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Volume 28

Gastrointestinal Physiology IV

Edited by
J. A. Young, D. Sc., M. D., F. R. A. C. P.

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Consultant Editor's Note

The first volume of the *International Review of Physiology* appeared in 1974, and since that time this new review series has become an important part of physiological literature. One of its most important purposes is to provide a comprehensive learning source for teachers and students of physiology throughout the world.

To explain the reasons for beginning this new publishing venture, we need to repeat once again the philosophy, the goals, and the concept of the *International Review of Physiology*. This Review has the same goals as all other reviews for accuracy, timeliness, and completeness, but it also has policies that we hope and believe will add important qualities often missing in reviews, especially integration of physiological mechanisms and instructiveness. To achieve these goals, the publishing format provides for 1200 to 1500 pages per year, divided into physiological subspecialty volumes organized by experts in their respective fields. This extensive coverage allows consideration of each subject in depth. And to make the review as timely as possible, a new volume in each area of physiology is normally published every two years. In addition, occasional volumes will be published at appropriate times on such topics as the liver, the eye, and other physiological subspecialty areas that might not warrant a new volume every two years.

To help in achieving the goals of the *International Review of Physiology*, special editorial policies have been established. A simple but firm request is made to all authors that they utilize their expertise and judgement to sift from the mass of publications those new facts and concepts that are important to the progress of physiology; that they make a conscious effort not to write a review consisting of an annotated list of references; and that the important material that they choose be presented in thoughtful and logical exposition, complete enough to convey full understanding as well as being woven into context with previously established physiological principles. Hopefully, these processes will bring to readers a series of treaties that they will use not merely as a reference but also as an exercise in refreshing and modernizing their whole store of physiological knowledge.

Since publication of Volume 20 of the *International Review of Physiology*, I have reduced substantially my active participation in the selection and review of the material published in these volumes, excepting of course, the Cardiovascular Physiology volumes for which I serve as volume editor as well as series editor. The study of physiology, like the physiological literature itself, now encompasses so many diverse areas that only an established specialist in a given field can presume to pass upon the merits of this or that contribution. Accordingly, I feel that the interests of the *International Review of Physiology* and its readers are better served by relying upon the expertise of the individual volume editors, whose selection is made by the publisher, subject to my approval in each instance, and to whom I continue to offer my support and advice whenever needed.

Arthur C. Guyton

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

Publisher's Note

The *International Review of Physiology* remains a major force in the education of established scientists and advanced students of physiology throughout the world. It continues to present accurate, timely, and thorough reviews of key topics by distinguished authors charged with the responsibility of selecting and critically analyzing new facts and concepts important to the progress of physiology from the mass of information in their respective fields.

Following the successful format established by the earlier volumes in this series, new volumes of the *International Review of Physiology* will concentrate on current developments in neurophysiology and cardiovascular, respiratory, gastrointestinal, liver, endocrine, kidney and urinary tract, environmental, and reproductive physiology. New volumes on a given subject generally appear at two-year intervals, or according to the demand created by new developments in the field. The scope of the series is flexible, however, so that future volumes may cover areas not included earlier.

University Park Press is honored to continue publication of the *International Review of Physiology* under its sole sponsorship beginning with Volume 9. The following is a list of volumes published and currently in preparation for the series:

Volume 1: **CARDIOVASCULAR PHYSIOLOGY** (A. C. Guyton and C. E. Jones)
Volume 2: **RESPIRATORY PHYSIOLOGY** (J. G. Widdicombe)
Volume 3: **NEUROPHYSIOLOGY** (C. C. Hunt)
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Stimulus-Secretion Coupling in Mammalian Salivary Glands

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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

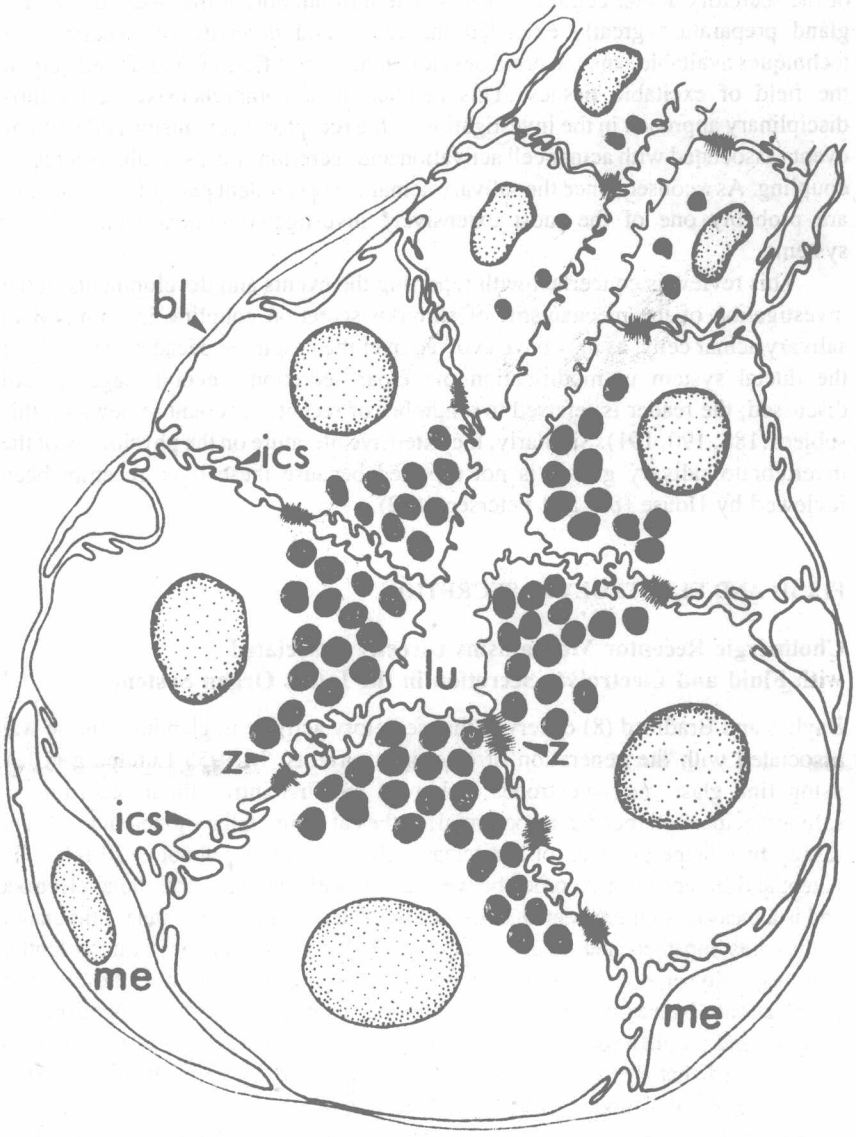

Figure 1. Schematic reconstruction of an endpiece of a typically serous gland such as the parotid, showing secretory canaliculi (*s*) opening into the lumen (*lu*). The canaliculi abut tight junctions (*z*) that separate them from the lateral intercellular spaces (*ics*). The canaliculi, in contrast 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.

of the secretory acinar cellular systems. The introduction of the isolated salivary gland preparation greatly extended the range and diversity of experimental techniques available, and the rapid developments in this field are paralleled only in the field of excitable tissues. This resulted in a comprehensive and multidisciplinary approach in the investigation of the receptor mechanisms and cellular events associated with acinar cell activation and secretion, i.e., stimulus-secretion coupling. As a consequence the salivary acinar tissues, rodent parotid in particular, are probably one of the most extensively investigated nonexcitable cellular systems.

This review is concerned with reporting the events and developments in the investigation of the mechanisms of stimulus-secretion coupling in mammalian salivary acinar cells, as they have evolved over the past three decades. The role of the ductal system in modification of acinar secretion (second stage) is not discussed; the reader is referred to a number of recent, excellent reviews on this subject (188, 190, 191). Similarly, the extensive literature on the physiology of the invertebrate salivary glands is not reported because these have recently been reviewed by House (80) and Petersen (132).

FLUID AND ELECTROLYTE SECRETION

Cholinergic Receptor Mechanisms Directly Associated with Fluid and Electrolyte Secretion in the Intact Organ System

Bayliss and Bradford (8) observed that secretory activity in glandular tissue was associated with the generation of electrical currents. In 1955 Lundberg (102), using fine glass microelectrodes, achieved the first intracellular recording of salivary acinar cell membrane potential, in the cat submaxillary gland in vivo. The resting membrane potential of the acinar cells was typically about -20 mV. The potential difference was recorded between an intracellular and extracellular electrode and is a measure of the potential across the basolateral acinar membrane. Stimulation of the parasympathetic nerves to the gland resulted in a change in membrane potential, seen as a hyperpolarization of about 20 mV. Lundberg termed these hyperpolarizations "secretory potentials." These secretory potentials were abolished by atropine and are therefore mediated by muscarinic cholinergic receptors. Lundberg also reported that there were some cells characterized by a much higher resting membrane potential (about -80 mV). The response in these cells to stimulation of the parasympathetic nerves was a depolarization. Lundberg concluded that these cells were nonacinar, most probably ductal cells. This study had then established that in salivary glands, as in excitable tissues, neurotransmitter activation of the effector cells was associated with the generation of transmembrane electrical currents. These electrical currents, generated by movement of ions across the plasma membrane, assume particular significance in epithelia associated with electrolyte and water transport because by observing these stimulus-induced electrical currents one is monitoring the transmembrane ion fluxes that are most probably associated with the net solute transport of secretion.

In subsequent electrophysiologic studies on the cat sublingual gland in vivo, Lundberg (103–105) recorded only the low "acinar" membrane potentials (about −30 mV). Again the stimulus-evoked secretory potentials were hyperpolarizing in nature. In one study Lundberg (104) used double-barreled microelectrodes: one barrel was the recording electrode and the second was used to inject current into the cells. By monitoring the amplitude of electronic potentials generated by injection of current pulses, a measure of the input resistance of the membrane was possible. Lundberg reported that the secretory potentials of the sublingual acinar cells were associated with only a modest reduction in membrane resistance, i.e., a slight increase in membrane conductance. Using the current injection facility Lundberg investigated the dependence of the secretory potentials on the transmembrane potential difference. By injecting direct current the resting membrane potential could be altered. In this study the amplitudes of the secretory potentials were essentially independent of the transmembrane potential, i.e., they were hyperpolarizing at all levels of membrane potential.

Lundberg (106) determined the electrolyte composition of the sublingual gland and estimated the equilibrium (Nernst) potentials for sodium, potassium, and chloride as +29 mV, −97 mV, and −12 mV, respectively (see also Figure 2). None of these ions seemed to be in equilibrium at the resting potential of about −30 mV. The hyperpolarizing secretory response could then be explained by an increase in membrane permeability to potassium with a consequent efflux of potassium ions, by an active outward potassium transport, or by an active inward transport of anions. (The active transport mechanism for anions has to be proposed because the prevailing transmembrane electrochemical gradient favors an efflux rather than an influx of anions if there was simply an increase in passive membrane conductance for anions.) Burgen (17) had reported that stimulation of the parasympathetic nerves to the dog submandibular gland resulted in an efflux of potassium from the glandular tissue into both the venous blood and the saliva. Upon cessation of stimulation there was reuptake of potassium into the glandular tissue. Despite the evidence of Burgen that secretion was associated with an efflux of potassium, Lundberg rejected the possibility that the secretory potentials were due to an increase in passive membrane permeability because such a response should have shown a marked dependence on the transmembrane potential gradient, in contrast to the results of Lundberg's current injection experiments. The active outward transport of cations was rejected as unlikely because it would oppose secretion. Lundberg thus concluded that the stimulus-induced secretory potentials were due to activation of an active, inwardly directed transport mechanism for chloride ions.

Schneyer and Schneyer (163) determined the electrolyte composition of rat parotid and submaxillary glands. The equilibrium potentials for sodium, potassium, and chloride as estimated from these data for rat parotid are shown in Figure 2. They are in general agreement with Lundberg (106) in that none of these ions are in equilibrium at the resting membrane potential and again the intracellular chloride concentration is higher than can be explained by passive distribution of this ion.

Imai (82, 83; see also 187), using the perfused dog submandibular gland, monitored the electrolyte composition of the perfusate and intracellular ion

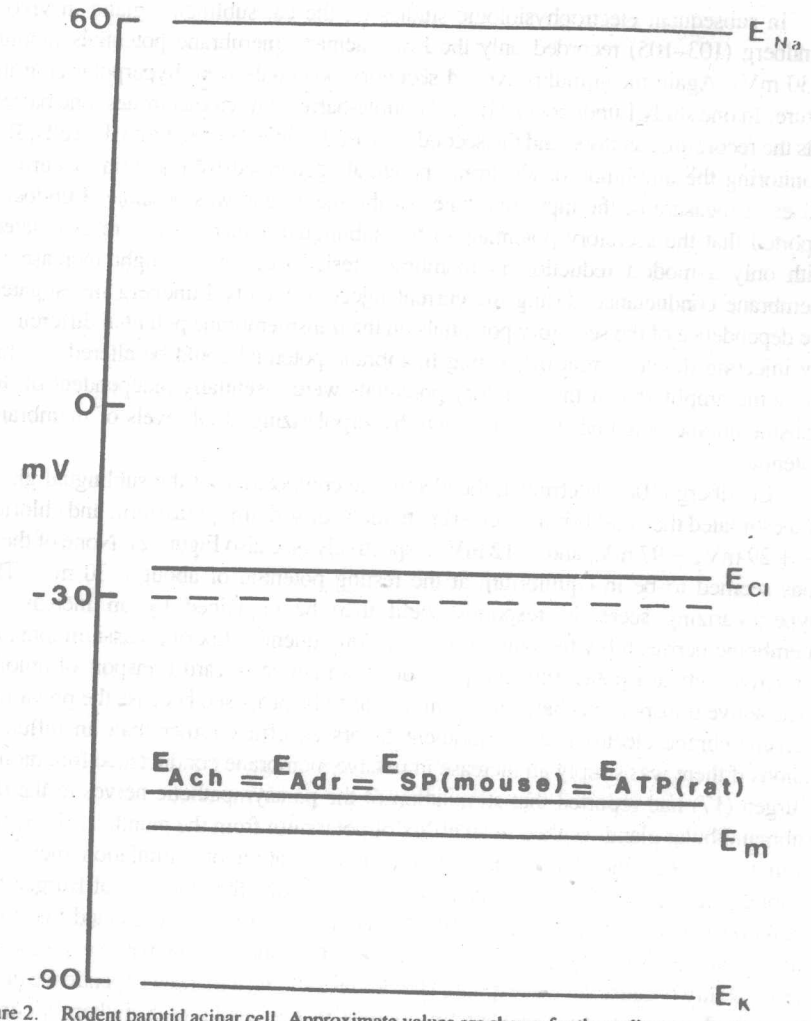

Figure 2. Rodent parotid acinar cell. Approximate values are shown for the sodium, potassium, and chloride equilibrium values. The true resting membrane potential is indicated (E_m) and the resting potential as determined in the early studies is shown by the broken line. The equilibrium or reversal potential 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

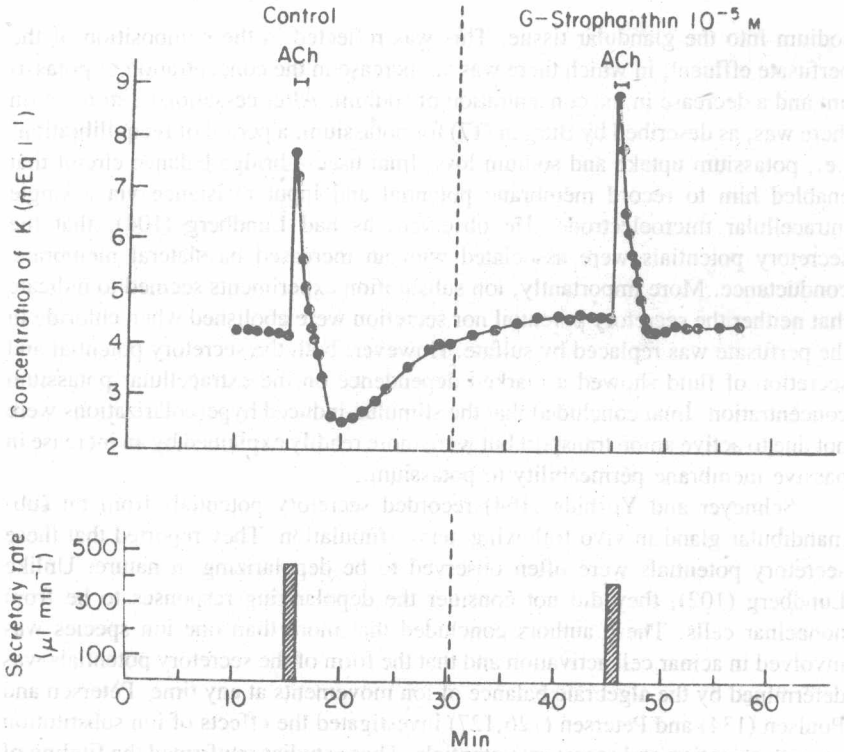

Figure 3. Isolated perfused cat submandibular gland. The effect of ouabain (G-strophanthin) on the acetylcholine-evoked changes in venous potassium concentration and salivary secretion is shown. Reprinted by permission from: Petersen, O. H. (1971). The ionic transports involved in the acetylcholine-induced change in membrane potential in acinar cells from salivary glands and their importance in the salivary secretion process. In: G. Giebisch (ed.), Electrophysiology of Epithelial Cells, pp. 207–221. Schattauer Verlag, Stuttgart.

depolarization, followed by a delayed hyperpolarization. Analysis of the amplitude and polarity of secretory potentials as a function of resting membrane potential in a population of cells indicated not only a marked transmembrane dependence of the initial potential change but a polarity reversal at about −50 mV (Figure 4). The delayed hyperpolarizing component of the biphasic responses did not undergo reversal, and Petersen suggested that it was an electrogenic manifestation of the activity of the sodium/potassium pump that operates to achieve ionic reequilibration in the period following the permeability changes.

Nishiyama and Kagayama (89, 118) simultaneously confirmed in rabbit and cat submandibular glands in vivo first that the resting membrane potentials were higher than had been revealed by earlier studies, and second, that the form of the secretory potential evoked by nerve stimulation was markedly influenced by the transmembrane potential in the manner described by Petersen (131). Nishiyama and Petersen (119), using isolated superfused preparations of mouse, cat, and rabbit submandibular glands, demonstrated the similar nature of the secretory

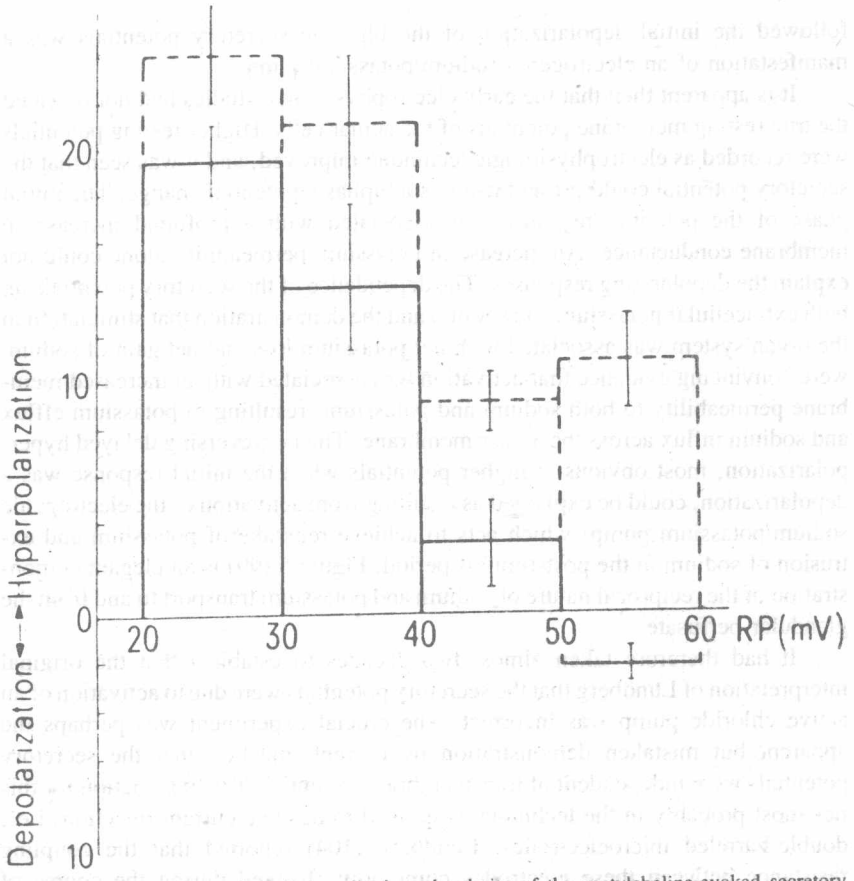

Figure 4. Histogram showing the amplitude and polarity of the acetylcholine-evoked secretory potentials at different levels of membrane potential (*RP*) in a population of cells in the mouse submandibular gland. The fully drawn columns represent the initial phase of the potential change and the columns outlined by the broken lines represent the second or delayed phase of the secretory potential. Reprinted by permission from: Petersen, O. H. (1973). Membrane potential measurements in mouse salivary gland cells. Experientia 29:160–161.

potentials evoked in each of these species by exogenous application of acetylcholine. These studies also demonstrated, using bridge balance current injection systems, that the secretory potentials were associated with a much more pronounced reduction in input resistance (i.e., increased conductance) than had been reported by either Lundberg (104) or Imai (83). In this study, mouse submandibular acinar cells were also loaded with sodium by superfusion with potassium-free media for several hours. Reintroduction of potassium resulted in a hyperpolarization of the membrane (about 30 mV) that was not associated with any change in input resistance. This was the first direct demonstration of the electrogenic capability of the sodium/potassium pump in this tissue, and was consistent with the proposal (131) that the nonreversing delayed hyperpolarization that

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