IMMUNOCYTOCHEMISTRY ON ACID HYDROLASES IN PREPUTIAL GLAND CELLS

R. Brands, R. Koninkx-Peeters, J.W. Slot and J.J. Geuze
Centre for Electron Microscopy, Med. School, University of Utrecht, The Netherlands

Present knowledge of the in situ localization of acid hydrolases is mainly based on enzyme-cytochemical observations on acid phosphatase and aryl sulfatase. In rat preputial gland cells, β-glucuronidase and acid phosphatase were demonstrated this way in secretory granule-like lysosomes (1). We have applied immunocytochemistry, which in principle allows the detection of proteins at intracellular sites where they are not (yet) enzymatically active.

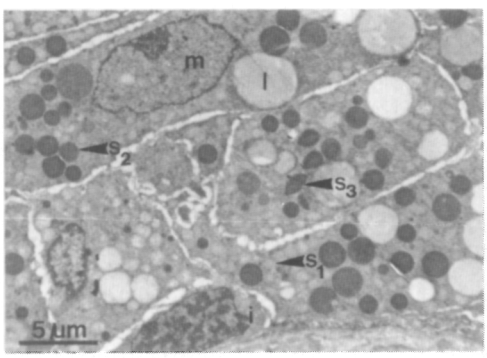
Acid hydrolases were purified from rat preputial gland (β-glucuronidase) and rat liver (aryl sulfatase, β-hexosaminidase and α-glucosidase) by column chromatography and preparative PAGE. Affinity purified goat and rabbit antibodies against these proteins were used for immunocytochemistry on thin frozen sections (2) of perfusion fixed preputial glands. The sections were indirectly labeled with rhodamine for fluorescence and with a 5 nm protein A/gold probe for electron microscopy.

The immunocytochemical approach allowed the localization of the proteins at their earliest sites of biosynthesis. The labeling pattern was essentially the same for all proteins studied. It was found throughout the RER, including the perinuclear space, the Golgi complex and several types of secretory-like granules (Figs.)

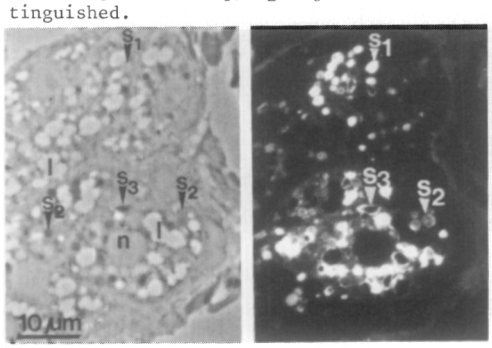
We conclude that acid hydrolases occur in all compartments of the secretory system in rat preputial gland cells.

1. Mesquita-Guimarães, J. (1974). Histochem. J. 6, 685-692.

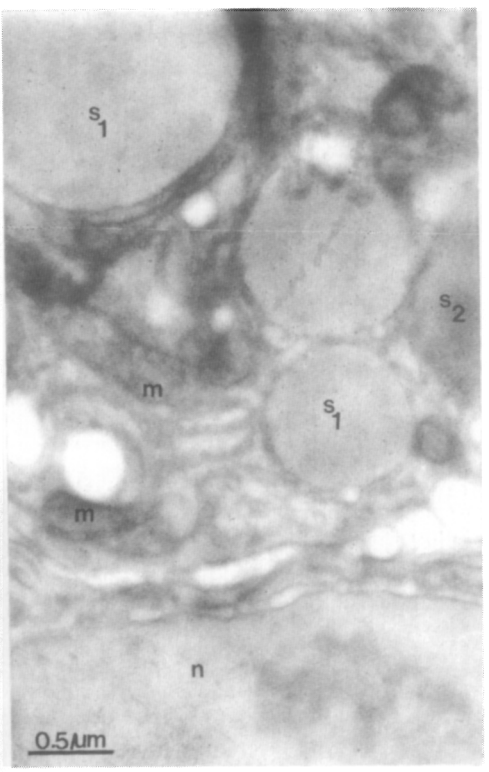
2. Tokuyasu, K.T. and S.J. Singer (1976). J. Cell Biol. 71, 894-906.



Epon section. Part of a rat preputial gland acinus with immature cells (i) and mature cells (m). Lipid droplets (l) and several types of granules (s_1, s_2, s_3) can be distinguished.



Phase contrast view of a semi-thin frozen section of rat preputial gland (left). *Immunofluorescent* (TRITC) demonstration of β-glucuronidase on the same section (right). Granule type s_1, s_2, and s_3 are labeled. The crystalloid in granule type s_3, nucleus and lipid droplets are not labeled



Ultrathin frozen section. Immunocytochemical demonstration of β-glucuronidase with 5 nm protein-A/gold particles. Label is seen over granules. Nucleus (n) and mitochondria (m) are not labeled.

0309-1651/80/080749-01/$02.00/0