Secretion of fluid lubricating the eye is an unconscious event, until some particle of dust or chemical touches its surface. Then, a profuse secretion of tears occurs that dilutes and washes away the foreign substance. Like the eye, the gut secretes fluid, analogous to tears, unbeknownst to most of us until some event triggers an outpouring of large volumes of fluid necessary to flush out a microbe or deleterious chemical. This host defense mechanism triggers our awareness by the onset of diarrheal symptoms.

Effector cells

How do “tears of the gut” or “enteric tears” form? They are produced continuously by immature cells called crypt cells, which, together with absorptive cells (villus and surface cells), form an epithelial cell lining in the intestine. Crypt cells take up chloride by an energy-requiring cotransporter protein that carries sodium, chloride, and potassium ions from the blood side or basolateral side of the cell to its interior (Fig. 1). Once chloride is in the cell, it diffuses into the lumen through pores or channels in the apical membrane down a gradient, established by electrical and concentration profiles of the cell. Sodium ions and water molecules join chloride in the lumen and make up the “enteric tears.” Thus chloride secretion with accompanying fluid accumulation, usually in conjunction with mucus secretion by specialized goblet cells, is one of the ways the intestine continuously lubricates itself, ensuring appropriate mixing and flow of digest along its length.

The enteric nervous system

The volumes of fluid produced by the intestine span a wide range from that necessary for lubrication, from ~1 liter/day to profuse secretion of 20 or more liters/day as a consequence of the actions of cholera toxin from the bacterium *Vibrio cholerae*. How is it possible to achieve such extremes of fluid secretion? This is mainly because the enteric nervous system functions with considerable independence from the brain. The enteric nervous system consists of at least two ganglionated plexuses embedded in the gut wall, the myenteric plexus (Auerbach’s plexus) and the submucosal plexus (Meissner’s plexus) (Fig. 1). In large mammals such as pigs and humans, the submucosal plexus is arranged in two or three layers, each differing in the types of neurons and their projections (12). For the most part, the two plexuses service different regions and structures of the intestinal wall. The myenteric plexus is responsible for ensuring appropriate contractile behavior of the smooth muscles, including the longitudinal and circular muscle, so that the digesta can be mixed and propelled. The submucosal plexus functions to control chloride secretion by crypt cells as well as blood flow to the intestinal lining. The functions of each plexus are not entirely exclusive, since there are neural projections between the two that are necessary for coordination of secretion, motility, and blood flow.

Intestinal reflexes

Chloride secretion is controlled predominantly by neurons in the submucosal plexus, which are arranged in reflex circuits. However, some neural secretory reflexes, particularly in response to certain bacterial toxins such as cholera toxin or STa toxin from *Escherichia coli*, may require the presence of myenteric ganglia for the full secretory response. Mechanical stimulation or cholera toxin causes activation of submucosal primary affenter neurons that synapse with other submucosal neurons and that transmit to myenteric ganglia either directly or indirectly via nicotinic cholinergic synapses (Ref. 4 and Fig. 1A). At least...
two types of myenteric secretomotoneurons project to the epithelium and may be important in mediating secretion through the myenteric plexus, although the specifics of synaptic connections to these neurons are unknown.

**Sensory cells**

A requirement for initiation of a reflex is a sensory cell that responds to mechanical or chemical stimuli. Sensory cells are not clearly defined but may be specializations of afferent nerve endings, endocrine cells, or other cell types. In the intestine, endocrine cells, which are called enterochromaffin cells, release serotonin, also called 5-hydroxytryptamine (5-HT), in response to mechanical or tactile stimuli, distension, and chemicals such as acid or glucose (9). In some species, subsets of enterochromaffin cells may contain other potential mediators such as guanylin, substance P, or neurotensin in addition to 5-HT. Enterochromaffin cells have a complex regulatory system due to expression of many surface receptors (Ref. 9 and Fig.1A). Some of these evoke 5-HT release (β2-adrenergic, cholera toxin, 5-HT3, muscarinic M3, and nicotinic receptors), whereas others are inhibitory (α2-adrenergic, adenosine A2, benzodiazepine, γ-aminobutyric acid A and B, histamine H1, 5-HT3, P2y purinergic, somatostatin, vasoactive intestinal peptide (VIP)/pituitary adenylate cyclase-activating peptide receptors) (Fig. 2).

Another sensory cell is a prostaglandin-secreting cell, which is triggered by mechanical stimuli or distension in some species (Ref. 3 and Fig.1B). The cell type is unknown but could be enterochromaffin cells, epithelial cells, fibroblasts, or other cells. Mast cells, which are a potential source of prostanoids, are not activated by mucosal stroking in noninflamed states.

**Intrinsic primary afferent neurons**

Afferent neurons when activated carry electrical signals in the form of action potentials to cell bodies of neurons in the enteric ganglia. Intrinsic primary afferent neurons consist of two types: those with cell bodies in myenteric ganglia (Fig. 3C) and those with cell bodies in submucosal ganglia (Fig. 3A). Intrinsic primary afferents whose cell bodies are in myenteric ganglia respond to chemical stimulation and tension and...
Synapse with other myenteric neurons necessary for control of motility patterns. Intrinsic primary afferents in the submucosal plexus of the guinea pig intestine contain substance P, acetylcholine, and glutamate (Refs. 2 and 5 and Figs. 1A and 3A). Submucosal primary afferents carry information from the intestinal lumen to submucosal ganglia and to myenteric ganglia (Refs. 2, 4, and 5 and Figs. 1A and 3A). These interconnections between both ganglionated plexuses may be necessary for orchestrating the coordination of motility, secretion, and blood flow.

Submucosal primary afferents in the guinea pig intestine are activated when serotonin released from enterochromaffin cells binds to 5-HT₁p receptors (Refs. 2 and 5 and Figs. 1A and 3A). Binding of the endogenous ligand triggers action potentials that propagate to the primary afferent neuron's cell body located in a submucosal ganglion (Fig. 1A). If the cell body has the appropriate conductance, the impulses will travel through it and along the axon, causing release of neurotransmitters at synapses with submucosal neurons and myenteric neurons (Fig. 1A). Substance P is released from submucosal primary afferents by mechanical stimulation (2). Whether acetylcholine or glutamate is released from these neurons as well is unknown.

Although not proven, submucosal primary afferents may participate in axon reflexes as well. Thus the action potential may also be propagated antidromically along collateral fibers near crypt cells. Released transmitter would act directly on epithelial cells to modulate chloride secretion (Fig. 3A). Thus the intrinsic afferent neuron also may have an efferent function.

In support of the axon reflex concept is the recent discovery of neurokinin (NK₁) receptor messenger RNA in isolated epithelial cells or crypt glands by reverse transcription-polymerase chain reaction and by in situ hybridization (2, 8). Results of ligand binding studies are consistent with the presence of NK₁ receptor protein, which is upregulated during inflammation (2, 8). Thus the epithelial expression of NK₁ receptors, in conjunction with cholinergic muscarinic M₃ receptors described in other studies, is consistent with a potential efferent role for submucosal primary afferents in regulating chloride secretion as a result of releasing either substance P or acetylcholine. It is unclear whether glutamate, also stored in submucosal primary afferents, is released or whether receptors for this excitatory amino acid are present on epithelial cells. To what extent submucosal axon reflexes, if present, contribute to the overall rate of chloride secretion during mechanical stimulation in healthy or diseased states is unknown.

**Interneurons and motoneurons**

Submucosal primary afferents synapse with motoneurons projecting to crypt cells or to arterioles (Fig. 3A). Although the term secretomotoneuron has often been used to refer to the

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**Figure 2.** Potential neuroactive mediators and receptors on submucosal neurons and sensory cells (information mostly from the guinea pig). ACh, acetylcholine; A₁ and A₂, adenosine; α₁ and β₁, adrenergic; AMPA, α-amino-3-hydroxy-5-methylisoxazole-4-propionic acid, with subunits GluR1, GluR2/3 and GluR4; BZ, benzodiazepine; CGRP, calcitonin gene-related peptide; CCK, cholecystokinin; CTx, cholina toxin; DYN, dynorphin; δ-ENK, enkephalin; EC cell, enterochromatin cell; GAL, galanin; 5-HT₁a, 5-HT₁b, 5-HT₄, 5-HT₅, 5-hydroxytryptamine; GABA, and GABA, γ-aminobutyric acid; GLU, glutamate; G, guanylin; H₁ and H₂, histamine; mGluR and mGluR5, metabotropic glutamate; NMDA, N-methyl-D-aspartic acid; M₁ and M₃, muscarinic; NK₁ and NK₃, neuropeptides; NMU, neuromedin U; NPY with Y receptor, neuropeptide Y; NT, neurtensin; N, nicotinic; NO, nitric oxide; PHI, peptide histidine isoleucine; PACAP, pituitary adenylate cyclase-activating peptide; P₂Y and P₂X, purinergic; SOM, somatostatin; SP, substance P; TRH, thyrotropin-releasing hormone; VIP, vasoactive intestinal peptide. *See NOTE ADDED IN PROOF and Ref. 7a.
motor innervation of epithelial cells, branches of secretomotoneurons may also project to arterioles and serve a vasomotor function. Secretomotoneurons of submucosal origin store VIP or acetylcholine as their main transmitter along with other substances whose functions are unknown (Fig. 2). In some cases, interneurons that contain acetylcholine or other potential neurotransmitters may be interposed between afferent neurons and motoneurons to integrate signals between these two types of neurons.

There is strong evidence that secretomotoneurons are depolarized predominantly as a consequence of receiving synaptic input from submucosal primary afferent neurons upstream in the reflex, rather than by mechanical distortion of their endings (3, 11). The reflex is most likely monosynaptic with submucosal primary afferent neurons synapsing directly with secretomotoneurons. Compelling evidence in support of this concept is the presence of NK$_1$ receptors, which bind substance P, on cholinergic secretomotoneurons (7). In the guinea pig, the absence of NK$_1$ receptors on VIP secretomotoneurons suggests that monosynaptic transmission from submucosal primary afferents to VIP secretomotoneurons, if it occurs, must utilize a receptor other than the NK$_1$ receptor if substance P is the transmitter at this synapse. Alternatively, primary afferents could transmit to postsynaptic receptors that bind glutamate or acetylcholine, both potential transmitters in submucosal primary afferents (Ref. 5 and Fig. 2).

Mechanical stimuli also activate a 5-HT-independent neural pathway by releasing prostaglandins (Refs. 3 and 10 and Fig. 1B). Recent studies suggest that the two sensory cell messengers, 5-HT and prostaglandins, regulate chloride secretion in concert via parallel neural pathways that converge on crypt cells (Fig. 1B). The prostaglandin-activated neural pathway includes cholinergic secretomotoneurons transmitting signals via muscarinic receptors or VIP secretomotoneurons projecting to the crypt cells.

Excitability in submucosal neurons comprising the secretory reflex in the guinea pig is modulated by excitatory input from myenteric 5-HT interneurons (5-HT$_3$ receptor) as well as...
inhibitory input from sympathetic fibers (α₂-adrenergic receptors), from myenteric interneurons containing enkephalins (δ-opioid receptors) or somatostatin (somatostatin receptors) and from adenosine (A₁ receptors) from unknown sources. Without such “physiological brakes” on reflexes that regulate chloride secretion, we most likely would be carrying around portable “potties” rather than cellular phones!

**Surveillance and input by the central nervous system**

How does the brain know when to modulate enteric nervous system function? The brain receives messages via vagal and spinal primary afferents, which sample the intestinal environment either directly or in conjunction with specialized sensory cells such as enterochromaffin cells. These vagal and spinal primary afferents have their cell bodies located in sensory ganglia outside (extrinsic) the gut wall in the nodose and dorsal root ganglia (Fig. 3B). Extrinsic primary afferents (vagal and spinal) contain the neurotransmitters substance P and calcitonin gene-related peptide. Flow of impulses along these neurons can ultimately lead to integration of signals in the spinal cord and brain and appropriate outflow via sympathetic and parasympathetic efferent pathways to the gut.

**Role of extrinsic primary afferents in axon reflexes**

Just as emotions can enhance secretion of tears, so do emotions affect “enteric tears” by modulating neural pathways that stimulate chloride secretion. Stress induces chloride secretion, which is often associated with gastrointestinal disturbances, including abdominal pain and diarrhea. Stress-induced chloride secretion may be an adaptive response to altered epithelial barrier function. Normally, the epithelium provides a functional barrier between the outside world and the host’s interior. The epithelial barrier prevents excessive penetration of macromolecules, such as foreign antigens derived from the food we eat or cell walls of invasive or resident bacteria. When the structural integrity of the epithelial barrier is compromised by stress or other factors, antigens from the lumen may readily penetrate this barrier to trigger immune cells such as mast cells (1). Mast cell-derived products stimulate chloride secretion, which plays an important role in the barrier-compromised intestine by production of enteric tears necessary to flush the lumen of deleterious antigens.

Barrier function appears to be dependent on extrinsic primary afferents. Chloride secretion is enhanced by stimulation of these extrinsic primary afferents only if the submucosal plexus is intact, and this is probably a consequence of activating cholinergic secretomotoneurons that express NK₁ receptors (13). The mechanism can be explained by an axon reflex that releases substance P from collaterals of extrinsic primary afferents associated with submucosal ganglia (Fig. 3, A and B).

Bacterial toxins provide additional insights into the role of axon reflexes involving extrinsic primary afferents in mediating host-defense mechanisms. Toxin A produced by the bacterium, *Clostridium difficile*, provides an interesting example. After spores are ingested, this opportunistic pathogen colonizes the colon when the indigenous floras have been suppressed by antibiotic therapy. Release of its toxins causes pseudomembranous colitis in humans, a condition characterized by increased epithelial permeability, inflammation, tissue necrosis, fluid secretion, and diarrhea. In rats, toxin A activates extrinsic primary afferents that release substance P both centrally in the dorsal horn of the spinal cord, where it may be involved in nociception, and also in the small intestine, where it stimulates mast cells and enteric neurons (8). Release of substance P and mast cell activation are key events that precede inflammation associated with neutrophil recruitment, altered permeability, and fluid secretion. These key events and their sequelae are abrogated by ablation of extrinsic primary afferents (8). This is an example of neurogenic inflammation caused by activation of an axon reflex involving extrinsic primary afferents. The excess fluid secretion probably results primarily from decreased absorption due to villous cell damage in conjunction with increased production of enteric tears by crypt cells, the latter mechanism being due to activation of the intrinsic neural reflex circuits amplified by newly recruited inflammatory mediators acting either directly on neurons or epithelial cells (6). This mechanism ensures prolonged flushing of the unwanted foreign microbe and its noxious toxin. The intriguing question is what factor or mediator triggers the extrinsic primary afferents? Is it something produced by bacteria or by epithelial, immune, or endocrine cells? Furthermore, why does activation of capsaicin-sensitive extrinsic primary afferents not always result in neurogenic inflammation? These are intriguing questions indeed.

In vivo, the output of the enteric nervous system is modified by input from the brain via sympathetic and parasympathetic efferent fibers that terminate in the enteric ganglia. The role of parasympathetic input to the gut is not well documented, although early studies suggested that parasympathetic stim-
ulation causes secretion. On the other hand, sympathetic discharge, which releases norepinephrine, hyperpolarizes cell bodies of VIP secretomotoneurons, attenuates the release of VIP, and reduces chloride secretion. Cholinergic secretomotoneurons appear to lack \( \alpha_2 \)-adrenergic receptors necessary for sympathetic inhibition of neural secretory reflexes. This so-called “sympathetic brake” on VIP secretomotoneurons is often a target for inhibition by inflammatory mediators such as interleukins-1 and -6, tumor necrosis factor-\( \alpha \), and platelet-activating factor. With the sympathetic brake inhibited, chloride secretion is free to escalate given the appropriate stimulus.

The regulation of chloride secretion or enteric tears is a result of complex interactions between neurotransmitters released by the enteric nervous system and extrinsic primary afferents in conjunction with a myriad of chemical messengers from epithelial, endocrine, and immune cells. Clearly, in the healthy individual, the bowel contents will provide the mechanical stimulus for activating submucosal neural reflexes and a low level of chloride secretion necessary for lubrication. During disease states, multiple mechanisms are targeted to maximize chloride secretion by exciting intrinsic and extrinsic neural reflex circuits and enhancing epithelial responsiveness. These are mostly designed to help flush out the intestinal contents in an attempt to preserve the epithelial barrier between the outside world and the interior of the host organism. In pathological conditions, flushing is often further facilitated by luminal fluid accumulation as a consequence of inactivation of other ion transport mechanisms as well. The challenge of the future is to understand the role of these diverse substances in integrated responses during health and disease.

**NOTE ADDED IN PROOF**

Since the writing of this article, a preliminary report suggests that a subset of cholinergic neurons contains calcitonin gene-related peptide. These neurons are thought to be a second type of afferent neuron in the submucosal plexus (7a).