Granule Alteration and Vacuole Formation, Two Primary Structural Features of the Histamine Release Process in Mast Cells

Mast cells are generally considered to release their cellular products by actively discharging their granules into the surrounding environment. A rather unique view on the nature of the secretory activity of the mast cell was forwarded by SMITH[1] who postulated that rat mesenterial mast cells may be caused to release histamine without shedding their cytoplasmic granules and without evidence of any other morphological changes in the cells. This postulate was based primarily on results of experiments in which toluidine blue was used to displace histamine from its binding sites in the mast cell granules. Toluidine blue will rapidly enter into, and spread throughout, the mast cell cytoplasm when applied locally to the cell surface[2]. The uptake and binding of this cationic dye to various chromotropes has been extensively studied[3,4].

To check the hypothesis of SMITH[1], rat peritoneal mast cells were exposed in vitro to varying concentrations of toluidine blue dissolved in a balanced salt solution. Released histamine was assayed fluorometrically[5] and the cells were studied by light microscopy as well as by scanning and transmission electron microscopy. In the presence of 0.9×10^{-3} *M* calcium and 6×10^{-5} *M* toluidine blue a 14% histamine release was noted; at 10^{-3} *M* dye concentration a maximum release of 65–80% was obtained. Spontaneous histamine release values were in the order of 4–5%. When calcium was omitted from the incubation medium histamine release was significantly reduced. For example, 10^{-3} *M* toluidine blue caused a release of only 34% histamine in the absence of calcium.

Contrary to earlier reports in the literature[1], toluidine blue does cause morphological changes in the mast cells, albeit peripheral expulsion of granules from the cell surface only takes place to a minor degree. At a dye concentration of 10^{-3} *M* (Figure 1), the most striking feature is the appearance of peripheral vacuolar structures containing altered mast cell granules. Small vacuoles containing single granules intermingle with large ones containing any number of granules. Granule fine structure, irrespective of degree of alteration also exhibits a characteristic feature. The fairly regular finely granular and/or filamentous substructure of the mast granules, is interrupted by irregularly distributed patches of some electron lucid material. In all probability this material represents some portion of the bound dye. Granules, apparently lying free in the extracellular medium, are also observed as are granules located within phagocytic vacuoles in the cytoplasm of leucocytes. When the concentration of toluidine blue is increased, the intracytoplasmic vacuolation is far more extensive than at lower concentrations and the granule alteration process involves most of the granule population. Extracellular altered granules are present although their number does not greatly exceed that of specimens treated with low concentrations of toluidine blue. Peripheral vacuoles may communicate freely with the extracellular milieu. Pores, through which this communication is sustained, are well demonstrated in the scanning electron microscope (Figure 2). The main effect on mast cell morphology of omitting calcium from the medium is a decreased capacity for vacuole formation.

The present findings indicate that the main effects of toluidine blue on mast cells are associated with the granules and their immediate cytoplasmic surroundings, effects which are accompanied by release of large amounts

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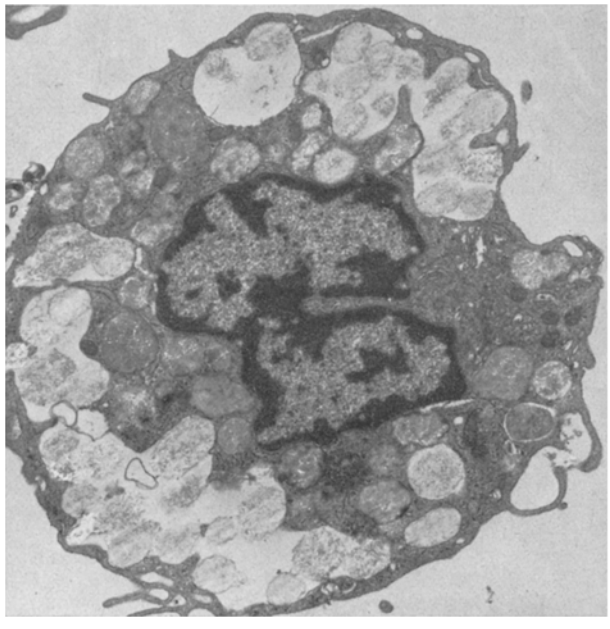

Fig. 1. Electron micrograph of a rat peritoneal mast cell treated with 10^{-3}*M* toluidine blue in the presence of calcium. Histamine release 65%. Note the numerous cytoplasmic vacuoles containing altered mast granules, the latter show a mottled appearance. ×10,000.

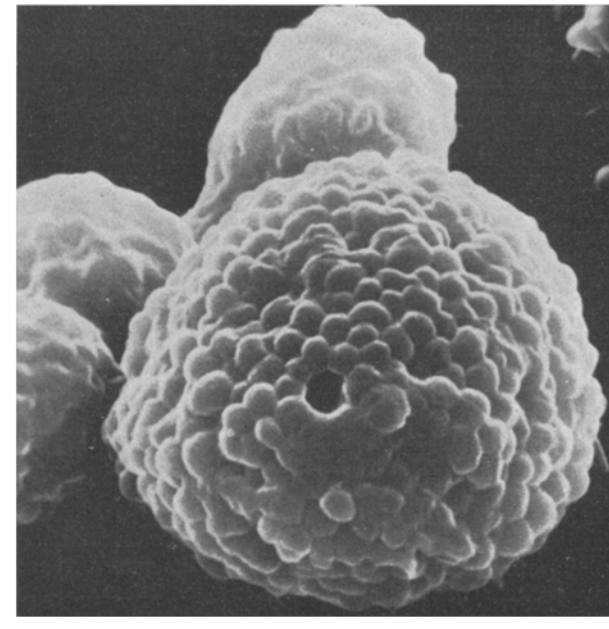

Fig. 2. Scanning electron micrograph of a rat peritoneal mast cell treated with 10^{-3}*M* toluidine blue in the absence of calcium. Histamine release 34%. Note the cellular pore in center of picture. ×9,000.

of histamine. As there is free communication between vacuoles containing altered granules and the extracellular medium, actual discharge of granules, viz. expulsion from the confinements of the cell, does not seem to be a prerequisite for the release of histamine from these cells. This is in agreement with previous observations on the effects of certain histamine liberating agents such as stilbamidine[6], compound 48/80[6,7], bee venom[8], ATP[9] and also the antigen/antibody reaction[10]. In the mast cell literature, the term 'degranulation' is often employed as a synonym to 'granule discharge'. In the authors' opinion a distinction should be made between the 2 terms, as 'degranulation' simply implies a decrease in numbers of cytoplasmic granules but does not specify if the granules are actively expelled out of the cell or if they simply disappear in some other manner, e.g. are dissolved at their original site. It seems evident that both these processes occur and must be taken into consideration when discussing the nature of the secretory activity of the mast cell.

Zusammenfassung. Die Histaminfreisetzung in peritonealen Mastzellen der Ratte, hervorgerufen durch Toluidinblau, wurde in vitro studiert und festgestellt, dass Toluidinblau vor allem auf die Feinstruktur der Mastzellgranula und deren unmittelbare Umgebung einwirkt, wodurch Cytoplasmabläschen entstehen. Diese setzen sich wahrscheinlich mit dem extra-zellulären Raum in Verbindung, woraus geschlossen wird, dass das Auswandern von Granula aus dem Zellbereich keine Vorbedingung für die Histaminfreisetzung darstellt.

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Localization of Sodium Ions in Dog Submandibular Gland Tissue by the Use of Potassium Pyroantimonate

Several sites in salivary glands have been proposed as having the capability for active sodium transport. For instance, it has recently been suggested that active sodium transport occurs at acinar cells in cat submandibular glands[1,2]. Other workers have put forth evidence for sodium transport in both intraglandular[3–7] and excretory duct cells[8–10]. A reliable histochemical technique for sodium localization would be a valuable tool to differentiate among these possible sodium transport sites. The technique of using potassium pyroantimonate for the histochemical localization of sodium was first proposed by KOMNICK[11] and has recently been applied to a variety of tissues[12–17]. This paper reports the results of experiments designed to test the applicability of the pyroantimonate method to canine submandibular tissue.

Materials and methods. The fixatives used were 6.25% glutaraldehyde and 2% osmium tetroside either unbuffered or buffered with 100 m*M* potassium phosphate (pH 7.4). Sufficient potassium pyroantimonate (K & K Laboratories, Plainview, New York) was added to each of the fixatives to yield a final concentration of 2%. Some heating was generally required for dissolution of the potassium pyroantimonate. Controls were run using fixatives prepared without pyroantimonate.

Submandibular glands of adult mongrel dogs were fixed in two ways. In some experiments glands were removed from dogs anesthetized with sodium pentobarbital given i.v. at a dose of 30 mg/kg. The glands were chopped into small pieces and placed into 1 of the 4 fixative solutions described above. In other experiments a 2% potassium pyroantimonate solution was retrogradely perfused into the duct system for 15 min using methods previously described[6]. The gland was then perfused with 1 of the fixatives described above for 10 min. Parts of the perfused glands were removed, cut into fine pieces and placed in fresh fixative. All tissues were dehydrated in alcohols, embedded in epoxy, stained with uranyl acetate and viewed in either an RCA EMU 3G or an AEI EM 801 electron microscope.

Results. Precipitate localization patterns of tissues fixed by immersion in pyroantimonate-containing fixative depended on whether the fixative contained osmium or glutaraldehyde. The precipitate in either acinar or duct cells was primarily extracellular when tissues were fixed in glutaraldehyde. In glutaraldehyde fixed acinar tissue the precipitate was found in areas betweeen cells, the periphery of acini, around myoepithelial cells, in the extracellular space, and in the acinar lumen. There were only occasional intracellular precipitate particules which appeared to be localized mainly around storage granules. Fixation in osmium resulted in a greater number of intra-

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