CC/NUMBER 14
APRIL 4, 1983

This Week's Citation Classic™

Smith R E & Farquhar M G. Lysosome function in the regulation of the secretory process in cells of the anterior pituitary gland. *J. Cell Biol.* **31**:319-47, 1966. [Dept. Pathology, Univ. California Sch. Med., San Francisco, CA]

By electron microscopy and staining for acid phosphatase we investigated the importance of enzyme alterations relevant to the role of the Golgi apparatus and lysosomes in the secretory process of mammotrophic hormone-producing cells (MT) in pituitary glands from lactating rats and in involving MT cells induced by cessation of lactation. [The *SCI*® indicates that this paper has been cited in over 795 publications since 1966.]

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January 31, 1983

"My fascination with the anterior pituitary gland first began in undergraduate study at Earlham College and was further kindled while a graduate student at Indiana University by Fernan-dus Payne. While a medical student there, I was asked to take over, as a guardian of sorts, an RCA-II electron microscope (EM) which provided me an opportunity to undertake EM studies of the mouse anterior pituitary. As I recall, a paramount question was: what are these round structures 500 mμ in diameter in the gonadotrophic cells without cristae, not mitochondria? With a few 'good' micrographs (1959 vintage) and many questions, I wrote to Marilyn Farquhar, which culminated with an invitation for me to work in her laboratory. In February 1962, as a student at the University of California, San Francisco, and working with Farquhar, we were the first to distill the magic fixative, glutaraldehyde, and stained for acid phosphate which resulted not only in lead phosphate deposition in the 500 mu granules but also around some secretion granules and Golgi cisternae of the various cells. It was evident that there was a critical need for better morphology which precipitated development of the Smith-Farquhar Tissue Sectioner. In the spring of 1962, R. Marion Hicks convinced us that we were staining lysosomes, and a letter from Alex Novikoff expressed elation over our micrograph showing acid phosphatase staining of Golgi cisternae and vesicles in rat anterior pituitary cells.

"But what were lysosomes doing in cells with little phagocytic capability? Based on my light microscopic trichrome studies with Payne, I posed the question: what do pituitary cells do with secretion granules that cannot be released? Stimulation of mammotrophic cells by suckling and abrupt removal of nursing young was normal but produced an exaggerated physiologic condition ideal to study the role of the Golgi apparatus and lysosomes in the secretory process. It had been shown that when suckling young were removed, prolactin levels in the serum of the mother rat dropped quickly; however, assays of the glands revealed more hormone, which disappeared slowly in two to three days. Two years later, the theory of granulophagy (subsequently defined by de Duve as crinophagy) could be considered an alternative to secretion granule release whereby cells disposed of excess or outdated secretory material by an internal mechanism. The mechanism has been observed and studied morphologically, cytochemically, and biochemically in various types of secretory cells, while the cardinal events remain essentially the same; the principal reason I believe the paper has become a *Citation Classic*. However, the paper has also been referenced frequently because of its contribution to understanding the polarity and compartmentalization of the Golgi cisternae and their relation to the formation of Golgi vesicles, lysosomes, and the condensation of secretory material into granules.

"I recognized we were only staining for a marker enzyme, and in reality proteases were the enzymes essentially controlling crinophagy. I later worked with J. Ken McDonald to develop synthetic peptide substrates for anterior pituitary proteases. Then, with a move from Stanford VA Hospital to Eli Lilly and Company, my interest shifted to fluorogenic protease substrates designed to study the conversion of proinsulin to insulin and for the assay of thrombin and plasminogen activator.[1] In 1975, at Lawrence Livermore National Laboratory, I approached the study of proteases in the pituitary by flow cytometry[2] and then diverted my interests in the direction of clinical application of proteases and assisted in the development of the American-Dade Protopath and other clinical systems.[3,4] I am now back into the basics of cell biology, studying glandular kallikrein in the cat submandibular gland and saliva with John R. Garrett,[5] and defining isoenzyme patterns of cathepsin B relevant to limited proteolysis in the cells of the good old rat anterior pituitary gland."

1. **Smith R E & Van Frank R M.** The use of amino acid derivatives of 4-methoxy-β-naphthylamine for the assay and subcellular localization of tissue proteinases. (Dingle J T & Dean R T. eds.) *Lysosomes in biology and pathology.* Amsterdam: North-Holland. 197S. Vol. 4. p. 193-249.
2. **Smith R E & Dean P N.** A study of acid phosphatase and dipeptidyl aminopeptidase II in monodispersed anterior pituitary cells using flow cytometry and electron microscopy. *J. Histochem. Cytochem.* **27**:1499-504, 1979.
3. **Huseby R M & Smith R E.** Synthetic oligopeptide substrates—their diagnostic application in blood coagulation. fibrinolysis, and other pathological states. (Mammen E F. ed.) *Seminars in thrombosis and hemostasis.* New York: Thiemc-Stratton. 1980. p. 173-314.
4. **Pearson K W, Smith R E, Mitchell A R & Bissel E R.** Automated enzyme assays by use of a centrifugal analyzer with fluorescence detection. *Clin. Chem.* **27**:256-62, 1981.
5. **Garrett J R, Smith R E, Kidd A, Kyiacou K & Grabske R J.** Kallikrein-like activity in salivary glands using a new iripeptide substrate, including preliminary secretory studies and observations on mast cells. *J. Histochemistry* **14**:967-79, 1982.