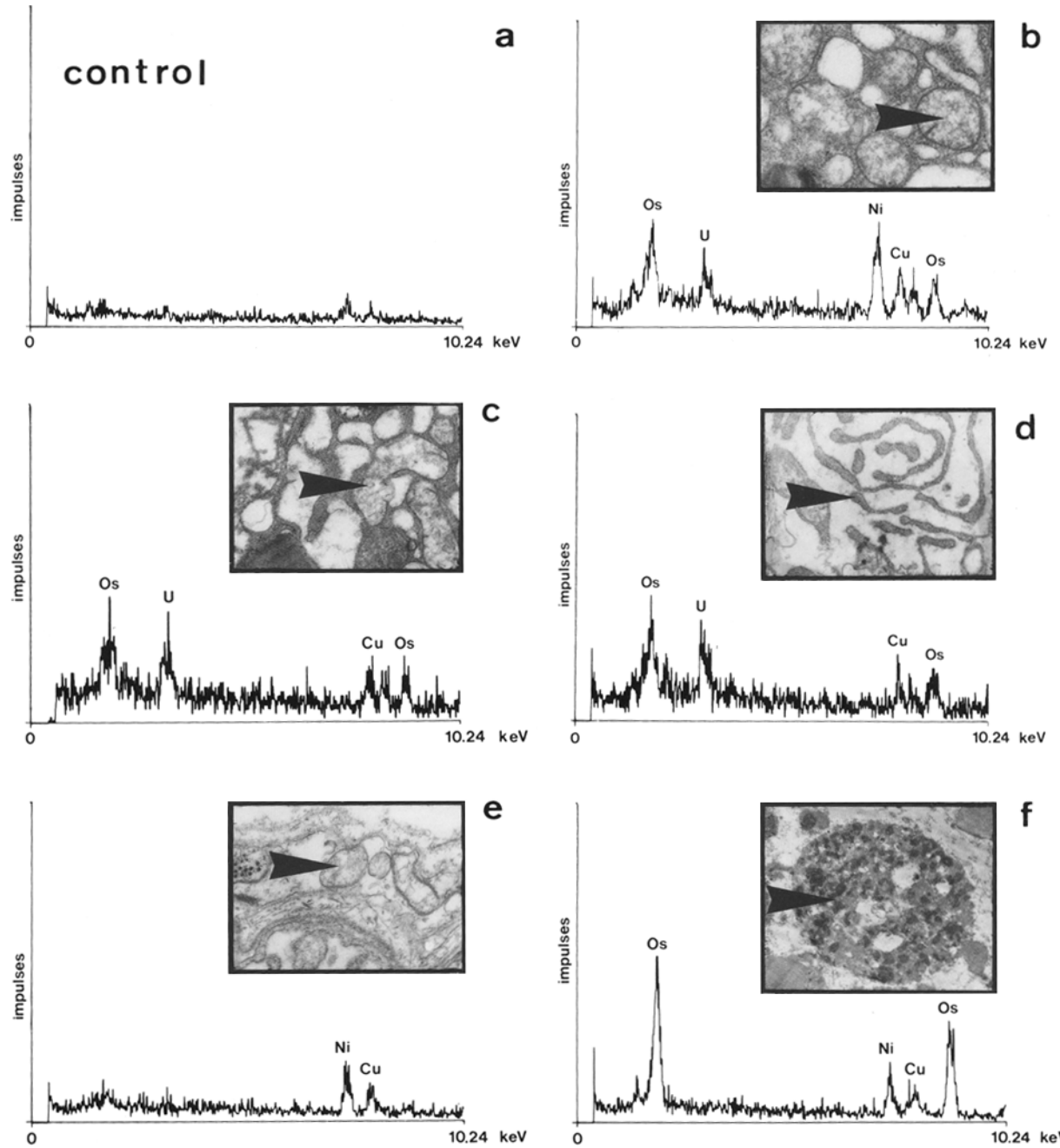


Fig. 13a–f. Element spectra of the epithel of the midgut gland of *Nautilus macromphalus*. **a** Control measurement in a chief cell section. Grid material: plastic; **b** point of measurement: cisterna of the RER of a basal cell III. Grid material: nickel; **c** point of measurement: cisterna of the RER of a basal cell III. Grid material: plastic; **d** point of measurement: ribosomes of a basal cell III. Grid material: plastic; **e** point of measurement: vesicle in the blood space. Grid material: nickel; **f** point of measurement: dense body of a basal cell III. Grid material: nickel

ments, which indicates the beginning of a general karyolysis (Fig. 10, 12). In the same way we can see a plasmolysis, particularly characterized by an enlargement of the RER cisternae. A large number of them seems to be fused to one single entire area subdivided into many irregular compartments containing high quantities of the above-mentioned ring-shaped molecules. The cytoplasm is reduced to small irregular bars with a high density of ribosomes and contains dispersed dense bodies and lipid droplets (Fig. 12). In the contact area of the cells with the blood space, an increased tendency of cell decay can be found and cellular fragments with RER cisternal seem to pass the loosened lamina basalis into the blood sinus.

3. Atomic absorption spectroscopy analyses

The total copper content of the midgut gland tissue was initially determined in three specimens of *Nautilus macromphalus* by means of atomic absorption spectroscope (AAS). The resultant values, which indicate a high but variable copper content, are summarized in Table 1.

4. Results obtained with analytical scanning transmission electron microscopy

AAS revealed a significantly high copper content in the midgut gland tissue. ASTEM based on the energy-dispersive X-ray microanalysis was suitable for localizing the copper within certain cells or intra- or extracellular structures. The spectrum of elements within the different compartments of the cells could thereby be measured. Tests have demonstrated an increased copper content in the basal cells or, more specifically, in the *synthesized* products of these cells.

The investigations revealed a significant localization of copper in the extended cisternae of the granular ER of the basal cells (Fig. 13b, 13c) and in the vesicles within the blood space near the terminal alveolus (Fig. 13e). Even the dense bodies that can also be found in the basal cells show traces of copper (Fig. 13f). In the area of ribosomes a copper peak could also be recorded (Fig. 13d).

Controls taken outside the basal cells, the chief cells and tissue-free araldite indicate no copper-peak in the diagram (Fig. 13a). Ni peaks, if present, can be attributed to the use of nickel grids.

D. Discussion

1. Localization and cytological aspects of the glandular cells

As the Tetrabranchiata, unlike the coleoida, do not have a hemocyanin-synthesizing branchial gland (Naef 1913), it is interesting to note that, within the terminal alveoli of the voluminous MGG tubules of *Nautilus macromphalus* and *Nautilus pompilius*, the existence of a special glandular cell type likely to have a hematopoetic function was consistently confirmed. These cells are found beyond the base of the chief cells (Schipp et al. 1985) and always in immediate contact with the adjacent blood sinus, and are therefore known as basal cells.

Apart from the close topical relation of the basal cells to the blood space, a series of cytological particularities that have a close correlation to the hemocyanin-synthesizing cells of the branchial gland of coleoids could be demonstrated (Dilly and Messenger 1972; Schipp et al. 1973; Muzii et al. 1974). Therefore, these basal cells also have an ovoid nucleus, numerous sharply distinct nucleoli and abundant RER, all of which is characteristic of a highly active RNA and protein synthesis.

In the nautiloids examined these basal cells appear in a configuration that is different and could perhaps be regarded as an expression of a physiological cycle or cytomorphological turnover of one and the same cell type. The RER cisternae seem to be increasingly extended, eventually fusing to a more or less unified cisternal space in the basal cells of stage IV. A similar cycle has also been described in the branchial gland cells of *Sepia officinalis* (Linné, 1758), in which the RER turns increasingly into a ribosomeless SER (Schipp et al. 1972). In the cells of both organs the karyoplasm tends to assume a more homogeneous consistency during this cycle, i.e. the nucleoli regress to a certain extent, disappearing in ever greater numbers as, on the other hand, the cells approaching the blood space show an increasing marginal decay. In stage IV this degeneration seems to affect the complete cell and lead to a nuclear pyknosis as well as a general lysis and an elimination of the cells through a cell-moulting process.

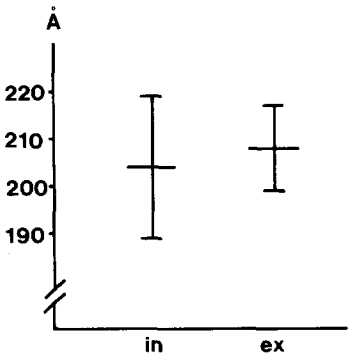


Fig. 14. Histogram of the diameter of ring-shaped intracisternal (*in*) molecules and extracellular (*ex*) hemocyanin molecules

The hypothesis that the basal cells synthesize hemocyanin is backed up by the comparison between the shape and size of the intracisternal ring-shaped macromolecules that appear as a dominating synthesis product in large numbers in the RER, especially in the cell stages II–IV, with the free hemocyanin molecules within the blood space. The two structures are practically identical in diameter (204 Å or 208 Å; Fig. 14). These values fall within the same dimensions as the corresponding parameter of the ring-shaped molecules within the glandular cells of the ligamentum branchiale (Saure et al. 1987, *Nautilus macromphalus*, 203 Å) and of the hemocyanin molecules of coleoids, which are likewise ring shaped (Dilly and Messenger 1972, *Octopus*, 250 Å; Ghiretti and Ghiretti-Magaldi 1972, *Loligo*, 240 Å; Schipp et al. 1973, *Sepia*, 170 Å; Muzii 1981, *Eledone*, 250 Å). This coincidence is also reflected in the corroborative sedimentation coefficients (50–58.7 S) (Bonaventura et al. 1981).

The form of the secretion in ring-shaped molecules also brings the basal cells of *Nautilus* and the branchial gland of coleoids, as well as the hemocyanin-producing "pore cells" of gastropods (Sminia 1977; Sminia and Vlugt van Daalen 1977), cytologically into line. In all the previously mentioned glandular cells, cisternal vacuoles of the RER obviously released the ring-shaped Hcy-molecules by means of exocytosis. In the basal cells of nautiloids the secretory products must pass through the broad lamina basalis, limiting the acinus to blood sinus.

In the area of these cells the lamina basalis appears loose and incomplete. The question that remains unanswered however, is whether or not the free ring-shaped molecules, larger cisternal vacuoles with such molecules or even larger parts of decayed cells in stage IV or whole cells in the sense of a holocrine secretory mechanism could surmount this barrier.

The intracisternal myelin bodies frequently observed in basal cells II–III probably provide lipids to produce new cisternal membranes during these secretion processes.

2. The copper content of the midgut gland

The element copper undoubtedly plays a crucial role in the synthesis of the respiratory pigment hemocyanin. Consequently, in animals (Xiphosura, Crustacea, Gastropods, Cephalopods) with blood containing hemocyanin it is mainly the midgut gland that is reponsible for storage, irrespective of the actual localization of hemocyanin synthesis.

Table 2. Comparison of the copper concentrations of some organs of coleoids and initial measurements of a nautiloid midgut gland

Species	Organ	Method	Cu concentrations		Authors
			µg/g wet weight	µg/g dry weight	
Octopus vulgaris (adult animals)	MGG	Microchemical analysis		2550	Ghiretti-Magaldi et al. (1958)
	Branchial heart			93	
	Blood			2450	
	MGG	MGG		1276–1948 (ppm)	Ghiretti and Violante (1964)
	MGG	AAS	–	2500± 700	Miramand and Guary (1980)
	Branchial heart	AAS	–	500± 40	
	MGG			1267–1948	Ghiretti and Violante (1964)
	MGG	Microchemical analysis	–	4880±2549 (ppm)	Noviello and Rocca (1969)
	Blood	Photometrical analysis	183±5		Rögener (1987)
Eledone moschata	MGG	Microchemical analysis	–	1900	Henze (1901)
Architeuthis dux	MGG	Microchemical analysis	–	845	Schmidt-Nielsen and Flood (1926)
Loligo opalescens	MGG	AAS	–	5350±3210	Martin and Flegal (1975)
Ommastrephes bartrami	MGG	AAS	–	195± 212	
Symplectoteuthis oualaniensis	MGG	AAS	–	1720± 151	
Sepia officinalis (adult animals)	MGG	Microchemical analysis	–	1200	Wang-Tai-Si (1928)
(juvenile animals)	MGG	AAS	100±49	298±131	Schipp and Hevert (1981)
	Branchial gland		22± 7	122± 43	
	Branchial heart		20± 5	111± 28	
	Blood		158±27	1182±277	
Nautilus macromphalus (adult animals)	MGG	AAS	473±79 851 (max) 249 (min)		(Present investigation)

Table 2 compares the copper concentrations of the midgut gland and some other organs of coleoids with our first measurements of the nautiloid MGG. The Nautilius concentrations seem to be high compared with the values of juvenile *Sepia officinalis*, even bearing in mind that they are referred to wet weight (Schipp and Hevert 1981); it must be assumed that the values of adult *Sepia officinalis* are also considerably higher. Corresponding to our findings in *Nautilus*, it is also evident that coleoids have strong individual variability of concentrations (see, for instance, the standard deviations of the MGG value of *Loligo;* Martin and Flegal 1975). With these initial results in view it can be concluded that in both nautiloids and coleoids the MGG constitutes the most important storage organ for copper and that no principal differences exist in the concentration of species of two systematical groups.

Nevertheless, further research based on a larger number of individuals and taking account of age, size and specific samples from different organ areas is necessary.

3. Cytological localization of copper within the basal cells

The results of the ASTEM analyses also support the hypothesis that hemocyanin synthesis takes place in the basal cells. The measurements taken in different compartments of the chief cells revealed no significant copper content, whereas the appropriate diagrams for the cisterns of the RER and the dense bodies (as in the case of the free hemocyanin molecules and released vesicles within the blood sinus) showed clear Cu peaks. Investigations of this nature in coleoids have yet to be carried out; however, the histochemical and AAS results so far obtained in Octopus vulgaris (Lamarck) and *Sepia officinalis* seem to indicate that the MGG cells storing copper and other metals, as well as the branchial gland cells responsible for hemocyanin synthesis, have a certain Cu content. This content lies below the values acquired for tissues of the MGG and the blood, leading to the conclusion that in these species the site of hemocyanin synthesis is obviously not identical with the most important sites of Cu storage (Schipp et al. 1973; Schipp and Hevert 1978).

Not only the copper peaks but also the uranium peaks, which occur with remarkable regularity are worth mentioning. As these samples were not stained with uranyl, it can be assumed that such radionucleotides can be enriched in the MGG of *Nautilus*, as is the case in the MGG and branchial heart complex of coleoids (Guary et al. 1981; Nakahara et al. 1979).

Acknowledgements. The authors wish to express their appreciation for the technical assistance kindly given by Dr. Doell, Strahlenzentrum, Dipl. Ing. Brückel, Institut für Arbeits- und Sozialmedizin, University of Giessen, as well as by S. Beckermann, A. Bleichner, A. Hudel, R. Lawson and H. Schmidt. The research was supported by the Deutsche Forschungsgemeinschaft (West German Science Foundation). The work reported in this paper was performed in partial fulfullment of the requirements for a higher academic degree.

References

Bonaventura C (1981) Hemocyanin of the chambered *Nautilus:* structure – function relationship. 211:589–598

Dilly PN, Messenger JB (1972) The branchial gland: a site of haemocyanin synthesis in *Octopus*. Z Zellforsch 132:193–201

Ghiretti F, Violante U (1964) Ricerche sul metabolismo del Rame in *Octopus vulgaris*. Boll Zool 31:1081–1092

Ghiretti F, Ghiretti-Magaldi A (1972) Respiratory proteins in molluscs. In: Florkin M, Scheer BT (eds) Chemical Zoology. 7:201–217

Ghiretti-Magaldi A, Guiditta A, Ghiretti F (1958) Pathways of terminal respiration in marine invertebrates: I. The respiratory system in cephalopods. J Cell Comp Physiol 52:389–429

Guary JC, Higgo JJW, Cherry RD, Heyraud M (1981) High concentrations of transuranics and natural radioactive elements in the branchial hearts of the cepholopod *Octopus vulgaris*. Marine Ecol 4:123–126

Henze M (1901) Über den Kupfergehalt der Cephalopoden-Leber. Hoppe – Seyler's Z Physiol Chem 33:417–425

Humphrey CD, Pittman FE (1974) A simple methylene blue-azure II-Basic fuchsin stain for epoxy-embedded tissue sections. Stain Technol 49:9–14

Joubin L (1980) Recherches sur l'appareil respiratoire des nautiles. Rev Biol Nord France 11:409–429

Mangold-Wirz K, Fioroni P (1970) Die Sonderstellung der Cephalopoden. Zool Jb System 97:522–631

Martin JH, Flegal AR (1975) High copper concentrations in squid livers in association with elevated levels of silver, cadmium and zinc. Marine Biol 30:51–55

Messenger JB, Muzzii EO, Nardi G, Steinberg H (1974) Haemocyanin synthesis and the branchial gland of *Octopus*. Nature 250:154–155

Miramand P, Guary JC (1980) High concentrations of some heavy metals in tissues of the Mediterranean *Octopus*. Bull Environ Contam Toxicol 24:783–788

Muzii E, Nardi G, Steinberg H (1974) Blocco della sintesi in vivo della emicianina dopo distruziare della ghiandola branchiale in *Octopus vulgaris* Lam (Cephalopoda). Boll Zool 41:508–509

Muzii E (1981) Intracellular polymerized haemocyanin in the branchial gland of a cephalopod. Cell Tissue Res 220:435–438

Naef A (1913) Studien zur generellen Morphologie der Mollusken: 2. Das Coelomsystem in seiner topographischen Beziehung. Fortschr Zool 3:329–462

Nakahara M, Koyonagi T, Ueda T, Shimizu C (1979) Peculiar accumulation of cobald-60 by the branchial heart of *Octopus*. Bull Jpn Soc Sci Fish 45:539

Noviello L, Rocca E (1969) Ricerche sul rame dell' epatopancreas di *Octopus vulgaris* Lamarck. Pubbl Staz Zool Napoli 37:237–260

Rögener W, Renwrantz L, Uhlenbruck G (1987) Analysis of *Octopus vulgaris* hemolymph containing a glycoprotein with blood group A-like properties. Comp Biochem Physiol [B] 86:347–351

Saure H, Schipp R, Magnier Y (1987) Morphological and functional aspects of the branchial complex in *Nautilus* (Tetrabranchiata, Cephalopoda). Zool Jb Anat 116:39–62

Schipp R, Höhn P, Ginkel G (1973) Elektronenmikroskopische und histochemische Untersuchungen zur Funktion der Branchialdrüse (Parabranchialdrüse) der Cephalopoden. Z Zellforsch 139:523–269

Schipp R, Hevert F (1978) Distribution of copper and iron in some central organs of *Sepia officinalis*. A comparative study by flameless atomic absorption and electron microscopy. Marine Biol 47:391–399

Schipp R, Hevert F (1981) Ultrafiltration in the branchial heart appendage of dibranchiate Cephalopods: a comparative ultrastructural and physiological study. J Exp Biol 92:23–25

Schipp R, Ruth P, Magnier Y (1985) Zur Cytomorphologie und Histochemie der Mitteldarmdrüse von *Nautilus* (Tetrabranchiata, Cephalopoda). Verh Dtsch Zool Ges 78:316

Schmidt-Nielsen, Flood (1926–28) After: Noviello L, Rocca E (1969) Ricerche sul rame dell' epatopancreas die *Octopus vulgaris* Lamarck. Pubbl Staz Zool Napoli 37:237–260

Sminia T (1977) Haemocyanin-producing cells in gastropod molluscs. In: Bannister JV (ed) Structure and function of haemocyanin. Springer Verlag, Berlin Heidelberg New York, pp 279–288

Sminia T, Vlugt van Daalen JE (1977) Haemocyanin synthesis in pore cells of the terrestrial snail *Helix aspersa*. Cell Tissue Res 183:299–301

Taki I (1964) On the morphology and physiology of the branchial gland in cephalopoda. J Fac Fish Anim Husb Hiroshima Univ 5:345–417

Wang-Tai-Si (1928) Recherches sur le cuivre, le fer, le manganèse et le zinc chez les mollusques. Thesis, Paris

Received July 3, 1987