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Gastrointestinal Physiology IV

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Preface

The physiology of the gastrointestinal tract is extraordinarily diverse, embracing major sub-branches of physiology such as smooth muscle, autonomic nervous system, epithelial transport, exocrine secretion, and endocrine secretion, as well as digestion, absorption, and motor control. Reviews appearing in the preceding volumes in this series have covered most of these topics, and the present volume is intended to supplement the earlier ones in the series by the introduction of two new topics in the chapter "*Brunner's Glands,*" by I. M. Lang and M. F. Tansy, and the chapter "*Formation and Metabolism of Chylomicrons,*" by T. G. Redgrave. In addition, an integrative chapter, "*Stimulus Secretion Coupling in Mammalian Salivary Glands,*" by D. V. Gallacher and O. H. Petersen, has been included to broaden the coverage of this subject as compared with that attempted in the previous volumes and, although the chapter is limited in scope to salivary glands, it should provide insight into the process of stimulus-secretion coupling in all exocrine glands. The remaining two chapters, "*Gastric Acid Secretion in Response to Food,*" by M. Mignon, J. Vatier, A. Ruskoné, M. Merrouche, and S. Bonfils, and "*Immune System of the Gastrointestinal Tract,*" by G. McCaughan and A. Basten, have been included because of rapid expansion of knowledge in these particular areas, and aim to provide new perspectives. As mentioned by the editor of the previous volume in the series (*Gastrointestinal Physiology III*), many other topics, including comparative physiology, cell renewal and mucosal growth, and iron transport, still await review, and the rapid expansion of our knowledge of the gastrointestinal hormones will soon necessitate a recapitulation of advances in that area.

J. A. Young

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Salivary glands are compound organs that secrete electrolytes and protein as an aqueous fluid (saliva) into the oral cavity. The glandular tissue is comprised of specialized groups of cells, acinar cells, arranged as endpieces surrounding a small central lumen that opens into a narrow ductule, the striated or intercalated duct (Figure 1). These fine ducts open in turn into a branching system of larger ducts that ultimately converge into a main excretory duct, which drains into the oral cavity. Salivary glands are characterized by a structural heterogeneity that is manifest not only between different species but also between the major glands in any one animal. This structural heterogeneity is associated with functional diversity, as seen by the marked variation in the end product saliva from different species in response to an equivalent stimulus. Salivary secretion is a reflex response, controlled by both parasympathetic and sympathetic secretomotor nerves. The important physiologic stimulus for secretion is the presentation and ingestion of food; the quantity and quality of the secretion vary with the nature of the food. Salivary function has thus developed to subserve the particular dietary habits of the individual species. As John Young stated (188), "as one might expect the findings are as divergent from one another as are the appearances and behavior of the individual species."

In the first half of this century it was the stimulus-evoked end product saliva that was most extensively studied in the investigation of the mechanism of salivary secretion. No standard experimental model was adopted, and so profound were the species variations that no general concept of secretion could be developed that would stand extrapolation between the species. In 1954, however, Thaysen and colleagues (177; see also 178) advanced a hypothesis that ascribed separate roles to the acinar and ductal components in salivary secretion. This was a two-stage model of secretion in which the acinar cells, or endpieces, secreted an isotonic plasmalike primary fluid that was subsequently modified in its passage along the ductal system by secretion or reabsorption of electrolytes. The development of micropuncture techniques has made it possible to sample endpiece fluid, and the near-isotonic and constant composition of the primary fluid has been confirmed in a number of species (see 188, 190). This was the first unified concept of secretion.

An important consequence of this hypothesis is the knowledge that one group of cells, the acinar cells, is responsible for the secretion of the isotonic fluid. This realization then made it possible to investigate secretory mechanisms at a cellular level rather than in the intact organ system. At the same time that this concept was evolving a number of advanced techniques were being developed in the investigation of excitable cell systems, and these were adapted and applied in the study

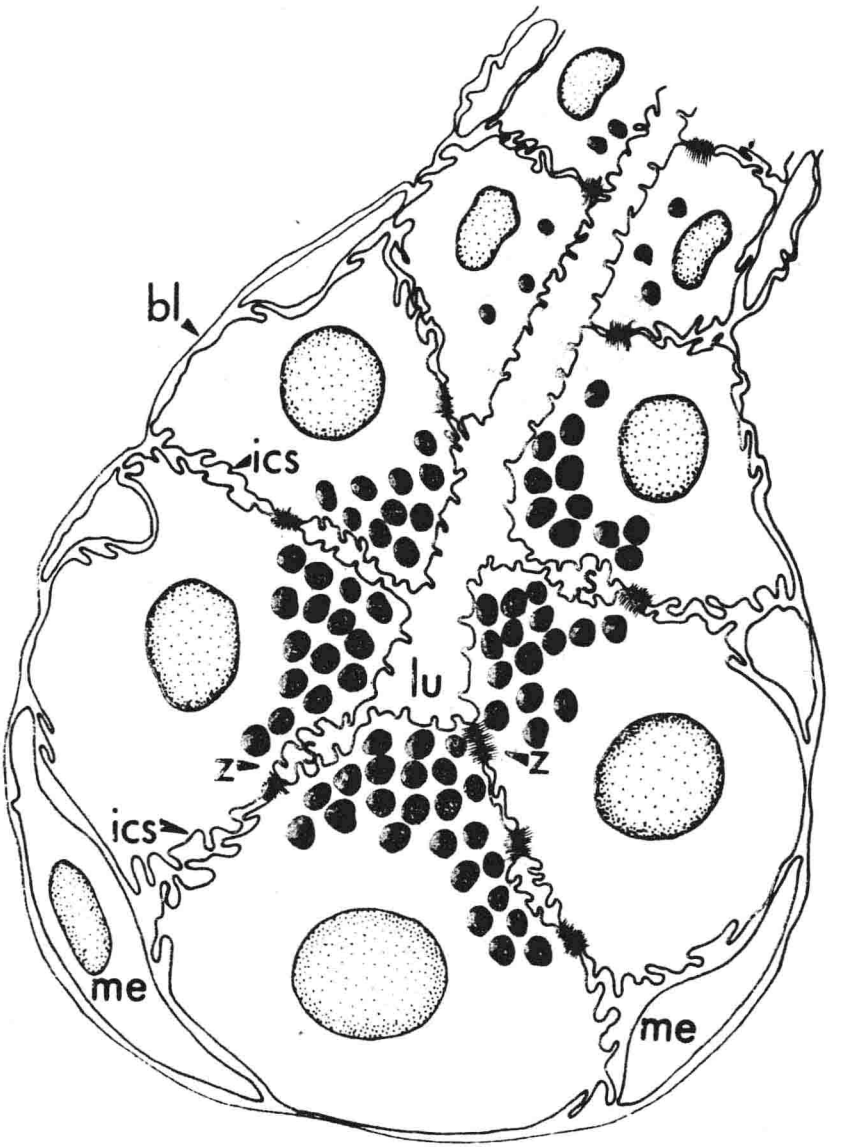

Figure 1. Schematic reconstruction of an endpiece of a typically serous gland such as the parotid, showing secretory canaliculi (*s*) opening into the lumen (*lu*). The canaliculi abut tight junctions (*z*) that separate them from the lateral intercellular spaces (*ics*). The canaliculi, in contrast to the intercellular spaces, do not surround the cell on all sides and are seen only occasionally in any particular section. The adjacent acinar cells are coupled by gap junctions (not shown) that permit transcellular exchange of ions and small molecules, including cyclic nucleotides. The functional and electrical unit is therefore the acinus rather than the individual acinar cells. In addition to the secretory cells, myoepithelial cells (*me*) are shown. Reprinted by permission from: Young, J. A., and Van Lennep, E. W. (1978). Morphology of Salivary Glands. Copyright © (1978) Academic Press Inc. (London) Ltd.

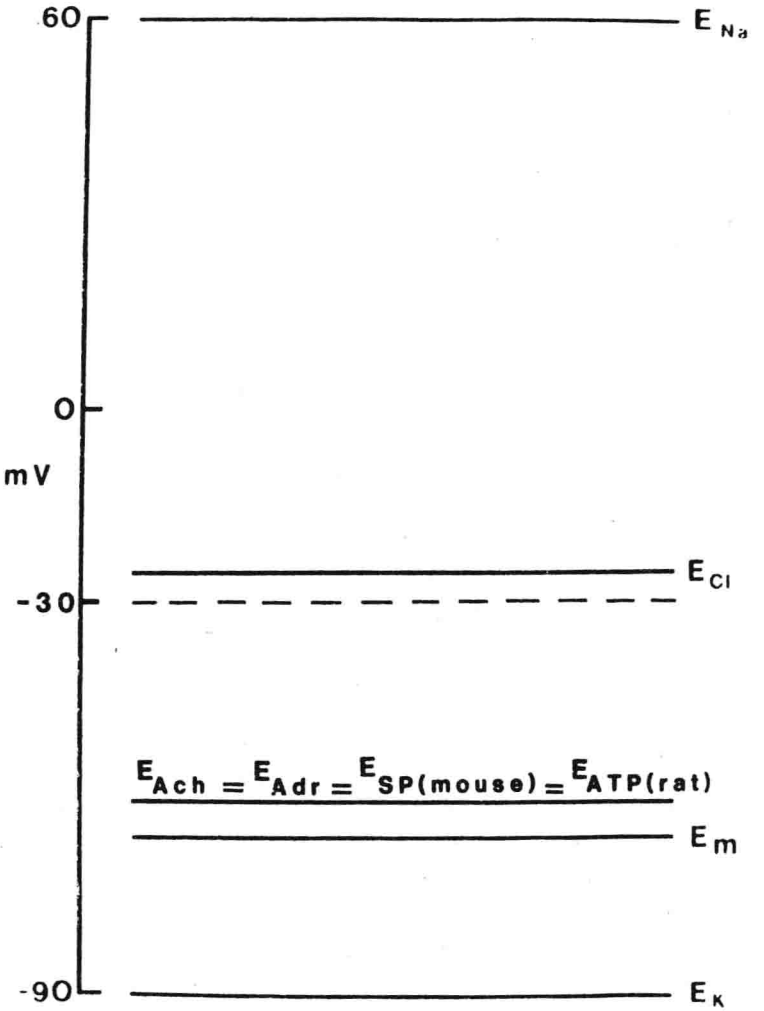

Figure 2. Rodent parotid acinar cell. Approximate values are shown for the sodium, potassium, and chloride equilibrium values. The true resting membrane potential is indicated (E_m) and the resting potential as determined in the early studies is shown by the broken line. The equilibrium or reversal potential of the stimulus-permeability–coupled agonists is also shown. All four receptor types have not to date been demonstrated in any single species and the figure is a composite of rat and mouse parotid glands. Reprinted by permission from: Petersen, O. H. (1980). Electrophysiology of Gland Cells. Monographs of the Physiological Society. No. 36. Copyright © (1980) Academic Press Inc. (London) Ltd.

concentrations of glandular tissue after stimulation of the parasympathetic nerves to the gland. He also investigated the effects of ion substitution on the stimulus-induced secretory potentials and the simultaneous secretion of saliva. It was reported that stimulation was associated with a loss of potassium and an uptake of

sodium into the glandular tissue. This was reflected in the composition of the perfusate effluent, in which there was an increase in the concentration of potassium and a decrease in the concentration of sodium. After cessation of stimulation there was, as described by Burgen (17) for potassium, a period of reequilibration, i.e., potassium uptake and sodium loss. Imai used a bridge balance circuit that enabled him to record membrane potential and input resistance via a single intracellular microelectrode. He observed, as had Lundberg (104), that the secretory potentials were associated with an increased basolateral membrane conductance. More importantly, ion substitution experiments seemed to indicate that neither the secretory potential nor secretion were abolished when chloride in the perfusate was replaced by sulfate. However, both the secretory potential and secretion of fluid showed a marked dependence on the extracellular potassium concentration. Imai concluded that the stimulus-induced hyperpolarizations were not due to active anion transport but were more readily explained by an increase in passive membrane permeability to potassium.

Schneyer and Yoshida (164) recorded secretory potentials from rat submandibular gland in vivo following nerve stimulation. They reported that these secretory potentials were often observed to be depolarizing in nature. Unlike Lundberg (102), they did not consider the depolarizing responses to be from nonacinar cells. These authors concluded that more than one ion species was involved in acinar cell activation and that the form of the secretory potentials was determined by the algebraic balance of ion movements at any time. Petersen and Poulsen (134) and Petersen (126,127) investigated the effects of ion substitution on both secretion and secretory potentials. These studies confirmed the finding of Imai that secretory potentials were not abolished in the absence of extracellular chloride. Secretion was totally abolished if chloride was replaced by the impermeant anion sulfate, but only reduced if nitrate was substituted for chloride. Petersen demonstrated that these processes showed a marked dependence of both extracellular sodium and potassium concentrations, and suggested that acinar cell activation was associated with changes in passive membrane permeability to both sodium and potassium. The poststimulus reuptake of potassium and extrusion of sodium (17, 83) he suggested was due to activation of an active sodium/potassium pump. Sodium/potassium ATPase activity had been demonstrated in salivary glands (161, 162; see also 73). In 1971 Petersen (129, 130) demonstrated that the cardiac glycoside strophanthin G (ouabain) abolished this reuptake without affecting the stimulus-induced potassium efflux (Figure 3), confirming that the reuptake process was an active mechanism.

In 1973 Petersen (131), using isolated superfused segments of mouse parotid and submandibular glands, found that the resting membrane potentials were higher than previously reported. He observed that the form of the secretory potential evoked by application of exogenous acetylcholine showed a marked dependence on transmembrane potential. At the lower resting potential the cells exhibited hyperpolarizing secretory potentials, but those cells with high resting potentials responded with biphasic potential changes, the initial component of which was a

followed the initial depolarization of the biphasic secretory potentials was a manifestation of an electrogenic sodium/potassium pump.

It is apparent then that the early electrophysiologic studies had not revealed the true resting membrane potentials of the acinar cells. Higher resting potentials were recorded as electrophysiologic technique improved, and it was seen that the secretory potential could present itself as a biphasic potential change. The initial phase of the potential response was associated with a profound increase in membrane conductance. An increase in potassium permeability alone could not explain the depolarizing responses. The dependence of the secretory potentials on both extracellular potassium and sodium and the demonstration that stimulation in the organ system was associated with net potassium loss and net gain of sodium were convincing evidence that activation was associated with an increased membrane permeability to both sodium and potassium, resulting in potassium efflux and sodium influx across the acinar membrane. The nonreversing delayed hyperpolarization, most obvious at higher potentials when the initial response was a depolarization, could be explained as resulting from activation of the electrogenic sodium/potassium pump, which acts to achieve reuptake of potassium and extrusion of sodium in the poststimulus period. Figure 5 (99) is an elegant demonstration of the reciprocal nature of sodium and potassium transport to and from the glandular perfusate.

It had therefore taken almost two decades to establish that the original interpretation of Lundberg that the secretory potentials were due to activation of an active chloride pump was incorrect. The crucial experiment was perhaps the apparent but mistaken demonstration by current injection that the secretory potentials were independent of transmembrane potential. The explanation for this lies most probably in the technique employed to achieve current injection, i.e., double-barreled microelectrodes. Lundberg (104) reported that the coupling resistance between these electrodes commonly changed during the course of experiments. Although such measurements were discarded when obvious, it is likely that the findings of Lundberg can be explained by the development of potentials across these electrodes, and the recorded potentials would not therefore reflect true transmembrane electrical gradient. It was primarily this observation of Lundberg combined with the low resting potentials of the early electrophysiologic studies that led people to accept only with caution the mounting evidence of an increase in passive membrane permeabilities to potassium and sodium.

Development of the Isolated, Superfused Salivary Preparation

The electrophysiologic experiments detailed in the previous section were the first investigations of secretory mechanisms in terms of cellular systems as opposed to organ system. The demonstration by Petersen (131) and Nishiyama and Petersen (119) that the secretory potentials evoked by application of exogenous agonists in superfused isolated salivary gland segments were the same as those evoked in vivo or in perfused organ systems following nerve stimulation (89) freed the investigator from the restraints of the intact organ system. Moreover, the isolated

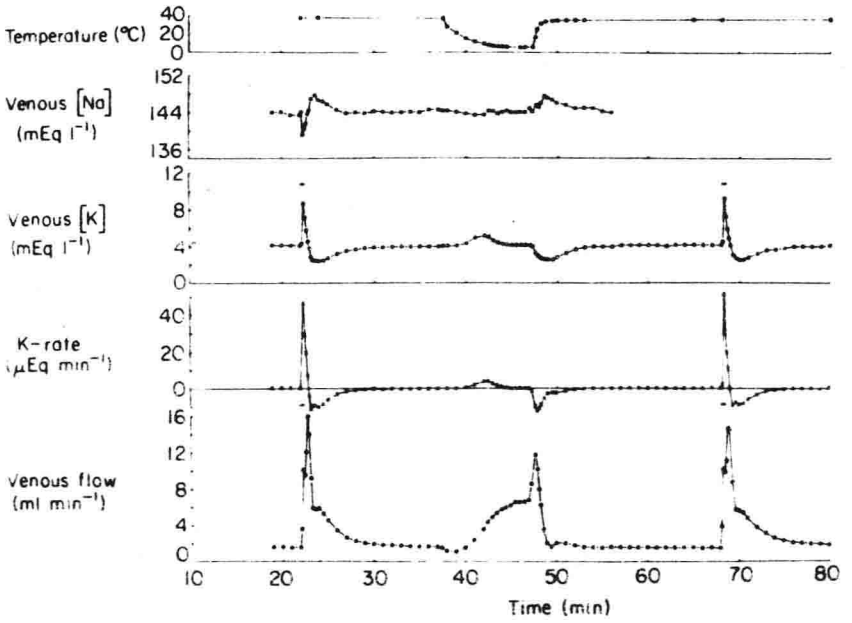

Figure 5. Isolated perfused cat submandibular gland. The effect of acetylcholine and cooling on potassium metabolism. K-rate is the rate of loss of potassium to the perfusion fluid; uptake is negative. The short horizontal bars indicate stimulation by acetylcholine (10^{-5}M). Reprinted by permission from: Laugeson, L. P., Neilsen, J. O. D., and Poulsen, J. H. (1976). Partial dissociation between salivary secretion and active potassium transport in the perfused cat submandibular gland. Pflügers Arch. 364:167–173.

preparation extended the range of experimentation possible, and isolated salivary glands (rodent parotid in particular) have been extensively utilized by researchers in many disciplines, both in the investigation of the mechanisms of secretion and as a model system in the investigation of receptor mechanisms in nonexcitable tissues in general.

Bdolah and Schramm (9, 10) had developed an in vitro superfused preparation of rat parotid slices in the investigation of amylase release (see later sections). This isolated slice preparation, similar to the isolated parotid segments, provides a near-homogeneous tissue composed of some 80% of a single cell type (the serous acinar cell) and remains viable in vitro, as determined by intracellular ion concentrations, even after prolonged incubation (101). Batzri et al. (6) reported that measurable potassium release (measured by ion-selective electrodes or atomic absorption spectrophotometry) could be evoked from the parotid slice preparation by application of exogenous agonists. The usefulness of this preparation in the investigation of ion fluxes was greatly extended when Putney (143) reported that rubidium could substitute for potassium in this tissue and that, by preloading acinar cells by incubation with trace amounts of ^{86}Rb and monitoring the rate of efflux of the radioligand, one could indirectly monitor the changes in membrane permeability to and movement of potassium. Similar protocols have