X-Ray Microanalysis of Mast Cells in Rat's Muscle

Mast cells are characterized by numerous large metachromatic granules which appear electron microscopically as dense, membrane enclosed, cytoplasmic bodies. Their function consists of elaboration and/or storage of several substances which can be released rapidly and which elicit powerful pharmacological effects.

Although heparin, histamine and several other compounds have been isolated from the granules, little is known about their in vivo composition and metabolism[1-4].

Electron microscopic X-ray microanalysis[5] is particularly well suited for the study of mast cells in their natural habitat of connective non-homogeneous tissue. This technique provides information on intracellular chemistry with a high degree of ultrastructural and elemental resolution[5].

Tissues from tongues of healthy albino rats weighing 120 g were used. (In the course of another study of tongue musculature, this material was found to contain many mast cells). Fixation was by a 10-min perfusion and 1 h immersion in 2.5% glutaraldehyde buffered with 0.1 *M* cacodylate. No post fixation was done, and after rapid alcohol dehydration and propylene oxide, the tissues were embedded in Epon 812. The metachromasia of the mast cell granules facilitated selection of suitable areas for analysis from 1 μm thick toluidine blue stained samples. 120 nm thick sections (gold) were then cut and collected on formvar or collodion and carbon-coated nickel grids. They were examined unstained, with the EMMA-4 (AEI, Manchester, UK). Crystal diffraction (LiF for zinc and PET for calcium) and energy dispersive detectors (Si/Li Kevex) were used. The accelerating voltage was 40 kV. The beam current was around 200 nA. The probe diameter was 200–300 nm.

In the energy dispersive analysis, which was the main detecting method employed, the elemental spectra of various spots were photographed after 50 sec of probing. Integral counts of peak and peak minus background were recorded from many areas of interest and the relative mass fractions of phosphorus, sulphur, calcium and zinc could be calculated by using the 'white' radiation count (from an X-ray emission zone devoid of special peaks) as a relative measure of specimen mass (after making an adjustment for supporting material).

The use of glutaraldehyde as the sole fixative resulted in specimens of poor contrast but the 120 nm section thickness and the low accelerating voltage made visual orientation and probe localization possible. The various types of cells, their boundries, nuclei cytoplasmic granules and other organelles were easily recognizable.

The mast cells varied in size and in number of their granules which were very electron dense. The granules differed slightly from each other in size and opacity, but even with higher magnifications none showed any discernible structural details in the unstained sections.

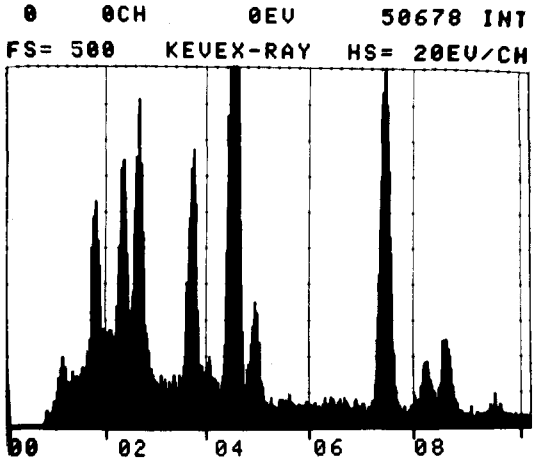

Fig. 1. Elemental spectrum from a mast cell granule. (50 sec analysis). There are prominent peaks of sulphur (2.30 keV), chlorine (2.62 keV), calcium (3.69 keV) and zinc (8.63 keV). The other peaks are due to instrumentation and supporting materials. The abscissa shows the energy of the X-ray lines.

[1] U. Bergqvist, Acta physiol. scand. Suppl. *330*, 24 (1969).
[2] A. A. Horner, J. biol. Chem. *240*, 231 (1971).
[3] P. G. Kruger and G. D. Bloom, Experientia *29*, 329 (1973).
[4] J. Padawer, Expl. molec. Path. *20*, 269 (1974).
[5] T. A. Hall, in *Physical Techniques in Biological Research*, 2nd edn. (Academic Press, New York 1971), p. 203.

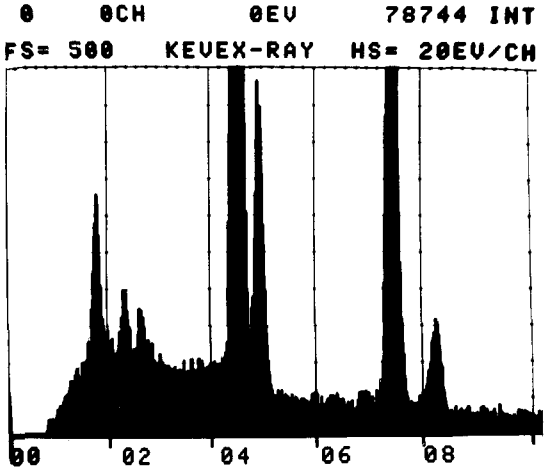

Fig. 2. Elemental spectrum from mast cell cytoplasm. Small sulphur and chlorine peaks are present, there are no visible calcium and zinc peaks.

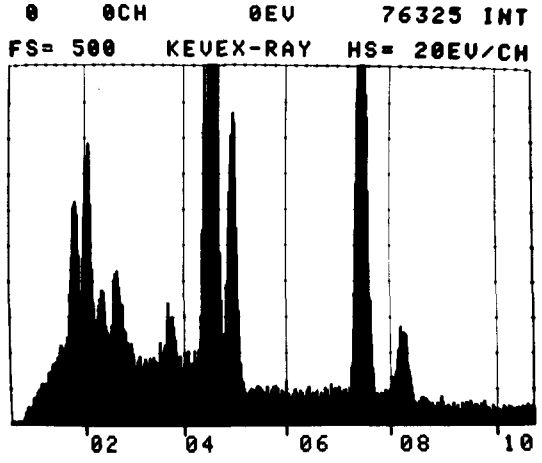

Fig. 3. Elemental spectrum from mast cell nucleus. Phosphorus (2.01 keV) is the main peak. Sulphur, chlorine, calcium and a little zinc are also detected.

Relative concentrations ($R \times 10^3 \pm$ SEM) of various elements in mast cells

	Zn	Ca	S	P
Mast cell granules	456 ± 26 (8)	1117 ± 69 (8)	1200 ± 81 (8)	0
Mast cell cytoplasm	57 ± 40 (3)	275 ± 167 (3)	384 ± 60 (3)	43 ± 32 (3)
Mast cell nucleus	632 ± 190 (4)	1811 ± 414 (4)	170 ± 50 (4)	1558 ± 200 (4)
Myofibre sarcoplasm	0	120	234	0
Erythrocyte	82	45	389	0
Epon only	0	0	320	0

In brackets are number of spots analyzed. The readings of nearby areas areas are given for comparison.

The elemental spectra from various intracellular spots are shown in Figures 1–3. The X-rays detected in interstitial areas devoid of structures were mainly from instrumentation and supporting materials. These extraneous radiations include the lines of silicon (1.74 keV), titanium (4.51 keV) and nickel (7.47 keV). When the probe was placed on a granule of a mast cell, the elemental spectrum became very distinctive (Figure 1). High peaks of sulphur (2.30 keV) chlorine (2.62 keV) calcium (3.69 keV) and zinc (8.63 keV) appeared. The spectra were similar in the numerous granules of several mast cells examined. The abundance and exact localization of elements in the granules was further confirmed by analyses of mast cell cytoplasm, nuclei (Figures 2 and 3) and nearby structures (Table). In the cytoplasm, there were small sulphur and chlorine peaks, while in the nuclei phosphorus (2.01 keV) predominated.

The Table shows the relative concentrations of various elements present in different areas of a mast cell and nearby structures. The high sulphur, calcium and zinc concentrations in the granules are vey striking ,the sulphur especially is noteworthy and constant. The calcium and zinc proved more variable, but usually occured in concentrations resembling the high nuclear content of these substances.

The examination of mast cells by combined transmission and X-ray emission electron microscopy proved more informative than the previously reported X-ray analysis in the surface scanning electron microscope[6]. Elements localized precisely to granules could be detected and their concentrations could be compared with those inside and outside mast cells. The good emission peaks after relatively short probing times (50 sec) were probably due to the high beam current used, but also to the minimal processing of the material.

Omitting buffer rinses and secondary osmification rendered visualization less satisfactory but obviously prevented much of the 'wash-out' of elements. Nevertheless, it remains uncertain whether the elemental concentrations recorded here represent in vivo values, since, as previously reported, even brief fixation with glutaraldehyde affects cell composition[7,8].

The sulphur emission from the granules is undoubtedly due to the sulphated polysaccharides of heparin[1–3]. The high level of sulphur has also been noted with scanning X-ray analysis and it has even been suggested as a 'marker' of mast cells in a mixed cell population. Zinc is known to occur in mast cell granules, and it has been previously detected there by X-ray analysis[9]. It appears to be involved in binding histamine to heparin[10].

The high calcium content of mast cell granules has been overlooked by previous researchers because they used calcium-rich glass as the specimen support[6]. Calcium has been detected by microanalysis in several kinds of granules and electron opaque areas of different cells[9,11]; it is often associated with phosphorus. No phosphorus appeared here which may indicate that the mode of calcium activity is different in mast cell granules than elsewhere. Its involvement in histamine release is known but incompletely understood[3,4].

Further EMMA examinations would be especially useful in studies dealing with dynamic physiological and pathological processes of granule release in mast cells.

Summary. Mast cells from rat's tongue muscles, fixed with glutaraldehyde only, were examined with an electron microscopic microanalyzer – the EMMA-4. With the preparatory method used, high emissions of a number of elements were obtained in various intracellular spots. The granules of mast cells were found to contain strictly localized high concentrations of sulphur, calcium and zinc.

R. YAROM, C. A. MAUNDER, T. A. HALL and V. DUBOWITZ[12]

Department of Paediatrics and Neonatal Medicine and the Jerry Lewis Muscle Research Centre, Hammersmith Hospital London (England), the Electron Microscopy Section, Cavendish Laboratory, Free School Lane, Cambridge CB2 3RQ (U.K.) and the Pathology Department, Hebrew University, Hadassah Medical School, Jerusalem (Israel),
9 June 1975.

[6] J. PADAWER, J. cell. Biol. *61*, 641 (1974).
[7] A. PENTTILA, H. KALIMO and B. F. TRUMP, J. cell. Biol. *63*, 197 (1974).
[8] K. E. RASMUSSEN, J. Ultrastruct. Res. *46*, 87 (1974).
[9] R. YAROM, A. WISENBERG, P. D. PETERS and T. A. HALL, Proc. Microprobe Meetg., Las Vegas (1975), in press.
[10] M. CHVAPIL, S. L. ELIAS, J. N. RYAN and C. F. ZUKOSKI, Int. Rev. Neurobiol. Suppl. *1*, 105 (1972).
[11] R. J. SKAER, P. D. PETERS and J. P. EMMINES, J. cell. Sci. *15*, 679 (1974).
[12] This work was supported by a grant from the MRC. Funds for the purchase and running cost of the instrumentation were provied by the SRC.