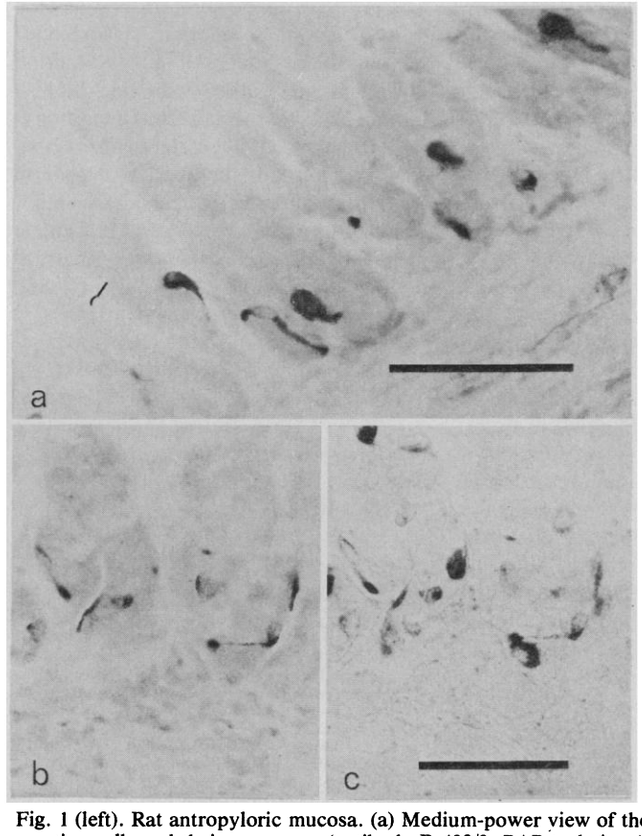
Somatostatin Cell Processes as Pathways for Paracrine Secretion

Abstract. *Somatostatin is produced by gastrointestinal endocrine cells that have long, nonluminal, cytoplasmic processes. Such processes terminate on other cell types, including gastrin-producing and hydrochloric acid-producing cells, whose functions are profoundly affected by somatostatin. The findings suggest that somatostatin cells control the functions of other cells through local release of the peptide by way of cytoplasmic processes. Also, certain other types of gastrointestinal endocrine cells have similar cytoplasmic prolongations, which may have analogous local (paracrine) regulatory functions.*

Somatostatin, a growth hormone release inhibiting peptide first isolated from the ovine hypothalamus (*1*), suppresses the secretion of other hormones such as insulin, glucagon, and gastrin and affects gastric and pancreatic exocrine secretion (*2*). Immunological studies show that somatostatin is not restricted to hypothalamic neurons, but also occurs in endocrine-like (D) cells of the gut and pancreas (*3*). These cells belong to the system of diffusely distributed endocrine (paracrine) cells of Feyrter (*4*), who suggested that in addition to endocrine functions, they exert paracrine effects on neighboring epithelial cells. Since somatostatin is a potent inhibitor of secretion from many different endocrine and exocrine cells, such local paracrine effects represent an attractive possibility. Somatostatin has a very short half-life in blood and somatostatin-secreting tumors produce only very mild clinical symptoms (*5*), suggesting that its paracrine actions may be more important than its endocrine actions. For instance, in the stomach somatostatin could be an important physiological regulator of gastrin and hydrochloric acid release. Although D cells occur in the same zone of the gastric mucosa as gastrin (G) cells, there is a considerable distance between the two cell types. By use of sensitive immunocytochemical techniques (*6*), we have now been able to show that the somatostatin cells have long, nonluminal processes, which come in contact with numerous putative effector cells, including gastrin cells. Our studies suggest that the release of gastrin and hydrochloric acid from the stomach may be regulated by local release of somatostatin from such processes.

Rats (N = 10) were killed by decapitation and human (N = 7) material was obtained at surgery for peptic ulcer or carcinoma or through peroral biopsies. Specimens from the antropyloric (human, rat) and oxyntic (rat only) mucosa were frozen in melting Freon-22, freeze-dried, fixed with paraformaldehyde vapor, embedded in paraffin, and sectioned at 3 to 10 μm. Sections were reacted with one of three rabbit antibodies against somatostatin (R 37/3, R 213/3, and R 433/3) at dilutions in buffer of 1:800, 1:3200, and 1:1600, respectively, for 24 hours at 4°C. The antibodies were prepared with synthetic ovine somatostatin covalently coupled to bovine serum albumin with carbodiimide.

The immunization scheme was identical to that described for cholecystokinin (*7*). The site of antigen-antibody reaction was revealed by the peroxidase-anti-



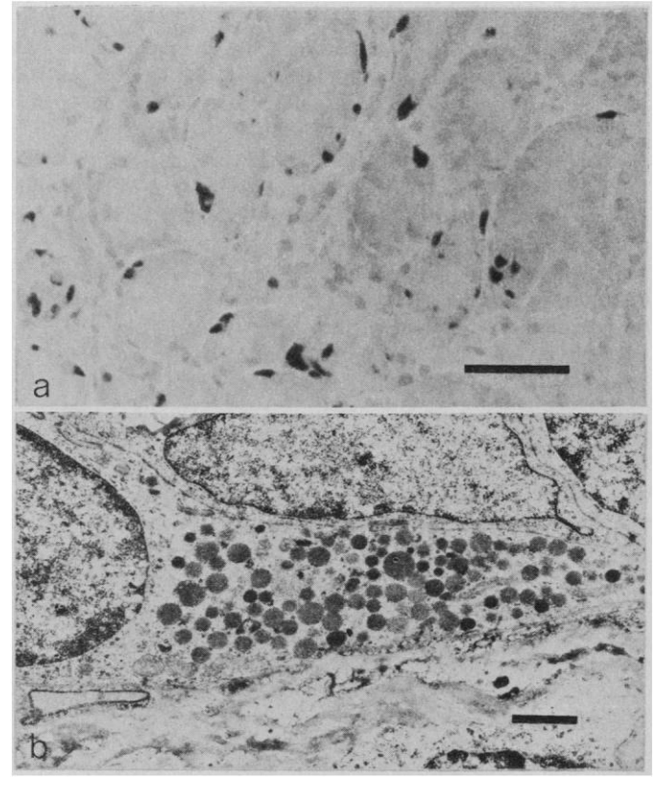


Fig. 1 (left). Rat antropyloric mucosa. (a) Medium-power view of the mucosa, showing the frequency and extent of the somatostatin immunoreactive cells and their processes (antibody R 433/3, PAP technique). (b) Somatostatin cell of rat antropyloric mucosa emitting a long process terminating in a bulbous swelling. (c) The same section after elution of somatostatin antibodies and restaining with gastrin antibodies. The somatostatin cell process (brown in original photomicrograph) terminates on a gastrin cell (blue-black in original photomicrograph). Scale bar, 50 μm. Fig. 2 (right). Human antropyloric mucosa. (a) Paraffin section showing numerous somatostatin cells (antibody R 433/3, PAP technique; see Fig. 1), emitting short blunt processes. Scale bar, 50 μm. (b) Electron micrograph showing a somatostatin (D) cell with a granulated cytoplasmic process. Scale bar, 1 μm.

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peroxidase (PAP) technique (*6*) and peroxidase activity was demonstrated by the 3,3′-diaminobenzidine (DAB) technique (*6*), which gives a brown reaction product. Results were identical with all three antibodies. Occasionally, antibodies and PAP complexes were eluted from the preparations by the method of Tramu *et al.* (*8*), and the sections were poststained with gastrin antiserums 4562 or 4710, which detect the COOH terminal and middle regions of gastrin-17, respectively (*9*). The site of antigen-antibody reaction was again demonstrated by the PAP technique, but the peroxidase activity was now revealed by the 4-Cl-1-naphthol technique, which gives a blue-black reaction product that contrasts well with the brown color of the DAB reaction (*8*). Controls included conventional staining controls (*6*) as well as absorption against synthetic somatostatin (Beckman), synthetic human gastrin-17 (ICI), pentagastrin (ICI), and the synthetic midportion (*6-13*) of human gastrin-17. The peptides were added at concentrations of 5 to 100 μg per milliliter of diluted antiserum. The absorptions confirmed the specificity of the antiserums and excluded their cross-reactivity with unrelated peptides. For electron microscopy studies, specimens were fixed by immersion in a mixture containing 3 percent formaldehyde and 2 percent glutaraldehyde for 1 hour at 4°C, osmicated, and embedded in Epon 812 (*10*). Sections were cut on an LKB III ultramicrotome and contrasted on grids with lead citrate and uranyl acetate.

The somatostatin immunoreactive cells had long, nonluminal processes, extending from the epithelium along the basement membrane of the glands. In the rat, virtually every somatostatin immunoreactive cell showed a process. The processes were long, slender, and sometimes divided into two branches. Cells with two processes were occasionally seen. In some sections it could be seen that the processes were much longer than the cell bodies (Fig. 1). Thus, many processes were more than 40 μm long. In the human, the processes were shorter, thicker, and blunter. Most rat and human gastric somatostatin cells had processes. Occasionally, however, the human somatostatin cells showed the triangular shape of typical endocrine cells.

In contrast to the cell bodies, the somatostatin cell processes came into contact with a great many different glandular epithelial cells. In the rat, but not the human, the processes often terminated in bulbous swellings, which abutted on nonimmunoreactive epithelial cells. Double staining experiments (Fig. 1) demonstrated that many somatostatin cell processes ended on G cells in the antropyloric mucosa and on parietal cells in the oxyntic mucosa. Other types of cells also appeared to be in contact with somatostatin cell processes or their terminals. Such cells may include unidentified (nonimmunoreactive) endocrine cells as well as exocrine cells.

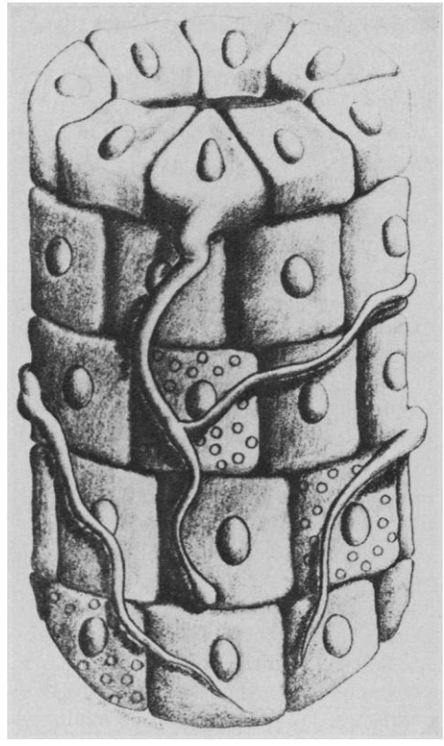

Fig. 3. Schematic drawing showing a three-dimensional arrangement of the somatostatin cells and their processes in an antral gland.

Electron microscopy confirmed the immunocytochemical observations in that cross-sectioned as well as longitudinally sectioned (Fig. 2) D (somatostatin) cell processes were frequently encountered. The processes were easily identified by their characteristic D cell granules (*3, 5*). However, the sensitive immunocytochemical methods used on thick paraffin sections were superior in revealing the cell processes. Once their presence had been established, techniques such as electron microscopy offered supportive evidence and showed the localization of the processes between the epithelium and the basement membrane (Fig. 2). The somatostatin cell processes are illustrated schematically in Fig. 3, which shows an antral gland surrounded by processes traveling along the base of the epithelium.

Its many and varied inhibitory activities make somatostatin a prime candidate for local paracrine control. In the pancreas, somatostatin cells occur in the vicinity of endocrine (insulin and glucagon) cells, whose functions are affected by this neurohormone. Consequently, processes of islet somatostatin cells are usually thick and short. In the gut, however, there is a considerable distance between somatostatin cells and their putative effector cells. These distances represent, at the cellular level, a considerable diffusion barrier for somatostatin. The presence of somatostatin-containing processes that end on putative effector cells suggests a way in which somatostatin may directly affect the secretion of gut hormones and enzymes.

We previously observed similar long processes emanating from other endocrine cells of the gut epithelium. In the rat ileum, an endocrine cell type, which shows COOH terminal gastrin immunoreactivity, appears to emit long basal processes that reach other cells (*11*). Similar processes are seen in enterochromaffin cells and other unidentified endocrine cells in the large intestine. These processes may reflect local (paracrine) release of secretory products from these cells. Cells similar in morphology to the endocrine cells described here have been identified in the accessory genital gland in *Aplysia* and it has been suggested that they represent primitive sensory-motor neurons (*12*). Since somatostatin as well as gastrin immunoreactivity has been localized to both nerves and endocrine cells and since the endocrine APUD (amine precursor uptake and decarboxylation) cells have been claimed to develop from neurally competent ectoderm (*13*), it is of interest to consider whether somatostatin cells represent intermediates between (neurosecretory) neurons and endocrine cells.

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Galactosemia: Alterations in Sulfate Metabolism Secondary to Galactose-1-Phosphate Uridyltransferase Deficiency

Abstract. *Cultures of nonmutant as well as galactokinase-deficient fibroblasts incorporate 20 percent more [^{35}S]sulfate when galactose is substituted for glucose in the medium; galactose-1-phosphate uridyltransferase-deficient cells incorporate 65.5 percent less. In addition to incorporating less [^{35}S]sulfate, the uridyltransferase-deficient cells showed significant accumulation of intracellular galactose-1-phosphate within 4 hours after galactose exposure. Under the same conditions, no difference in [^{3}H]uridine incorporation was observed. This metabolic alteration, occurring in response to galactose exposure, may be related to the pathophysiology of classical galactosemia.*

Galactokinase and galactose-1-phosphate uridyltransferase deficiencies are well recognized autosomal recessive forms of galactosemia (*1*). Lenticular cataracts are a common symptom in both disorders. The conversion of excess galactose to galactitol by lens aldose reductase causes an osmotic imbalance, the underlying mechanism in cataract formation (*1*). Other symptoms of uridyltransferase deficiency include vomiting, diarrhea, hepatomegaly, and mental retardation. This form of galactosemia can even be fatal. The factors responsible for the more severe manifestations are not well understood (*1*).

Krooth and Weinberg (*2*) were probably the first to demonstrate that fibroblast cultures derived from patients with galactosemia failed to grow in a galactose medium. Uridyltransferase activity is not detectable in cells cultured from galactosemic patients (*3*), and Mayes and Miller (*4*) demonstrated that such cells accumulate galactose-1-phosphate within 2 hours after galactose exposure. The underlying toxic mechanisms that produce the internal organ and neurological pathology are probably secondary to the intracellular accumulation of galactose-1-phosphate or a manifestation of the absence of uridyltransferase activity. Therefore, such toxic mechanisms might be identified by searching for biochemical alterations in cultured mutant cells challenged with galactose.

Our report demonstrates that when challenged with galactose, cells deficient in uridyltransferase activity (GALT cells) exhibit significantly reduced incorporation of [^{35}S]sulfate into acid-insoluble macromolecules.

Five different strains of GALT cells, one galactokinase-deficient (GALK) strain, one Hurler strain, and eight control strains were used (*5*). Galactokinase and uridyltransferase activities were measured as in (*3*). Cells were grown in minimum essential medium (MEM; Gibco or KC Biologicals) containing 16.6 percent fetal calf serum (Gibco or KC Biologicals) for routine passage and maintenance, with feeding every 3 to 4 days. Hexose-free MEM (Gibco) was used to prepare all experimental media. Concentrates (100 times) of glucose (Mallinckrodt) or galactose (Pfansteil) were added through 0.22-μm Millipore filters to hexose-free MEM containing 16.6 percent dialyzed fetal calf serum to yield the desired final concentrations. All media contained penicillin (50 U/ml) and streptomycin (50 μg/ml).

Glucose, galactose, and galactose-1-phosphate were measured enzymatically (*6*), using a fluorometer (Aminco model SPF 125) with limits of detection around 0.1 μg/ml. Hexokinase, glucose-6-phosphate dehydrogenase, galactose dehydrogenase, alkaline phosphatase, oxidized nicotinamide adenine dinucleotide (NAD^+), and oxidized nicotinamide adenine dinucleotide phosphate ($NADP^+$) were obtained from Boehringer Mannheim and the Sigma Chemical Company.

The cell cultures were labeled by adding $H_2{}^{35}SO_4$ (New England Nuclear; 10 μCi/ml) to the experimental medium in 25-cm^2 disposable Corning flasks containing a confluent cell sheet. The labeled cells were harvested by ethanol or trichloroacetic acid (TCA) precipitation. The ethanol precipitation method was described by Fratantoni *et al.* (*7*). The final washed precipitate was dissolved in 0.5 ml of 10 percent NaOH. Portions of 50 or 100 μl were counted in a Packard scintillation spectrometer (model 3385), 10 ml of Instagel (Packard) being used as the scintillation medium. Protein concentration was measured by the method of Lowry *et al.* (*8*); bovine serum albumin dissolved in 10 percent NaOH was used as the standard.

Alternatively, cultures were labeled with [^{35}S]sulfate or [5, 6-^{3}H]uridine (New

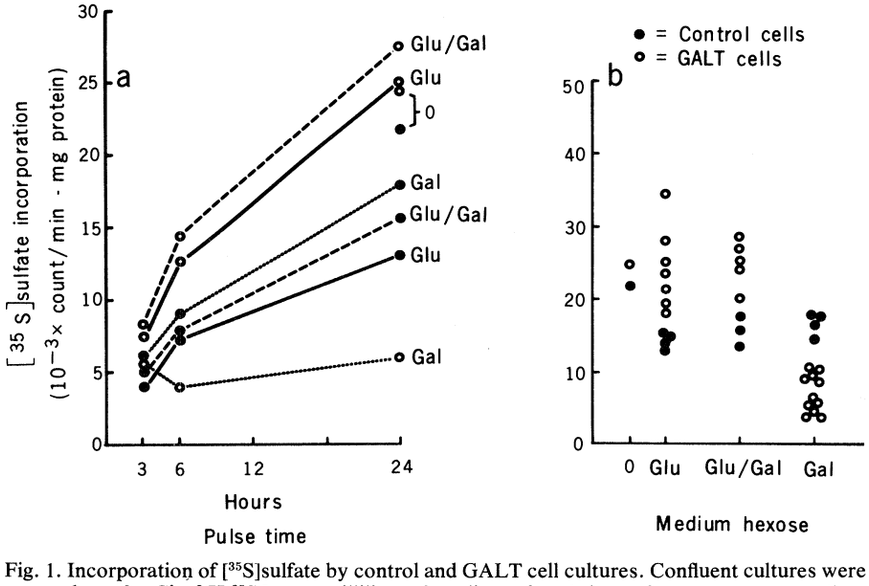

Fig. 1. Incorporation of [^{35}S]sulfate by control and GALT cell cultures. Confluent cultures were exposed to 10 μCi of $H_2{}^{35}SO_4$ per milliliter of medium after 6 days of exposure to a medium containing no hexose (*0*), 100 mg/dl glucose (*Glu*), 100 mg/dl galactose (*Gal*), or 50 mg/dl of hexose mixture (*Glu/Gal*). (a) The amount of isotope incorporated during 3-, 6-, and 24-hour exposures. (b) The amount of isotope incorporated by individual cultures during a 24-hour exposure.

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