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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[6] G. D. BLOOM, B. LARSSON and D. E. SMITH, Acta path. microbiol. scand. *40*, 308 (1957).
[7] G. D. BLOOM and Ö. HAEGERMARK, Expl Cell Res. *40*, 637 (1965).
[8] G. D. BLOOM and Ö. HAEGERMARK, Acta physiol. scand. *71*, 257 (1967).
[9] P. G. KRÜGER, G. D. BLOOM and B. DIAMANT, to be published.
[10] G. D. BLOOM and N. CHAKRAVARTY, Acta physiol. scand, *78*, 410 (1970).
[11] The authors are indebted to Miss MARIANNE BORG for technical assistance and the Swedish Medical Research Council and the Magnus Bergvall Foundation for grants. We also gratefully acknowledge the use of the Stereoscan S-4 scanning electron microscope which is a gift to the University of Umeå from the Knut and Alice Wallenberg Foundation.

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-

[1] O. H. PETERSEN, Phil. Trans. R. Soc. Lond. B. *262*, 307 (1971).
[2] J. R. MARTINEZ and O. H. PETERSEN, Experientia *28*, 167 (1972).
[3] J. R. MARTINEZ, H. HOLZGREVE and A. FRICK, Pflügers Arch. ges. Physiol. *290*, 124 (1966).
[4] J. A. YOUNG and E. SCHOGEL, Pflügers Arch. ges. Physiol. *291*, 85 (1966).
[5] J. A. MANGOS, G. BRAUN and K. F. HAMANN, Pflügers Arch. ges. Physiol. *291*, 99 (1966).
[6] I. A. SIEGEL and R. ZENDZIAN, Arch. oral Biol. *17*, 389 (1972).
[7] J. R. MARTINEZ, J. Pharmac. exp. Ther. *178*, 616 (1971).
[8] L. H. SCHNEYER, Am. J. Physiol. *217*, 1324 (1969).
[9] J. A. YOUNG, Pflügers Arch. ges. Physiol. *303*, 366 (1968).
[10] J. A. YOUNG, E. FROMTER, E. SCHOGEL and K. F. HAMANN, Pflügers Arch. ges. Physiol. *295*, 157 (1967).
[11] J. KOMMICK, Protoplasma *55*, 414 (1962).
[12] G. I. KAYE, J. D. COLE and A. DONN, Science *150*, 1167 (1965).
[13] E. TANI, T. AMETANI and H. HANDA, Acta Neuropath. *14*, 137 (1969).
[14] H. R. M. TORACK, Acta Neuropath. *12*, 173 (1969).
[15] R. E. BULGER, J. Cell Biol. *40*, 79 (1969).
[16] C. C. TISHER, W. J. CIRKSENA, A. V. ARSTILA and B. F. TRUMP, Am. J. Path. *57*, 231 (1969).
[17] S. M. SUMI, J. Histochem. Cytochem. *19*, 591 (1971).

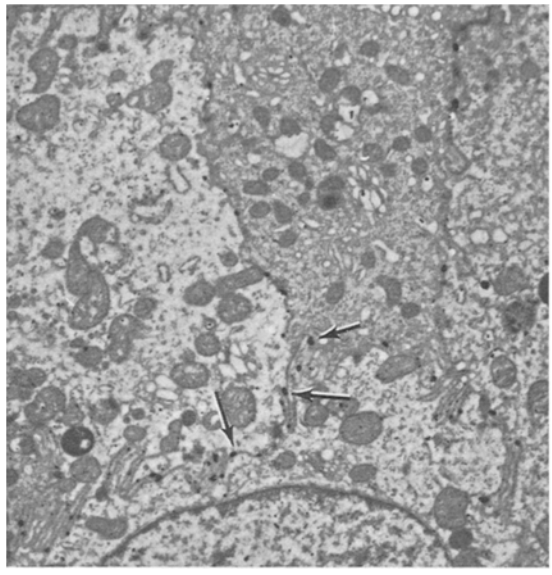

Fig. 1. Duct cells fixed in pyroantimonate and glutaraldehyde. ×6800. Arrows point to pyroantimonate deposits in both figures.

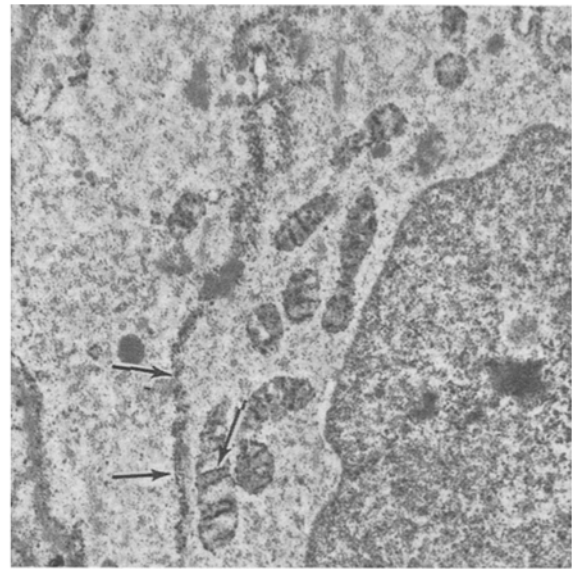

Fig. 2. Duct cells fixed in pyroantimonate and osmium. ×12,800.

cellular precipitate particules than were noted after glutaraldehyde fixation. These intracellular particules were throughout the cell in osmium-fixed tissue, but there was some degree of localization to the periphery of the storage granules.

When duct cells were fixed in glutaraldehyde opaque precipitates were seen primarily in the interstitium, the lumen, and between lateral membranes of the adjacent cells (Figure 1). The deposit in the lateral space was distributed along the entire length of the area of tight junctions. The precipitate in this space appeared to be associated either with the external surface of the cell or to fill the area between cells. The precipitate pattern in osmium fixed duct cells was more diffuse. In addition to the pattern described above for glutaraldehyde fixed cells, deposits could also be found along the membranes of mitochondria, in the nucleus, and throughout the cell sap (Figure 2). In contrast to the extracellular localization noted in glutaraldehyde-fixed material, the deposits appeared to be primarily on the intracellular border of the cell membranes. No differences were noted between buffered and unbuffered fixative in either acinar or duct cells.

The pyroantimonate deposits were localized to the lumen of the ducts when glands were first perfused with 2% potassium antimonate and initially fixed by perfusing with either osmium or glutaraldehyde. Tissue treated in this manner did not have pyroantimonate deposits in gland cells or the interstitial space.

Discussion. These results using submandibular glands are similar to those reported for rat cerebral cortex[17] and rat kidney[16] in that the localization of pyroantimanate precipitate appears to vary according to the method of fixation and type of fixative employed. Furthermore, under appropriate conditions precipitation reaction between pyroantimonate and divalent ions and biological amines can occur[15,18,19]. Therefore, although the technique may indicate the presence of sodium ions, we believe that definition as to the actual in vivo location of sodium using the pyroantimonate technique must be made with extreme caution[20].

Résumé. Des glandes sousmaxillaires de chiens ont été coupées en morceaux et mises dans un fixatif composé de 2% $KSB(OH)_6$ et soit de 6.25% d'aldéhyde glutarique, soit de 2% d'osmium et préparées pour l'examen au microscope électronique. Quand les tissus sont fixés à l'aldéhyde glutarique, le précipité est principalement extracellulaire. Par contre, avec le fixatif à l'osmium, le précipité est plus diffus et se trouve partout dans la cellule. Cependant, on ne peut pas se fier à cette méthode pour localiser des ions de sodium in vivo.

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[18] S. Shin-Ichi, V. Mizuhira, T. Amakawa and Y. Futaesako, J. Histochem. Cytochem. *20*, 65 (1962).

[19] R. L. Klein, S. Yen and A. Thureson-Klain, J. Histochem. Cytochem. *20*, 65 (1972).

[20] This work was supported by grants No. DE 06200 and No. DE 01701 from the National Institutes of Health.

On the Activity of Neurosecretory Cells in the Flesh-Fly *Sarcophaga bullata*

The hormones secreted by the neurosecretory cells of the insect brain are clearly known to be implicated in certain metabolic activities[1]. These hormones, it has been indicated, may also influence the egg production in the female[2]. Again, from their studies on female insects, some workers[3–5] conclude that the hormones of the neurosecretory cells of brain and also of other ganglia appear to be involved in the maturation and production of eggs. However, to our knowledge, no information is on record about the involvement of these hormones in the