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ELEMENTAL MAPPING OF CRYOSECTIONS FROM CNIDARIAN NEMATOCYTES

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Abstract

The distribution of elements in stinging capsules containing cells called nematocytes is shown by pseudocoloured maps representing the X-ray intensity collected from freeze-dried cryosections. This method provides a distinct overview in addition to the quantitative evaluation of single X-ray spectra. Selected examples illustrate the elemental compartmentation in various cnidarian animals. In particular the matrix of capsules in Hydra vulgaris contains high amounts of K in comparison to the tubule, the surrounding capsule wall and the cytoplasm, whereas in Actinia equina capsules have either high concentration of Ca or Mg, the latter accompanied by S.

KEY WORDS: actina equina, cnidaria, cryosection, elemental mapping, hydra, ion compartmentation, nematocyst, nematocyte, stinging capsule, X-ray microanalysis.

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Introduction

A common characteristics of all cnidarian animals is the existence of nematocytes, which are cells containing stinging capsules called nematocysts. On appropriate chemical or electrical stimulation the nematocysts are discharged from the animal by ejecting a stylet with an attached tubule (Tardent and Holstein, 1982; Holstein and Tardent, 1984). Nematocytes are located mainly in tentacles and in acrorhagi of the animals as depicted in Fig. 1. Whereas nematocysts in the tentacles are used for the capture of prey, those in acrorhagi are used to keep distance to neighbours. Fig. 2 shows a drawing of a typical undischarged nematocyst. In principle, the nematocyst which is a secretory product of the nematocyte, consists of a spherical, oblong or cylindrical capsule containing a tubule of varying length. According to the type of nematocysts, the tubule is equipped with associated structures such as stylet, barbs and spines which in the course of tubule evagination (Tardent and Holstein, 1982) help to overcome the integuments of either prey or predator organisms. The intracapsular liquid filled space is called matrix and contains more than 30 different soluble components amongst which are toxins, cations and anions, in particular poly-γ-glutamates (Weber, 1990, 1991).

The mechanism of this exocytotic process is not yet elucidated. One of the current hypotheses supposes that high osmotic pressure in the matrix of the nematocyst to be the source for the discharge force (Lubbock and Amos, 1981; Weber, 1989). This would imply high ion concentration differences between the nematocyst matrix and the surrounding cytoplasm. Consequently, this problem was studied by X-ray microanalysis several years ago (Lubbock et al., 1981). These authors found very high concentrations of Ca in the acrorhagial nematocysts of the sea anemone Rhodactis rhodostoma and Anthopleura elegantissima. Most of this Ca was found to be released from the capsule matrix by inducing nematocyst discharge. X-ray microanalytical measurements of cryosections from Hydra vulgaris revealed high concentrations of K in the range of 2 mol/kg dry mass in the capsule matrix before as well as after the nematocyst discharge (Zierold et al., 1989). Recently,

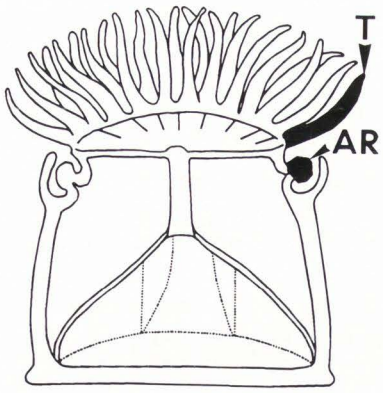

Fig. 1: Schematic drawing of the cnidarian animal Actinia equina showing tentacles (T) and acrorhagi (AR) where nematocytes are located.
From: Tardent et al. (1990) with permission.

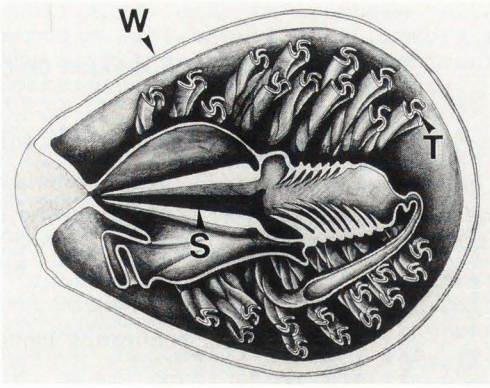

Fig. 2: Schematic drawing of a nematocyst of the stenotele type. S = stylet, T = tube, W = capsule wall.
From: Tardent and Holstein (1982), with permission.

Recently, Tardent et al. (1990) reported data on the wide variety of cation accumulation in nematocysts among the cnidarian species. Here, we intend to demonstrate the capacity of elemental mapping in elucidating element compartmentation in cnidarian nematocytes, by example from Hydra vulgaris and Actinia equina.

Material and Methods

Cryofixation was done as described previously (Tardent et al., 1990). Tentacles of Hydra vulgaris and acrorhagial parts of Actinia equina were dissected from the animals placed on gold freeze-etch planchettes and frozen by plunging into liquid propane cooled by liquid nitrogen. Hydra tentacles were electrically stimulated to induce nematocyst discharge as described by Zierold et al. (1989). From the frozen tissue approximately 100 nm thick cryosections were prepared, freeze-dried and cryotransferred into a scanning transmission electron microscope (Siemens ST 100F) equipped with a cryo stage and an energy dispersive X-ray microanalysis system (USC nuclear semiconductor and Link Systems multichannel analyzer). X-ray spectra were recorded during irradiation with an electron beam of 100 kV and 1.4 nA. Spectra were collected within 100 s live time either in a spot mode or in a scanning mode over the area of interest. Quantitative evaluation of X-ray spectra was done according to the Hall continuum method.

Elemental mapping was done by recording X-ray counts corresponding to energy windows of 120 eV width around characteristic X-ray peaks. The spatial resolution was 128 x 128 pixels with a dwell time of 10 ms resulting in a recording time of about 3 min per frame. Usually, between 5 and 10 frames were superimposed. In order to avoid specimen drift, contours marked on the electron microscope screen were used to check shifts of the selected area after each frame and to correct if necessary. X-ray intensities were represented as pseudocoloured maps. Link Systems computer programs were used for quantitative evaluation of X-ray spectra and for image processing to improve the presentation of the measured element distribution.

Results

The element concentrations measured in the nematocyst matrix and in the sourrounding cytoplasm of Hydra vulgaris and Actinia equina are compiled in Table 1. Obviously, nematocysts of Hydra accumulate K, whereas nematocysts in Actinia equina contain high amounts of Mg and S in tentacles and Ca in acrorhagi.

Fig. 3 shows a set of elemental maps from a cryosection obtained from Hydra after stimulation for nematocyst discharge. In the corresponding scanning transmission electron microscope (STEM) image the nematocyst matrix with a part of the tubule, the nematocyst wall and the cytoplasm can be clearly distinguished. The pseudocolour scale indicates X-ray intensity from low (black, dark blue) to high (red, white) for the elements Mg, P, S, K.

Fig. 4 shows elemental maps from a cryosection from Actinia equina. Due to the closely packed tubule the undischarged nematocysts appear too dense for identification of any intracapsular structures. X-ray intensity maps were processed by intensity slicing and then superimposed in binary mode as coloured maps of Mg, S, K, and Ca to the corresponding STEM image. By intensity slicing only high elemental levels are represented by a colour, whereas low elemental levels close to the continuum intensity are cut away. By this procedure two kinds of cysts were identified: In one kind (typical for tentacles) Mg and S are accumulated together, whereas in the other kind

Table 1

Elemental composition of the nematocyst matrix and surrounding cytoplasm as found by quantitative evaluation of X-ray spectra in mmol/kg dry mass ± standard deviation according to the data from Zierold et al. (1989) and Tardent et al. (1990). n = number of measurements.

	Hydra vulgaris		Actinia equina			
			tentacles		acrorhagi	
	nem.	cytoplasm	nem.	cytoplasm	nem.	cytoplasm
n	19	11	16	4	9	2
Na	60± 10	9± 15	5± 12	33±11	57±45	0; 2
Mg	22± 16	43± 16	1495±592	43±11	100± 93	82; 27
P	11± 15	302±104	197± 11	251±33	191± 42	404; 242
S	123± 67	173± 39	2071±344	386±27	388±126	601; 421
Cl	13± 8	9± 3	62± 40	257±49	49± 42	353; 222
K	2203±905	92± 59	43± 24	235±85	18± 9	355; 119
Ca	6±9	23± 17	24± 12	10± 4	951±261	29; 17

(typical for acrorhagi) Ca is concentrated. K is located in the surrounding cytoplasm.

Discussion

The elemental mapping procedure used in this paper is based merely on the X-ray intensity recorded in defined energy windows surrounding the characteristic X-ray peaks. Therefore, the maps shown in Figs. 3 and 4 represent the elemental distribution in a qualitative or semiquantitative manner in comparison to the data compiled in Table 1.

Recently, techniques were developed for the acquisition of fully quantitative elemental maps. Those maps require background correction and quantitative evaluation for each pixel and therefore remarkably long dwell times to get sufficient X-ray counts per pixel. Saubermann and Heyman (1987) acquired elemental maps of 64 x 64 pixels with a dwell time of 4 s from 500 nm thick cryosections. By comparison of the digital information obtained from each pixel in the freeze-dried state with the continuum radiation measured in selected areas in the frozen-hydrated state, they succeeded in providing quantitative maps in terms of the elemental concentrations per kg dry weight and wet weight. Wong et al. (1989) described quantitative elemental imaging of cryosections from rat parotid gland tissues. Ingram et al. (1989) generated quantitative elemental maps from freeze-dried cryosections of frog skeletal muscle with a pixel resolution of 65 to 130 nm.

The main problems in elemental mapping of cryosections with high spatial resolution are low X-ray intensity per pixel and insufficient stability of the specimen in the electron beam as previously noted by Somlyo (1984). For elemental mapping of nematocyte cryosections we used an electron beam of 1.4 nA at a magnification of 5.000 x corresponding to a pixel resolution of 120 nm per pixel. With the dwell time of 10 ms per pixel the irradiation dose in each frame was 6×10^3 e/nm^2. In completely freeze-dried cryosections this irradiation dose - or even the tenfold dose of 6×10^4 e/nm^2 due to superimposing 10 frames - caused only moderate or no mass loss and deformation of the section. Fully quantitative mapping requires a dwell time of at least 1s/pixel, in ultrathin sections rather 5 s/pixel. This considerably higher irradiation dose often caused not only specimen drift but also non-uniform deformation of section components such as shrinkage, twisting and rotation. These non-uniform deformations are not easy to correct as necessary for fully quantitative mapping. Therefore, we were not yet able to provide fully quantitative maps from ultrathin freeze-dried cryosections.

In our experience elemental mapping does not provide any quantitative information on element distribution which is not achievable by single X-ray spectra. The specific advantage of elemental mapping is the easily conceivable visual representation of the element distribution in cells and tissues such as elemental compartmentation or gradients. For example the few Mg containing cysts among the majority of K accumulating cysts in Hydra could be easily detected by mapping. The existence of two kinds of nematocysts in Actinia equina, one accumulating Mg together with S, the other accumulating Ca, is proved more clearly by maps than by single X-ray spectra. Thus, we recommend characteristic X-ray intensity maps as a useful method for element localization in addition to X-ray microanalysis of single spectra for quantitative studies.

From the biological point of view the elemental maps (Figs. 3 and 4) agree with previous X-ray microanalytical data obtained from cnidarian nematocytes. The high K accumulation in the matrix of Hydra nematocysts before and after discharge agrees with the data reported by Zierold et al. (1989). Obviously, K concentration is lower in the tubule and the cysts wall in comparison to the matrix, as can be seen by the K map in Fig. 3. Recently, Weber (1990) showed that the major constituents of isolated Hydra nematocyst are poly(γ-glutamic acids) in a concentration as high as 2 M. These polyanions probably attract cations from the environment of the cyst followed by water thus forming a high internal

osmotic pressure of 12.5 MPa which is supposed to be responsible for the force driving nematocysts discharge (Weber, 1989). Gerke (1989) showed that in isolated nematocysts the cation content could be exchanged by Mg, Mn, Co and Na as depending on the composition of the surrounding solution. In vivo most nematocysts accumulate K with the exception of a few Mg-containing cysts shown in Fig. 3.

The enhanced content of S found in various structures of cnidarian nematocysts such as tubule, stylet und capsule wall can be attributed to collagen-like proteins which are linked by S double bonds. The data obtained by elemental mapping of Actinia equina nematocytes (Fig. 4) reveal Ca containing cysts similar to the microanalytical observations of acrorhagial tissue from Anthopleura elegantissima by Lubbock et al. (1981). Remarkable differences in the cation content of nematocysts were found between acrorhagi and tentacles of Anthopleura elegantissima, Actinia equina and Anemonia viridis, whereas in Calliactis parasitica nematocysts in tentacles and acrorhagi are similar in their elemental content (Tardent et al., 1990). As both kinds of nematocysts were found in the elemental study shown in Fig. 4, the analyzed section presumably represents an intermediate region between tentacles and acrorhagi.

The wide variety in cation accumulation in cnidarian nematocysts is surprising and makes a unique explanation of the underlying biological reason difficult. We intend to contribute to the solution of these extraordinary biological phenomena of specific cation accumulation and nematocysts discharge by future microanalytical experiments on cnidarian nematocytes after time controlled cryofixation during exocytosis.

Acknowledgement

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Discussion with Reviewers

J.A. Morgan: You state that the K concentration in the nematocysts of Hydra is high both before and after discharge. How, therefore, do you explain the process of discharge in terms of osmotic pressure? Do you envisage that the K is re-

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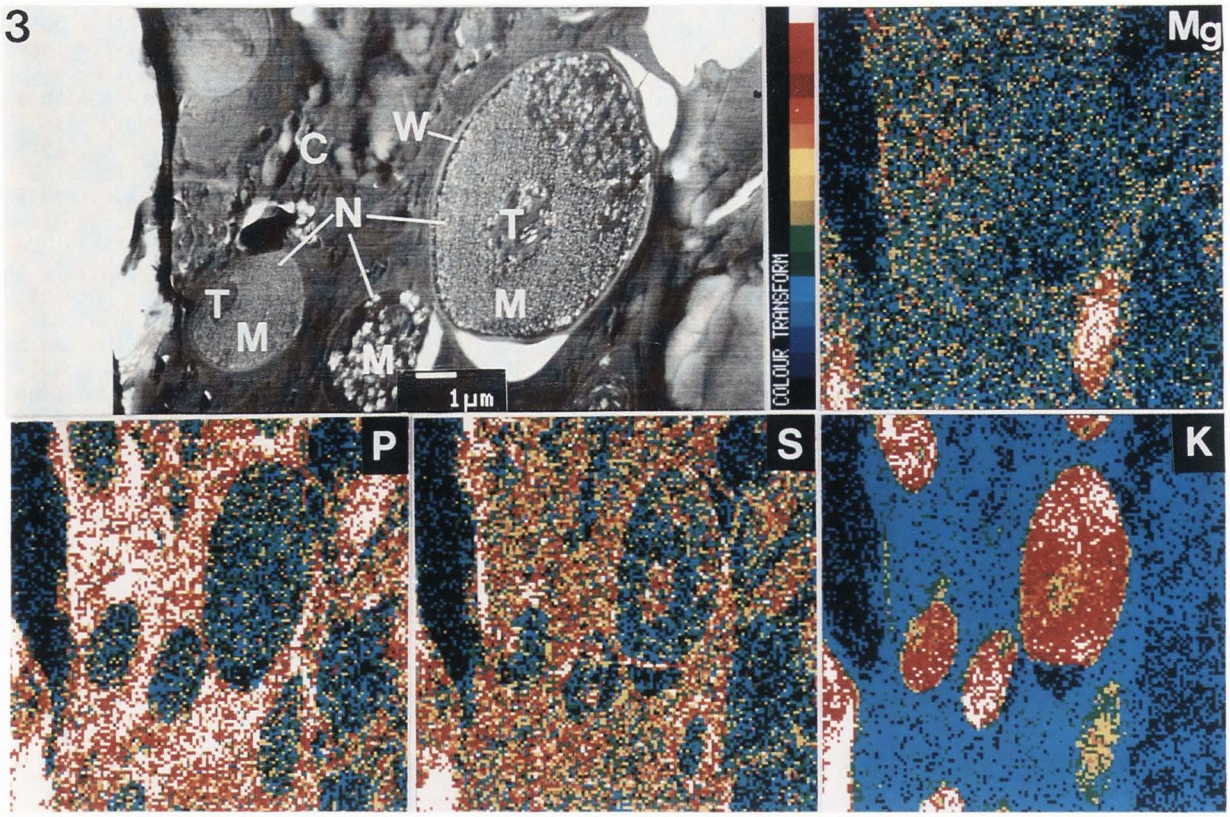
Fig. 3: Elemental mapping study of Hydra vulgaris, with discharged nematocysts.
The scanning transmission electron micrograph shows intracellular structures: C = cytoplasm surrounding the nematocysts (N), confined by the nematocyst wall (W). Within the matrix (M) of the nematocysts parts of the tubule (T) remaining after discharge can be identified. The colour scale indicates X-ray intensity from low (black, blue) to high (red, white). Corresponding maps of Mg, P, S and K are shown.
Fig. 4: Elemental mapping study of Actinia equina, with undischarged nematocysts.
The scanning transmission electron micrograph shows nematocysts (N). Corresponding maps of high X-ray intensity slices of Mg (green), S (blue), K (yellow) and Ca (red) are shown. The nematocysts content appears mass dense because of the closely packed tubule present in the undischarged state.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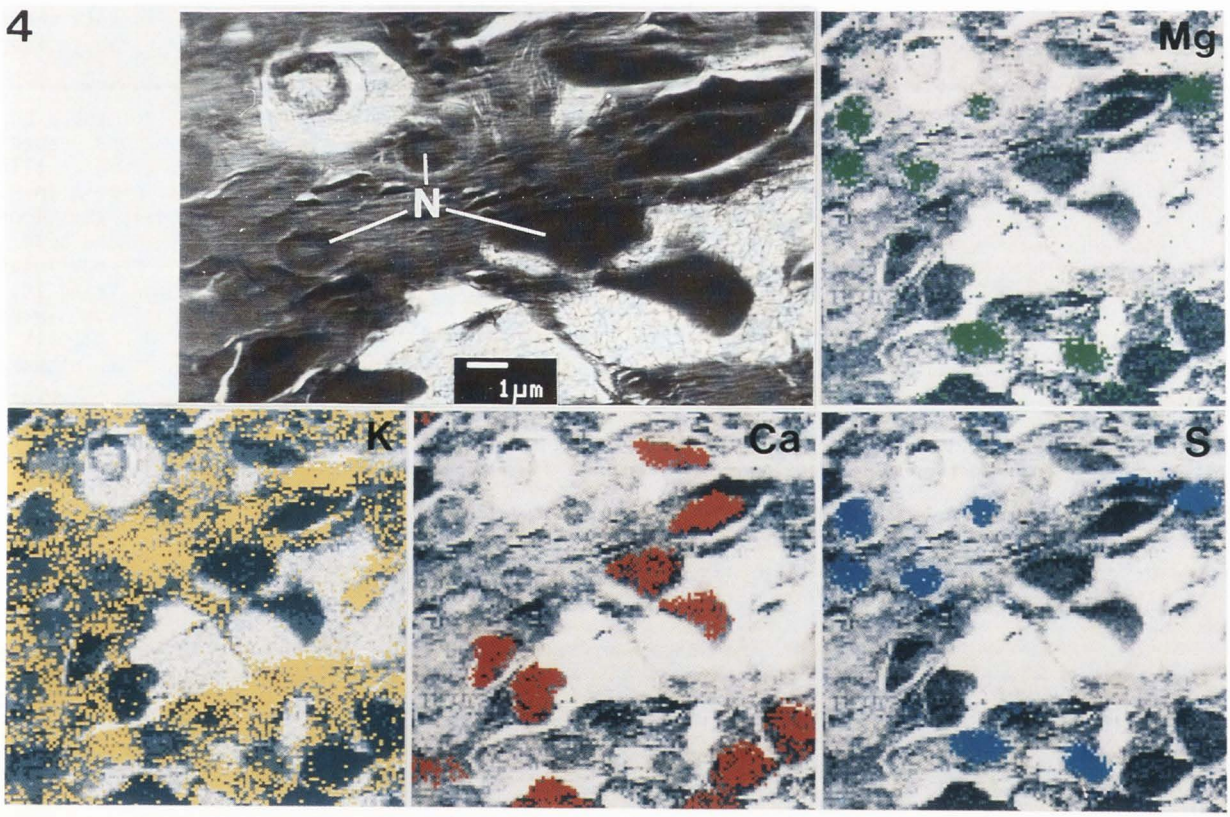
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leased from a non-osmotically active bound state to a free osmotically active state during stimulation?
Authors: The mechanism of nematocysts discharge is not yet known. The recent studies by Gerke (1989), Weber (1990, 1991) and Tardent et al. (1990) support the hypothesis that stimulation for discharge causes dissociation of polyanions and cations both present in high concentration within the cyst. The resulting high osmotic pressure causes water uptake of the cyst thus providing the force for discharge. However, this hypothesis needs to be proven by further experiments.

B.L. Gupta: There seems to be a difference in the scale of magnification between the STEM images and the corresponding X-ray maps. Is there any specific reason for this difference?
Authors: The magnification used for STEM imaging and X-ray mapping was the same. However, the rectangular STEM image (26 cm x 20 cm on the screen) corresponds to a quadratic map of horizontally and vertically equidistant 128 x 128 pixels. Thus, the vertical magnification in the maps is the same as in the STEM image, whereas the horizontal magnification of the maps is 77 % of the STEM image.

G.M. Roomans: The qualitative method of mapping used in this study requires high peak-to-background ratios and considerable concentration gradients. In the experiment of Fig. 3, what were typical peak-to-background ratios for K in the capsule and in the cytoplasm?
Authors: As can be seen from Table 1, peak-to-background ratios for K are an order of magnitude higher in the capsules than in the surrounding cytoplasm. Whereas analog mapping requires a peak-to-background ratio of about 3 for sufficient contrast in imaging, digital mapping combined with an appropriate colour scale allows to image almost every elemental gradient detectabel by X-ray microanalysis.

B.L. Gupta: What is the best lateral spatial resolution one can obtain in such maps without causing damage to the specimen? Would it for example be possible to map the distribution of ions in various parts of relatively large cells? I suppose a relatively low concentration of ions in the cells may preclude such mapping but it could conceivably be useful in addressing the highly topical question of looking for Ca stores where the focal dry-wet concentration in the stores may be quite high. Have you attempted this?
J.A. Morgan: Can you give a brief outline of the analytical conditions that provide for a maximum acceptable irradiation dose in ultrathin, freeze-dried, cryosections?
Authors: The spatial resolution achievable in X-ray mapping depends on the stability of the specimen in the electron beam. The stability of freeze-dried cryosections can vary remarkably depending on the actual contact of the section with the support film, on the amount of (bound) water remaining in the section after drying and on the vacuum conditions in the electron microscope (partial pressure of water and other possibly contaminating substances). Recently, we have acquired X-ray maps from 100 nm thick freeze-dried cryosections from epithelial cells with a pixel distance of 12 nm on the section. This distance is shorter than the X-ray microanalytical resolution of about 30 nm due to the thickness of the specimen. The corresponding irradiation dose was 6×10^6 e/nm^2. Mass loss and distortions were visible, but still acceptable. Unfortunately, this result was not reproducible in every section. Some sections showed tremendous distortions after a tenth of this irradiation dose. Based on these experiments, mapping can be considered as useful to detect elemental gradients or accumulations with a spatial resolution of 30-50 nm, implying an irradiation dose of 10^5-10^6 e/nm^2.

J.A. Morgan: What is the general nature of the corrections that are necessary for non-uniform deformations before fully quantitative elemental mapping can be successfully realized?
Authors: One could imagine a currently repeated correlation of the acquired pixel information to the measured area during section deformation, but we do not know how to realize this idea.

B.L. Gupta: What do you think is the immediate scope of improving the sensitivity and resolution of such mapping? The use of 500 nm thick sections might prove helpful?
Authors: Probably, thicker section would exhibit less mass loss related to their thickness and less spatial resolution. Therefore, thicker sections would be advantageous in experiments where a spatial resolution in the range of the sections thickness is considered to be sufficient.

B.L. Gupta: Can this semiquantitative method be also applied to frozen-hydrated sections, especially at the sort of magnifications you have illustrated? Such an application would be most invaluable in addressing some very basic questions for example on epithelial transport where both the intracellular distributions and pericellular distributions are important. Have you tried it, especially on carbon-coated sections? Even in Hydra the mechanism of maintaining high K levels in the body tissue is poorly understood and desperately needs a microprobe study.
Authors: We agree with your arguments, but due to the high mass loss usually observed during X-ray microanalysis of frozen-hydrated sections, we have not yet tried. Perhaps, protection of the frozen-hydrated section by an appropriate coat could help to reduce radiation damage.