PHARMACOLOGICAL REVIEWS
Vol. 30, No. 2

Printed in U.S.A.

Stimulus-Permeability Coupling: Role of Calcium in the Receptor Regulation of Membrane Permeability*

JAMES W. PUTNEY, JR.

Department of Pharmacology, Wayne State University School of Medicine, Detroit, Michigan

I. Introduction

A characteristic effect of agents acting on surface membrane receptors is an alteration in plasma membrane permeability. Because the locus of both receptor and effect are similar, it has been traditionally assumed that the receptors and permeability pathways are intimately associated in the membrane at the molecular level; one possibility is that the receptor and the structures forming the ion channels (i.e., the ionophore) exist as a complex. In such a scheme the number of reactions between receptor activation and the resulting permeability change are kept to a minimum, thus decreasing the likelihood of intervening control mechanisms. Undoubtedly, there are systems where simple, membrane-level coupling between receptor and ionophore occur. It may be that the nicotinic receptor at the vertebrate neuromuscular junction functions in this way.

Recently, evidence has been rapidly ac-

* Work from the author's laboratory was supported by Grants DE-04067, EY-01978, and GM-07176 from the National Institutes of Health and a grant from the Cystic Fibrosis Foundation.

cruing to suggest that receptor control of membrane permeability can also occur by more complex mechanisms. It now seems likely that in a number of tissues membrane permeability responses may be mediated by the actions of second messengers—most frequently, calcium. In other words, the primary effect of receptor occupation may be activation of Ca influx or release with a number of secondary permeability changes following, which are mediated by the rise in cytosolic Ca. The analogy with the concepts for control of contraction (excitation-contraction coupling, 33, 244) and secretion (stimulus-secretion coupling, 239, 240) suggests the term *stimulus-permeability coupling* to denote the sequence of events involved in these response mechanisms. Indeed, this analogy provides more than semantic convenience. It is becoming increasingly apparent that much of our accumulated understanding of contractile and secretory systems is also applicable to dissecting out the various steps involved in the permeability responses.

This review, therefore, will deal largely with systems in which drugs, hormones, or neurotransmitters affect membrane permeability and for which evidence is available for a role of Ca (or other coupling factor) in the response. Information concerning all of the steps linking agonist action to membrane permeability changes will be analyzed. Specifically, these steps include: 1) receptor occupation; 2) coupling of receptors to Ca channels or stores; 3) Ca influx or release; and 4) activation of secondary permeabilities or ion channels (usually K) by intracellular Ca. In a few cases, hypotheses as to the molecular nature of these processes will be considered. One such hypothesis that has been generally applied to such systems is Michell's suggestion that phosphatidylinositol breakdown may be involved in Ca channel activation by receptors.

Less emphasis will be placed on those systems in which control of membrane permeability by Ca is well documented but not primarily regulated by membrane receptors. In this category would fall most excitable tissues in which membrane potential is the controlling factor and also the erythrocyte, where the "physiological" mechanisms for control of membrane permeability are not known. Some discussion of these areas will be included in developing the historical basis for the field and, especially for the red cell, in defining basic principles relevant to other tissues. Two recent reviews emphasizing the control of membrane permeability by Ca in excitable cells have appeared (182, 183). A number of reviews have dealt with other specific aspects of this discussion from somewhat different perspectives (25, 59, 64, 74, 126, 144, 145, 174, 187, 209, 221, 225, 228, 234, 235, 248, 249, 254, 268).

II. Historical Development

Probably the earliest observations of a modification of membrane permeability by Ca were made on energy-depleted red cells. Poisoning by lead (122) or fluoride (78, 283) leads to a sizable K loss from the cells. The early interpretation was that the formation of some abnormal glycolytic intermediate might be involved in controlling membrane permeability and a good deal of research effort was channeled in this direction (174, 205, 284).

It was Gardos (104–106) who, by using chelating agents, first determined that Ca was an essential ingredient for the K permeability effect. In recognition of this pivotal observation, the activation of K permeability by Ca is often referred to as the "Gardos effect." It was Whittam (281), however, who first explained these observations by proposing that membrane permeability to K was in part controlled by the intracellular concentration of ionized Ca. The results of more recent experiments using ionophores (such as A-23187) or resealed ghosts have been largely confirmatory (174).

At about the same time that the Ca theory for the red cell was gathering support, Krnjevic and coworkers observed an increase in K conductance of cortical neurons in which oxidative phosphorylation had

been uncoupled by dinitrophenol (108, 109). Subsequently, they found that microinjection (iontophoretic) of Ca similarly increased K conductance and reasoned that this was the underlying mechanism for the effect of dinitrophenol (165). Almost simultaneously, Meech (181) and Meech and Strumwasser (184) demonstrated that pressure injection of Ca into invertebrate (*Aplysia*) nerve cells increased membrane K conductance. Recently, evidence has appeared suggesting that intracellular Ca may activate K conductance in the heart and may thus serve to regulate phase II of the cardiac action potential (17, 133–137, but see 193).

Concurrently, evidence that receptor-mediated changes in K permeability might also be due to changes in intracellular Ca began to appear. Schramm, Selinger, and coworkers observed that alpha-adrenergic activation of parotid slices stimulated a loss of cellular K and that Ca was required for this effect (18, 19). Later, they demonstrated that K release could also be obtained with a divalent cationophore, A-23187, and concluded that receptor activation must first stimulate Ca influx, which in turn triggers the increased K reflux (256).

These latter studies were important in demonstrating that intracellular Ca can control membrane permeability to K not only in excitable cells, but also in cells under control of autonomic receptor mechanisms. More importantly, however, they raised the issue of potential ubiquity of the Gardos effect; previously, the phenomenon was suspected of being a "red herring," characteristic only of "dying" erythrocytes. Consequently, investigators examining control of membrane permeability in a number of tissues began to invoke and test hypotheses that included Ca ion as a primary controlling factor. The results obtained suggest that such control systems are certainly widespread if not ubiquitous. In the next section, some of the situations are discussed in which there is evidence to suggest that intracellular Ca may be involved in regulation of permeability.

III. Specific Systems

A. The Red Cell

A number of reviews that address the nature of the Ca-regulated K permeability in the red cell have appeared recently (126, 127, 171, 172, 174). This review will concentrate on those aspects especially amenable for comparison with the receptor-controlled processes to be discussed later.

As mentioned above, the Gardos effect in the red cell was the first disclosure of the ability of internal Ca to regulate membrane permeability to K. However, despite some reasonable conjecture (15), the role of this mechanism in the normal functioning of the erythrocyte is still unknown. The result of this situation is that the mechanism can only be studied through rather "artificial" perturbations of cellular Ca. The varied nature of these methods has, as might be expected, led to some conflicting theories as to the basic mechanisms involved. Some discussion, therefore, of the procedures and results obtained seems warranted.

The earliest method employed for elevating intracellular Ca in red cells involved inhibition of active Ca extrusion. Since no specific inhibitors of this process are known, Ca pumping was prevented by depletion of the metabolic fuel for active transport, ATP. A glycolytic inhibitor, iodoacetic acid, and a metabolic substrate, adenosine (or inosine or glucose), are synergistic in this regard and reduced cellular ATP from 10^{-3} M to 10^{-6} M in 1 to 2 hours (172). Accordingly, after a short delay, a sizeable loss of K occurred. Experiments monitoring the time course of ATP depletion, Ca influx, and K loss suggested that it is the net increase in cellular Ca, rather than the decrease in ATP, which accounts for the increased K permeability (174). For example, during ATP depletion, Ca influx may be varied by changing external K. Under such conditions it is the rate of Ca entry, rather than ATP depletion, with which the rate of K loss is best correlated (170).

This is not to say that the Ca-mediated

K flux may not be affected by the loss of cellular ATP. Evidence described below suggests that ATP may serve to modulate the sensitivity of the K channel to Ca.

Resealed ghosts, used so successfully as tools for studying the Na,K pump, have not been extensively employed in studying the Ca-K interaction. One particularly detailed study has been described by Simons (260, 261). Resealed ghosts were prepared containing one of several Ca buffers so that intracellular Ca could be controlled over a wide concentration range. In order to prevent rapid extrusion of Ca, the ghosts were first depleted of ATP. With this preparation, Simons found that the kinetics of Ca activation of the K channel were complex. The simplest model called for two high affinity sites for activation and a lower affinity site that was inhibitory (260, 261). In agreement with an earlier report by Porzig (213), K efflux was activated half-maximally by about 0.4 μM intracellular Ca and was maximal around 1 to 5 μM. Besides K, Rb was transported similarly, Cs slightly, and Na not detectably. Sr, at higher concentrations, could substitute for Ca. The K efflux mechanism appeared passive, being linearly related to intracellular K (except at low concentrations). The total span of the Ca-sensitive K efflux was slightly less than two orders of magnitude, the rate constants ranging from 0.14 hr^{-1} to about 3.8 hr^{-1}.

These data provide for some interesting comparisons with the results obtained by Lew and Ferreira (173, 174) using the divalent cationophore A-23187 to control intracellular Ca. These investigators measured total cell Ca under conditions of varying ionophore and external Ca concentrations and were able to calculate (and thus control) the intracellular ionized Ca concentration over a wide range (90). With this method, the rate constant for ^{42}K equilibration under near steady-state conditions (activation of K exchange did not appear to desensitize with time) was measured as a function of intracellular Ca. When the concentration of ionophore was about 10 μM, the maximum increase in K permeability due to internal Ca was greater than that obtained with ATP-depleted cells or ghosts, spanning about three orders of magnitude (173). More importantly, the apparent K_m for activation of K flux by Ca was about 1 mM—much higher than previously reported (173). When the experiments were repeated with higher concentrations of ionophore (100 μM) or with ATP-depleted cells, a low K_m was obtained (μM range) similar to that reported previously with ATP-depleted cells or ghosts. In addition, the estimated K_m for Ca activation of the Ca pump (1 μM) agreed reasonably well with the earlier value obtained with resealed ghosts and was not affected by ionophore concentration (173). These observations indicate that the theoretical basis of the method by which internal Ca was determined is sound, and that the different affinities obtained for Ca activation of K flux represent real differences in sensitivity of the K channel to Ca. Lew and Ferreira (174) suggest that variations in the Ca sensitivity of the Ca channel may be of physiological significance, adding much versatility and independence to various functions responding to Ca as second messenger in the same cell. Some additional discussion of alteration in Ca sensitivity of the K channel is included in the section on salivary glands.

On first inspection, it is easy to criticize a proposal of a mechanism involving intracellular Ca with a K_m for Ca in the millimolar range. However, as Lew and Ferreira (173) point out, the magnitude of the Ca effect is so large that alterations of intracellular Ca in the micromolar range would still produce significant changes in membrane permeability to K. Also, as is discussed below, it is conceivable that in other cells where Ca permeability can be regulated by receptors or by membrane potential, Ca concentrations in the immediate vicinity of the surface membrane could be substantially higher than in more central regions of the cell.

Lew and Ferreira (173) also performed another experiment that may be relevant

to the mechanism of the altered sensitivity. In order to measure the Ca sensitivity of the K channel with no ionophore present, they first depleted cells of Mg in order to inactivate the Ca pump. Despite the maintenance of normal levels of ATP, this treatment also resulted in a condition of high Ca affinity. Alone, this observation could suggest that it is the activity of the Ca pump rather than the ATP level that controls the sensitivity of the K channel to Ca. This does not seem likely, however, in light of the observation that high ionophore concentrations do not affect Ca pumping (90) but do produce the high affinity state (173). Conceivably, some other ATPase activity could be involved (perhaps a membrane phosphorylation), thus explaining the requirement for both ATP and Mg. Obviously, further studies are required to determine the mechanism by which Ca sensitivity of these K channels is controlled.

As to the mechanism of the K channel activation itself, Lew and Beaugé (172) best summarized our current knowledge: "We know nothing about the actual nature of the K pathway but we disguise our ignorance with lively controversies." It is true that phenomena left unexplained are vacuums into which hypotheses are inevitably drawn. It is, therefore, not surprising that at least two proposed candidates for the molecular identity of the Ca-sensitive K channel have emerged.

The first is the suggestion by Hoffman and Blum that the K channel is an altered form of the Na,K pump (38, 126). This model was based largely on the partial inhibition of Ca-stimulated K efflux by inhibitors of the Na,K pump such as ouabain, oligomycin, furosemide, and tetraethylammonium. Lew (171), however, found that the effect of ouabain decreased as cellular ATP fell and reasoned that the cardiac glycoside, by inhibiting Na,K-ATPase, lessened the rate of decline of ATP and thus also lessened the rate of rise of intracellular Ca. However, inhibition could still be obtained when ouabain was present only during the K flux measurement (38, 126) and Lew (171) also found effects of ouabain on K flux with only barely detectable effects on ATP. Lew has raised the point, however, that small changes in the ATP content of red cells can drastically affect the K movements, not only by altering the accumulation of Ca, but also by affecting the sensitivity of the K channel to Ca (174).

Obviously, the issue is far from settled. Experiments using Lew's ionophore technique where ATP levels remain high might prove useful in disclosing an ATP-independent effect of ouabain. In the parotid gland (discussed later), ouabain does not affect the Ca-mediated K efflux, although the relevance of this observation would depend on the degree of similarity ascribed to the mechanisms in the parotid acinar cell and the erythrocyte.

Another possible mechanism by which Ca might activate an ion channel is through the stimulation of 1,2-diacylglycerol and phosphatidate synthesis that occurs when intracellular Ca increases in red cells (9). It is not surprising that Lew and Ferreira (173) were tempted to speculate about an association between these two membrane-localized, Ca-mediated phenomena: K channel activation and phospholipid breakdown. A theory similar in many respects has been put forth for smooth muscle by Abdel-Latif and is discussed later. In a recent study, Allan and Michell (7), using red cells from several species, measured the ability of the ionophore A-23187 and Ca to stimulate synthesis of diacylglycerol and phosphatidate, and to stimulate efflux of K. Cells from man, rat, guinea pig, and rabbit showed both the phospholipid and the K efflux effects, while cells from ox, sheep, chicken, and turkey showed neither effect. Red cells from the pig, however, showed no phospholipid effect but did demonstrate a Ca-mediated increase in K flux. The K loss was blocked by quinine, suggesting that the K permeability mechanism is similar in the pig cells to that in the more extensively characterized species.

It is important to emphasize that while these results argue against the increase in

diacylglycerol and phosphatidate as relevant to the K channel activation, they may not rule out the possibility entirely. The source of diacylglycerol and phosphatidate in the red cell may be polyphosphoinositides (8). Abdel-Latif et al. (2, 3, 5) have suggested that ion channel activation due to increased intracellular Ca in smooth muscle may result from breakdown of triphosphoinositide. In most tissues, the relative fraction of total membrane phospholipid represented by the polyphosphoinositides is low (2, 3, 49), and changes may thus pass undetected in measuring total phospholipid breakdown. Thus, as for the Na,K pump theory, the phospholipid theory must also remain an open question. It is hoped that continued investigations will resolve some of these controversies and help to explain the control of K channel activation in this important model system.

B. The Exocrine Glands

Appreciation of the exocrine glands as models for study of Ca and stimulus-permeability coupling mechanisms has developed almost entirely in the last 10 years. The permeability changes are of sufficient magnitude to ensure easy quantification. Also, the exocrine cells not only have the same advantage as does the erythrocyte in lacking voltage-dependent permeability parameters, but also afford the investigator the additional flexibility of studying any of several receptor mechanisms controlling Ca flux under physiological conditions (225). Some consideration of the functional role of ion movements will precede the discussion of Ca and control mechanisms.

1. Role of Ion Movements in Exocrine Gland Function. A number of theories have appeared from time to time attempting to explain the bulk transport of water by epithelial cells such as occurs in the exocrine glands. Berridge and Oschman (30) have outlined arguments against the theories of classical osmosis, active transport of water, and pinocytosis. The latter mechanism may be important in the transport of material by endothelial tissue (48, 158). Curran's studies of intestinal transport of water led to the development of a three-compartment double-membrane model that allowed for a "build-up" of osmotically active material in a central compartment delimited by membranes of different permeabilities (70–72, 159, 196). Kaye et al. (159), in studies of gallbladder ultrastructure, postulated that the central compartment might be the intercellular spaces. These observations and speculations were further refined by Diamond and Bossert (82, 83) and Diamond and Tormey (84, 85) into the *standing gradient hypothesis.* In this scheme, the intercellular spaces of epithelial tissues contain standing gradients of solute maintained by active transport. The apical end of the spaces contains the highest concentrations, which draw water osmotically from the cells. As the hypertonic solutions pass along the spaces, osmotic equilibration occurs, resulting in isotonic secretion.

It is beyond the scope of this review to detail the varied structural arrangements and models described in specific situations where the standing gradient hypothesis has been applied. The interested reader is referred to several recent reviews (30, 204, 251, 252). What is important to realize is that exocrine gland activity (with regard to fluid secretion) is regulated by alterations in membrane permeability to osmotically important electrolytes (Na, K, Cl, HCO_3), or by alterations in rates of active transport of these electrolytes, or a combination of these effects.

Because of the structural geometry of the exocrine glands, the locus of the standing gradients is not known with certainty, and thus models attributing varying degrees of significance to any of the major electrolytes or pumps can be constructed. At best, the ion fluxes detected in preparations in vitro, although probably functionally significant, are presently poorly understood as to their exact role in mediating water transport. These uncertainties, however, do not diminish the utility of these ion flux responses in the development of our basic understanding of control of membrane permeability by neurotransmitters and hormones.

2. The Salivary Glands—Receptors; Ca

and K Flux. The salivary glands have proved to be superb models for study of stimulus-permeability coupling mechanisms. The main hypotheses to be considered here are that 1) each of three independent receptors activates release of a common pool of Ca and activation of a common pool of Ca channels, 2) phosphatidylinositol breakdown may be involved in the Ca channel activation mechanism, 3) elevated intracellular Ca activates membrane permeability to K and Na, and 4) elevated intracellular Na may activate the Na,K pump. Various aspects and details of this overall scheme will provide a convenient model for discussion of similar mechanisms in other tissues.

Evidence that Ca may control transmembrane movements of other ions in the salivary glands has been available for some time. Basic studies of exocrine gland physiology were centered around the assumption that generation of transepithelial water flow must involve alterations in ionic permeability, transport, or both (56, 247). When Douglas and Poisner (86) showed that Ca was required for acetylcholine to stimulate salivary flow, the connection between Ca and ion flux was implicitly established. It was several years later, however, when Michael Schramm, Zvi Selinger and other members of the group at the Hebrew University in Jerusalem clearly demonstrated that Ca was an obligatory intermediate for agents affecting salivary cell membrane permeability to K (18–20, 248, 249, 254–256). These investigators found that agonists activating muscarinic or alpha-adrenergic receptors stimulated a sizeable net release of K from slices of rat parotid gland incubated in vitro. Stimulation of beta-adrenergic receptors, believed to cause exocytosis by a cyclic AMP-dependent mechanism, did not cause K release. The striking effect of agonists on K flux was an old observation (55). The novel contribution was the demonstration that this process (K release) was dependent on the presence of extracellular Ca. The ionophore A-23187 could also stimulate K release if extracellular Ca was present. Thus, they reasoned that activation of muscarinic or alpha-adrenergic receptors stimulated Ca influx and the elevated intracellular Ca acted to enhance membrane permeability to K (248, 249, 254). More recently, Rudich and Butcher (241) and Friedman and Selinger (96a) showed that a third receptor, activated by the undecapeptide substance P, produced a similar pattern of response. Subsequently, a number of investigators have demonstrated an enhancement of ^{45}Ca influx due to secretagogues in parotid cells and slices (153, 192, 219, 230). More recently, it has been shown that qualitatively similar mechanisms operate in the submaxillary (179, 180, 266) and sublingual (226) glands.

However, when Petersen and Pedersen studied the electrophysiological response of the parotid gland, they obtained a surprisingly divergent result (206, 210). These investigators obtained brief hyperpolarization (1–2 min) in response to muscarinic or alpha-adrenergic stimuli. The hyperpolarizations, which they reasoned resulted from enhanced K permeability, were not affected by removal of Ca even in the presence of a Ca chelating agent, ethylene glycol tetraacetic acid (EGTA).

Studies with isotopic techniques for measuring unidirectional efflux rates resolved this controversy. Figure 1 shows the response pattern obtained when efflux of ^{86}Rb from preloaded slices is monitored. The addition of a secretagogue to the incubation medium brings about a precipitous and immediate (<1 min) increase in isotope release [^{86}Rb and ^{42}K can be used almost interchangeably (218)]. This surge is somewhat transient, however, and in 2 to 4 minutes the efflux rate falls to a sustained* but still significantly elevated level. The important distinction suggesting two separate phases of the response is the pattern of Ca-dependency. When Ca is absent from the bathing medium, the later sustained phase is completely blocked while the early transient phase persists with only

* Actually, the "sustained" phase of the response in Figure 1 will eventually subside if the exposure to the agonist is maintained. This is discussed further later.

slight attenuation (218, Fig. 1). This pattern is obtained with any of the three receptor mechanisms that activate K efflux in the parotid (177, 218, 220, 221).

Thus, it seems likely that the transient Ca-independent phase of K release gives rise to the hyperpolarization observed by Petersen and Pedersen, while the Ca-dependent net loss of K observed by the Hebrew University group would correspond to the sustained Ca-dependent phase of release seen in the isotope efflux experiments.

What, then, is the mechanism of the Ca-independent, transient increase in membrane permeability† to K? Some insight into this question was obtained by studying the interactions of agonists acting on different receptors (muscarinic, alpha-adrenergic, substance P) in the parotid gland. It was found that in the absence of extracellular Ca (with EGTA present), only one transient response (K efflux) could be obtained (220). A second challenge failed to produce a response, even if an agonist acting on a different receptor was used. The term "cross receptor inactivation" was used to describe this phenomenon. The ability of the tissue to respond a second time could only be restored by an intermittent exposure to medium containing Ca (220).

Three conclusions were reached based on these results. First, it was suggested that the transient phase of the permeability response resulted from the receptor-mediated release of Ca from a pool inaccessible to EGTA (220). Second, it was suggested that this transient release of Ca might reflect the same molecular event as Ca channel activation, which results in the sustained phase of the response (220). In other words, perhaps bound Ca is released from the Ca channel when first activated. The only reasoning behind this proposal was the similarity in dose-response relationships for these two phases (218) and an attempt to begin with a somewhat conservative working hypothesis. The third suggestion, somewhat of a corollary to the second, was that since this pool of releasable Ca appeared accessible to all three receptors, then there must also be a single population of Ca channels that is regulated by all three receptors (220).

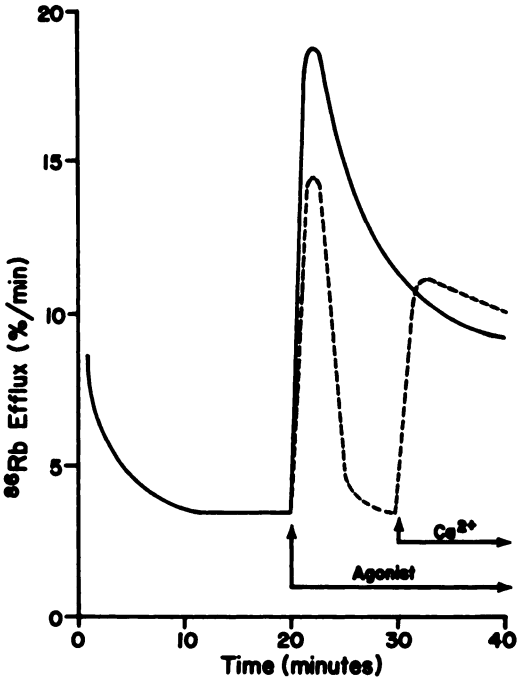

FIG. 1. Stylized kinetics of ^{86}Rb efflux from rat parotid gland in response to agonist and Ca. Two conditions are illustrated; the continuous *solid line* indicates the pattern obtained when agonist is present from 20 to 40 minutes in Ca-containing medium. The *dashed line* shows the response to agonist (20–40 min) when Ca is absent from the medium and the restoration of the response on reintroduction of Ca (30–40 min).

This overall scheme gained considerable support from a series of experiments designed to test independently the third conclusion (177). A protocol was employed as shown in Figure 1 (*dashed line*) whereby magnitudes of the transient (Ca release) and sustained (Ca influx) phases could be determined separately. Agonists were em-

† The term "permeability" is used here and other places in this review to denote the increase in efflux of ^{42}K or ^{86}Rb. In the major systems discussed, relevant conductance or voltage changes have been shown, indicating that the increased permeation rates reflect a rise in membrane permeability. This does not imply, however, that it is known whether these pathways are "pore-like" or "carrier-like" in character.

ployed alone, or in combination, always in supramaximal concentration. Under these conditions, the responses failed to summate in either phase, despite the fact that the Ca concentration employed (1.0 mM) was shown to be submaximal (177). This observation strongly supports the contention that the three receptors regulate a single population of Ca influx sites and strengthens (but does not prove) the suggestion that the transient and sustained phases are kinetically different manifestations of the same molecular event.

The status of information regarding the nature of the K channel in the salivary glands is similar to that for the erythrocyte. Virtually nothing is known. As for the red cell, it has been proposed that the K channel activation mechanism may have a relatively low affinity for Ca (232). The channel is also similar to that in the red cell in that high concentrations of tetraethylammonium chloride (TEA) will block it (222), and in that Sr, but not Ba, can substitute for Ca (112a). When measurements are made of unidirectional flux, the process is unaffected by ouabain (222). Alterations in extracellular K or Mg have no discernible effects nor does replacement of up to one half of extracellular Na or Cl (222). There is a ouabain-sensitive ^{86}Rb uptake, however, that is activated by autonomic agonists (see below). This rather clear separation of pumping from the K permeability effect suggests that the pump modification theory for the red cell is an improbable mechanism for the parotid gland. The possibility of a phospholipid mechanism again cannot be overlooked, although no evidence for such a phenomenon exists in the parotid gland. A rather sizable phospholipid effect definitely not related to K channel activation does occur that could obscure such changes (see below). In order to detect such effects, it may be necessary to utilize A-23187 to elevate intracellular Ca in order to avoid the receptor-related phospholipid effects (see below) and to use 2-deoxyglucose [after Abdel-Latif et al., (1)] to prevent relabeling of polyphosphoinositides.

After the fashion of the times, due consideration has been given to a possible involvement of cyclic nucleotide in stimulus-permeability coupling. In a number of tissues, stimulation of muscarinic or alpha-adrenergic receptors leads to an increased tissue level of cyclic 3′,5′-guanosine monophosphate (cyclic GMP) suggesting a role for this cyclic nucleotide in coupling mechanisms in general (110, 111). Cholinergic and alpha-adrenergic agonists both increase cyclic GMP levels in the parotid gland (60, 61) but substance P does not (241). The failure of substance P to increase cyclic GMP tends to deny an obligatory role for this cyclic nucleotide since the permeability responses to the three agonists are so similar (220, 221). Also, Butcher et al. (60, 61) found that 1-methyl-3-isobutylxanthine, a phosphodiesterase inhibitor, potentiated the cyclic GMP response, but not the K efflux response to submaximal concentrations of carbachol or phenylephrine. Taken as a whole, these observations render doubtful any role for cyclic GMP in the regulation of K permeability in the parotid gland.

One characteristic of the K channel in the rat parotid gland that clearly distinguishes it from the red cell channel is the phenomenon of desensitization to Ca (223). When slices of parotid gland were incubated with agonists in the presence of Ca, K permeability was seen to "fade," i.e., the response returned to control levels after 30 to 40 minutes (223). When fade was fully developed to one agonist, the response to an agonist acting on a different receptor was either absent or greatly diminished. When fade had developed to agonists, the response to A-23187 was diminished as well (author's unpublished observation). Within this same time frame, the rate of release of α-amylase, another Ca-dependent event (58, 169), remained constant.* Reintroduc-

* In a more recent report (115), longer exposures produced desensitizations of responses to amylase. These, however, were agonist-specific, suggesting that the prolonged exposures can cause receptor desensitization but not Ca channel desensitization.

tion of Ca to the medium after 30 minutes incubation in the presence of agonist but without Ca stimulated K efflux, suggesting that the continued presence of Ca and agonist together was required for this effect. These observations, taken together, suggest that the fade of the K permeability response in the parotid gland is not receptor-specific and results from a Ca-mediated loss of sensitivity of the K channel to Ca (223). By comparison, the responsiveness of the red cell channel to Ca remains constant with time, but appears labile to other conditions (see above). Heyer and Lux (125) have postulated a Ca-dependent inactivation of K channels in snail neurons. The relevance of this phenomenon to stimulus-permeability coupling in other systems is as yet undetermined.

With somewhat different protocols, other investigators have obtained evidence that receptor-specific desensitization can also occur in the parotid gland. Harper and Brooker (114, 115) found that amylase secretion and the increase in cyclic GMP due to carbachol faded with increased time of incubation. The elevated cyclic nucleotide level and secretion rate could be restored with alpha-agonists, but not with additional carbachol. Stritmatter et al. (269, 270) found that pretreatment with alpha-agonists decreased tissue responsiveness to a subsequent challenge with alpha-adrenergic but not muscarinic agents. Under these conditions, the number of alpha-adrenoceptors, assayed by ^{3}H-dihydroergocryptine binding, was also found to be decreased. Responsiveness could only be restored by incubating in medium containing elevated K. The significance of this latter observation is not altogether clear. It is unfortunate that the response measured was net K release, which would be sensitive to alterations in extracellular or intracellular K.

3. The Salivary Glands—Nature of the Ca Channel. Investigations into the nature of the Ca channel and its regulation by membrane receptors has proceeded along two rather independent lines of approach: the one primarily pharmacological, the other largely biochemical.

In the pharmacological studies, the actions of chemicals in the stimulus-permeability coupling sequence have been investigated in hopes of delineating the basic mechanism involved. When the actions of a number of reputed Ca-antagonists were investigated, the agents were found to act in one of two general ways (177). Neomycin, La, Co, and Ni all produced rather selective blockade of the Ca-dependent sustained phase of the response with little effect on the transient phase. This action is consistent with a selective and perhaps competitive inhibition of Ca influx by the agents, with little or no effect on receptor activation per se. The local anesthetics, procaine and tetracaine, as well as the methoxy derivative of verapamil, D-600, inhibited both phases of the response equally, suggesting that these agents affect the stimulus-permeability coupling mechanism in some manner other than direct antagonism of Ca influx (177). A direct inhibition of the K channel was ruled out when the response to the ionophore A-23187 was found to be insensitive to the effect. Since alpha-adrenergic and muscarinic agonists were equally affected, receptor blockade seemed unlikely. Strangely, however, the response to substance P was totally unaffected by the local anesthetic (177). This latter observation tended to argue against an inhibition of the Ca channel activation, since all three receptors presumably regulate the same channel (see above). Thus, it was proposed that the local anesthetics blocked the transduction mechanism by which occupied alpha-adrenergic and muscarinic receptors trigger the appropriate reactions that lead to Ca channel activation (177). For the substance P receptor, the transduction mechanism would have to be qualitatively distinct. More recently, however, it has been demonstrated that procaine can block the muscarinic receptor (as determined by radioligand binding) in parotid cells (author's unpublished observation). Whether

such an action explains the effect on the alpha-adrenergic response remains to be determined. If so, it is interesting that the peptide receptor would be so uniquely insensitive to agents that nonselectively interfere with muscarinic and alpha-adrenergic receptor binding.

At this point, a brief digression to discuss the actions of D-600 seems warranted. Were it not for the characteristic transient and sustained pattern of response in the parotid, the inhibitory action of D-600 and the local anesthetics might have been interpreted as a "specific" blockade of Ca channels. Clearly, D-600 has such actions in excitable cells (96, 225) and this discrepancy serves to point out the often ignored hazards of extrapolating from excitable to nonexcitable tissues. In fact, a specific Ca channel antagonist with demonstrated activity in nonexcitable cells unfortunately is as yet undiscovered. The only agents that effectively antagonize Ca movements in these cells are those that compete for Ca binding at virtually all membrane sites (heavy metals, aminoglycosides). Some examples of faulty hypotheses generated from misinterpreting the action of D-600 in nonexcitable cells will be mentioned later.

Returning to the original vein, the nature of the receptor-channel interaction has also been investigated biochemically. The studies of Michell and others along these lines have been based on the well-known, but until recently, unexplained effect of muscarinic agonists on phospholipid metabolism (129, 187). Virtually all tissues with muscarinic receptors and many others with receptor-controlled Ca channels show this effect, which Michell et al. (187–191) have suggested may be related to events involved in activation of Ca channels by receptors. In rat parotid gland slices, both alpha-adrenergic and muscarinic agonists (but not beta-adrenergic agonists) increase the labelling of phosphatidylinositol by ^{32}P (198) and diminish the fraction of total phospholipid present as phosphatidylinositol (150, 151). Most of the newly labelled phosphatidylinositol is associated with microsomes derived from rough endoplasmic reticulum leading some to doubt the relevance of the effect to membrane receptor phenomena (129, 198). Michell, however, suggests that the incorporation of ^{32}P into phospholipid is secondary to an earlier, net breakdown of phosphatidylinositol. This breakdown, he reasons, might represent an initial membrane event, perhaps related to the activation mechanism of the Ca channel (187–191). Consistent with this view is the considerable lag time that occurs between application of epinephrine to parotid slices and appearance of ^{32}P in microsomes (198).

It is the relationship of Ca to the phospholipid effect, however, which most strongly suggests an involvement in early events following receptor occupation. The effect is seen only with agents that activate endogeneous Ca channels (i.e., not with isoproterenol), but is independent of the external Ca concentration [and other electrolytes as well (151)]. Additionally, A-23187 can produce all of the Ca-dependent phenomena that occur subsequent to Ca channel activation including K release, exocytosis, and cyclic GMP synthesis (58), but not phosphatidylinositol labelling (198). These observations are consistent with a mechanism in which receptor activation triggers the breakdown of membrane phosphatidylinositol, a consequence of which is the activation of Ca channels (187–191).

The major problem preventing general acceptance of this scheme has been the failure to obtain phosphatidylinositol breakdown in a broken cell preparation. The simplest "unit" that will demonstrate this effect is the synaptosome (187). Synaptosomes retain most of the characteristics of intact cells, including the establishment of ionic gradients and membrane potentials (37). It is unlikely that ionic gradients or membrane potential are necessary for the phosphatidylinositol breakdown (151). One possible explanation is that some structural entity such as the submembranous cytoskeleton may be required for cou-

pling receptors to the appropriate enzymes. It is hoped that resolution of this important question will be soon forthcoming.

If the phospholipid effect does in fact represent a step preceding Ca influx, it would be interesting to know whether the reaction involves a molecular change in the Ca channel or whether the breakdown represents a mechanism by which receptors become activated and therefore capable of acting upon Ca channels. In other words, is the phosphatidylinositol breakdown a property intrinsic to receptors or to Ca channels? Michell et al. (189), arguing that early steps in a sequence of biochemical events must have the greatest apparent K_m values, showed that the phospholipid effect was a high K_m response, and coincided with the concentration dependence of receptor binding. Clearly, what is needed is a simultaneous determination of Ca channel activation by measurement of Ca influx. In the parotid gland, the dose-response relationships for receptor occupation by agonists lie well to the right of the curves for K efflux or ^{45}Ca influx (author's unpublished observation), suggesting that occupation of but a few muscarinic receptors is sufficient to activate the Ca channels maximally. In other words, it is suggested that a considerable quantity of spare muscarinic receptors exists in the parotid acinar cells. Unfortunately, the dose-response relationship for the effect of carbachol on phosphatidylinositol breakdown in the parotid gland is not yet available. The concentrations of acetylcholine required for maximal phospholipid effect are very high, however (150). This may suggest that it is receptor occupation rather then channel activation with which the phospholipid effect is associated.

Substance P and alpha-adrenergic agonists show a phospholipid effect in the same range of concentrations required for stimulation of K efflux (152, 198). Recent studies have shown that receptor occupation by alpha-adrenergic agonists and by substance P also occurs in this same concentration range (269, 270; author's unpublished observation); i.e., there do not appear to be spare alpha-adrenergic or substance P receptors. This is consistent with the idea that it is receptor occupation that best correlates with the phosphatidylinositol breakdown.

4. The Salivary Glands—Other Ionic Fluxes. Besides the Ca-regulated K fluxes discussed above, other equally interesting but less extensively characterized ionic events are associated with salivary gland activation. Data from a number of studies suggest that secretagogues also stimulate Na influx and ion pumping in the salivary glands (208, 214, 236). In electrophysiological studies using microiontophoretic application of secretagogues, Roberts and Petersen (236) found that the response to muscarinic or alpha-adrenergic stimuli consisted of two phases. The first was generally a hyperpolarization, but was occasionally a depolarization. By current injection, they found that the response had a reversal potential of −55 mV and was associated with a dramatic fall in membrane resistance. Thus, they concluded that this early phase resulted from an increase in membrane permeability to Na and K. The second phase, a slow hyperpolarization, was reduced by ouabain or low K media. This phase, they concluded, resulted from activaton of an electrogenic pump (236).

Similar conclusions were reached from studies of isotope fluxes. In parotid slices, carbachol, phenylephrine, and substance P each increase uptake of ^{22}Na without affecting uptake of the extracellular marker, ^{14}C-sucrose (227). Similar results have been obtained with dispersed parotid acinar cells (author's unpublished observations). Carbachol also stimulates ouabain-sensitive uptake of ^{86}Rb (227), an indicator of enhanced activity of the Na,K pump (42). Both of these effects require the presence of extracellular Ca. The increased pumping also requires the presence of Na in the medium. Furthermore, when Na is decreased from 125 mM to 5 mM, carbachol actually inhibits the uptake of ^{86}Rb. These results can best be explained by the following sequence of events. Receptor activation,

as described previously, would stimulate Ca influx, which would lead to an increase in the cytosolic Ca concentration. In some manner, intracellular Ca, well-known to affect membrane permeability to K, may also be able to increase Na permeability in the exocrine glands. Entry of Na would thus elevate intracellular Na and lead to a stimulation of the Na,K pump. In support of this, another means of increasing intracellular Na, omission of K from the incubation medium, similarly produced a stimulation of ^{86}Rb uptake (227).

A number of questions remain unanswered regarding the shifts in monovalent ions that result from secretagogue action in the salivary glands. It is not known, for example, whether movement of one or both of the cations (Na or K) is ultimately responsible for generating transepithelial water flow; theoretically, either could work (see above). It is also not known whether the Na and K channels are distinct from one another although in the lacrimal glands (see below) the evidence suggests that separate channels exist. In any event, the salivary glands obviously represent an intriguing and rather elegantly designed system where a single second messenger, Ca, can trigger increased permeability to Na and K as well as an increased activity of the Na,K pump. Even without knowing the particular locus of each relevant ionic shift, it is clear that Ca can provide all of the necessary ingredients for generating osmotic gradients and transepithelial water flow.

5. The Lacrimal Glands. The lacrimal glands have not been as extensively characterized as the salivary glands or pancreas, but are discussed here because of their marked similarity to the salivary glands. Actually, the exorbital lacrimal gland of the rat is at least equal to the parotid gland and pancreas in its utility as a model in vitro. Slices of exorbital gland are composed approximately 80% of a single type of acinar cell (231) and survive and respond quite well upon incubation in vitro (202, 203, 231). It may be noted in passing that these cells are excellent models for study of intracellular secretory protein distribution. This is because one of the packaged secretory enzymes is a peroxidase that is easily measured upon release in vitro, and, more importantly, is easily identified histochemically at the electron microscopic level (113, 124). The control of protein discharge is somewhat different from the parotid, however, there being no beta-adrenoceptor mechanism, and cyclic nucleotides having no apparent role (66, 123, 231).

The control of ion permeability in the exorbital lacrimal gland resembles that of the parotid gland in many respects. One difference is that the lacrimal fluid tends to have higher Na concentrations than saliva, such that this fluid is isotonic (6, 272). As for the salivary glands, the K concentration tends to be higher than in plasma, even in the primary secretion collected by micropuncture from the acinar-intercalated duct region (6). It has been known for some time that parasympathetic or muscarinic stimuli augment lacrimal flow (46, 47). The recent discovery of alpha-adrenoceptors associated with ion movements and protein secretions in the rat exorbital gland (66, 123, 142, 202, 203, 231) suggests that some control may be exerted by the sympathetic as well.

In slices, both alpha-adrenergic and muscarinic agonists increase ^{86}Rb efflux from lacrimal slices, but substance P and congeners have no effect (202). Histamine, serotonin, and cyclic nucleotide derivatives are also ineffectual (202). The K efflux response appears biphasic—an early transient phase persists in the absence of Ca while the later phase of the response has an absolute requirement for Ca (203, 229). In addition, the divalent cationophore, A-23187, can stimulate ^{86}Rb release if Ca is added to the medium (203). The alpha-adrenergic and muscarinic responses show cross-receptor inactivation; i.e., Ca is required to produce more than a single transient response (203). Finally, the Ca-dependent responses to carbachol and epinephrine do not summate even when the Ca concentration is submaximal (203). These results suggest that in the

lacrimal gland, a mechanism exists which is similar to that in the parotid gland. Thus, occupation of muscarinic or alpha-adrenergic receptors leads to activation of a single population of Ca channels. Associated with the activation is the release of a common pool of membrane-bound Ca. As for the parotid gland, both phases of the increased K permeability appear to reflect an elevation in cytosolic Ca.

The membrane potential of the rat lacrimal gland responds biphasically to agonists, the general pattern being hyperpolarization followed by depolarization (201). Thus, it is likely that permeabilities to ions other than K are altered as well. Both carbachol and epinephrine increase influx of ^{45}Ca and ^{22}Na into dispersed rat exorbital gland acinar cells. The responses to these drugs are blocked by atropine and phentolamine, respectively (161, 201). The Na influx response requires Ca in the medium and can be blocked by $CoCl_2$ in concentrations that also block Ca influx and the sustained phase of K efflux (201). Carbachol also stimulates ouabain-sensitive ^{86}Rb uptake, and this response requires both Ca and Na in the medium (229a). Again, the interpretation is that Ca-dependent Na influx activates the Na,K pump as was suggested for the parotid gland.

A detailed analysis of the electrical response of the mouse lacrimal gland to secretagogues has recently been provided by Iwatsuki and Petersen (142). By simultaneously impaling adjacent, coupled acinar cells, the effects of acetylcholine and epinephrine on membrane potential and input resistance were determined. These investigators found that the response to iontophoretic application of acetylcholine was invariably a hyperpolarization with a decrease in membrane resistance (about a 7-fold decrease, on the average). The equilibrium potential for the acetylcholine effect was somewhat less negative than the Nernst potential for K. Elevating external K largely prevented the potential change without affecting the resistance change. Epinephrine produced indistinguishable effects while isoproterenol was without effect. These results are consistent with those obtained by flux measurements. The effect of Ca on the permeability changes was not examined, however.

These investigators concluded that these agonists act "by opening up pathways mainly permeable to K, but also somewhat permeable to Na" (142), implying that a single species of channel is activated with only moderate selectivity for K over Na. In recent studies, however, it was found that amiloride, a K-sparing diuretic and antagonist of Na channels in epithelia (73, 74), antagonizes the Na influx in the exorbital gland, has no effect on Ca influx, and only slightly inhibits the efflux of K (201). This observation provides the only known evidence that the Ca-activated fluxes of Na and K occur through separate channels in the membrane. Teleologically, such an arrangement may be necessary in order for the altered ion permeabilities to produce the appropriate osmotic gradients necessary to drive water flow. It would be helpful to locate more precisely where on the cell surface the permeabilities to Na and K are affected.

One report has demonstrated that carbachol increases the incorporation of ^{32}P into total phospholipids in the lacrimal gland (66). More recently, Jones, Cockcroft, and Michell (personal communication) observed phosphatidylinositol responses due to muscarinic or alpha-adrenergic stimuli. The muscarinic effect did not require extracellular Ca.

Although the information is not complete at present, similarities between the salivary and lacrimal glands are apparent. A similar mechanism may then apply: receptor occupation, followed by Ca influx with elevated internal Ca activating specific channels for Na and K. The resultant increase in intracellular Na concentration may subsequently activate the Na,K pump. The involvement of phospholipids in this se-

quence has been documented only preliminarily, but a similar role here would seem quite likely as well.

6. The Exocrine Pancreas. The secretory mechanisms in the pancreas in many ways contrast and in other ways resemble those in the parotid and lacrimal glands. Schramm and Selinger (249) pointed out the differences primarily with regard to the mechanism regulating exocytosis. In this respect, the pancreas and lacrimal gland are somewhat alike, in that external Ca (rather than a cyclic nucelotide, as in the parotid gland) may modulate enzyme release. The vast majority of research in pancreatic physiology and pharmacology has been concerned with the mechanism of discharge of digestive enzymes (64). Indeed, it was the exocrine pancreas that served as the experimental model for much of the initial work on protein synthesis, transport, and secretion (200). Recently, information about stimulus-permeability mechanisms in the pancreas has appeared. Some of the newer work will be described here. In order to discuss control of Ca flux, however, considerable reference to enzyme secretion studies will be necessary.

One of the most striking differences between the pancreas and other exocrine glands is the lack of adrenergic control of the gland cells. Barlow et al. (16) found that adrenergic agonists inhibited pancreatic secretion even after alpha-adrenoceptor blockade to prevent effects of vasoconstriction. They concluded that ductal elements might be directly influenced by such agents. Significant adrenergic control of acinar cell function seems unlikely since neither slices (286) nor isolated cells (186) are affected by epinephrine. Legg (168) also failed to observe any adrenergic innervation of acinar cells in morphological studies.

The other rather unique feature of the exocrine pancreas is its failure to show a marked Gardos effect. Neither carbachol nor A-23187 causes net K release from pancreas slices (249), nor do they increase unidirectional efflux of K as measured with ^{86}Rb [author's unpublished observation; a moderate increase in ^{42}K efflux has been observed (63)]. It is generally agreed, however, that such agents activate amylase release by elevating intracellular Ca (63, 64, 185, 245, 246, 277, 285).

Until very recently, studies considering a role for Ca in modulating ion permeabilities in the pancreas were notably absent. This may be because of the rather prevalent supposition that while Ca may trigger exocytosis from pancreatic acinar cells, the physiological mechanism for fluid secretion involves a cyclic AMP-stimulated bicarbonate and Na transport system located in the ductal cells (63, 185, 249, 277). Two recent reports (appearing simultaneously) on studies with the perfused rat pancreas found that the actions of the peptide hormone, secretin, could be explained by such a mechanism, but that another mechanism also existed (155, 212). These investigators found that agonists known to stimulate exocytosis from acinar cells also stimulated the flow of pancreatic juice quite efficiently. Unlike the response to secretin, the responses to acetylcholine, pure cholecystokinin, or caerulin did not require the presence of bicarbonate in the perfusate, but required both Ca and Na ions. These agents, therefore, may produce a Ca-mediated water secretion by the acinar cells in contrast to secretin, which produced a cyclic AMP-mediated secretion by the centroacinar and terminal duct cells. Carbachol stimulates ^{22}Na uptake by pancreas of the baby rat (65) and dispersed pancreatic acinar cells (author's unpublished observation), but the Ca-dependency of this effect has not been demonstrated.

With some extrapolation from the parotid and lacrimal glands, a model can be proposed for receptor control of acinar cell water flow in the exocrine pancreas of the rat. Agonists such as acetylcholine or cholecystokinin may interact with receptors on the basal membrane of the acinar cells, which in turn activate Ca channels (or Ca release). The increase in cytosolic Ca may,

by some unknown mechanism, activate Na (and perhaps Cl) channels. In support of this contention, Poulsen and Williams (215) found that the Ca ionophore A-23187 produced a Na-dependent depolarization of pancreatic acinar cells. Intracellular injection of Ca produced a similar effect (139). Also, Schulz and Heil (250) demonstrated that the Na permeability of membrane vesicles from cat pancreas was activated by micromolar quantities of Ca. This effect of Ca demonstrated "sidedness"; i.e., Ca was most effective when applied to what appeared to be the cytoplasmic aspect of the membranes.

More speculatively, the Na entering the cell could stimulate the Na,K pump; the altered Na permeability and pumping could then act to generate transepithelial water flow. Since isolated pancreatic acinar cells can now be prepared with reasonable facility (11), these proposals can ultimately be tested with direct measurement of Na and Ca fluxes. Electrophysiological measurements have already confirmed that Na and Cl permeabilities are increased by acetylcholine while K permeability is little affected (138).

One aspect of this scheme about which some controversy exists is the manner by which the cytosolic Ca concentration is raised. Although the role of Ca in controlling acinar membrane permeability in the pancreas has received attention only recently, the regulation of Ca pertinent to control of amylase secretion has been extensively investigated (25, 63, 64, 185, 234).

First, the requirement for extracellular Ca has been somewhat controversial, and experiments carried out with different species and under various temporal protocols have added substantial confusion to the situation. It now seems likely that stimulation of exocytosis in the absence of Ca is a temporary condition, and the pools of Ca utilized are soon exhausted under these conditions (154, 211). This demonstrates at least a quantitative dependence on extracellular Ca.

Similarly, attempts to demonstrate (with slices) a stimulated Ca influx due to secretagogues had led to conflicting results (i.e., see 121, but also 67). The explanation for these discrepancies is not clear but a contributing factor could be a species difference. Thus, experiments utilizing fragments of mouse pancreas (67) and isolated acinar cells from the guinea-pig pancreas (102) failed to detect an enhancement of ^{45}Ca influx due to secretagogues. In two laboratories utilizing the rat pancreas as a model, cholinergic stimuli enhanced ^{45}Ca uptake in both fragments (121) and dispersed acinar cells (164). Gardner and Hahne (103) reexamined the procedure employed by Kondo and Schulz (164) and concluded that all of the extra ^{45}Ca uptake due to cholecystokinin occurred during the wash period when cells were being separated from radioactive medium. The difficulty in demonstrating Ca influx with these secretagogues may represent a technical problem rather than a biological phenomenon. Virtually all protocols reveal an effect of agonists on release (or efflux) of ^{45}Ca, however. In fact, if influx of Ca does occur, it must be small by comparison to Ca release, since the net effect of secretagogues on exchangeable Ca is to cause a net decrease in the tissue level of this cation under steady-state conditions (101). This observation, together with the transient nature of Ca-independent amylase release, suggests that the pancreas, parotid, and lacrimal glands may be qualitatively similar in mechanisms of Ca metabolism. All three, therefore, would act by releasing a bound pool of Ca followed by an influx of extracellular Ca to support continued responses. The bound pool in the pancreas, however, seems able to support responses for longer periods than in the other glands.

Regardless of the source of Ca, most investigators agree that agents stimulating exocytosis in the exocrine pancreas do so by elevating the concentration of ionized Ca in the cytoplasm. In support of this contention, Iwatsuki and Petersen (140, 141) found a marked depression of electrical coupling between cells to be a generalized response to secretagogues acting on pancreatic acinar cells. Such uncoupling could

be mimicked by intracellular Ca injections (139) and is, in general, considered to indicate a rise in intracellular Ca (175, 176, 237). The controversy involves the primary source of the Ca, however, and the relative contributions of extracellular and cellular pools of Ca will remain the subject of investigation for some time.

The use of Ca antagonists has not added much information in this regard. La is generally accepted as an efficient inhibitor, but there is not general agreement as to specificity of action (67, 121). Kondo and Schulz (164) have suggested that carbachol and pancreozymin activate different Ca channels since the muscarinic effect is blocked by D-600 and the peptide effect is not. High concentrations of D-600 (10^{-4} M) were required, however. It is likely that the effect seen by Kondo and Schulz represented the local anesthetic-like action of D-600 as described above for parotid gland. Again, the differential sensitivity of the muscarinic and peptide responses may signify differences in the receptor mechanisms as suggested for the parotid gland above. In support of this contention, under certain conditions tetracaine has been shown to inhibit secretion due to urecholine but not that due to cholecystokinin (194).

As was shown for the parotid, heavy metal Ca antagonists do not discriminate between cholinergic and peptide receptor mechanisms in the pancreas (140, 154). In order to determine whether the same or different Ca channels are utilized by these receptors, agonists would have to be tested for summation of responses (Na influx or amylase release) at limiting Ca concentrations.

The mechanism of Ca channel activation (or Ca release) in the pancreas may be similar to that in the parotid, since both show a similar phosphatidylinositol effect. Indeed, the initial observation of the phospholipid effect was made by Hokin and Hokin (130) using pancreas slices. Hokin (128) was also first to demonstrate that Ca was required for exocytosis but not for the phospholipid effect. This observation served to discourage hypotheses indicating that the phospholipid effect was related to secretion. Another discouraging observation was the discrepancy in dose-response relationships, concentrations approximately three orders of magnitude greater being required for the phosphatidylinositol effect as compared to the secretory effect (129, 191). Michell recognized, however, that the different sensitivities of these responses only served to suggest their sequence (189, 191). He reasoned that perhaps only a small quantity of occupied receptors and subsequent activated phosphatidylinositol breakdown would suffice to provide sufficient Ca intracellulary to saturate the secretory mechanism. The relevance of these arguments to the phosphatidylinositol effect in the parotid gland has been discussed above. Such a scheme is analogous to the "spare receptor" theory for smooth muscle, which also describes the situation when only a few receptors need be occupied to produce a maximal mechanical response (100, and see below). In point of fact, however, the rate-limiting step is not generally known and it is often difficult to state whether receptors, phospholipid metabolism, or Ca channels (if these be separate entities) are "spare" (present in excess). More information is available for the case of smooth muscle, and further discussion will be deferred to that section.

7. A General Scheme for Receptor Control of Permeability in Mammalian Exocrine Glands. Based on the studies outlined thus far, a general scheme for receptor mechanisms in exocrine glands, as related to ion permeability, can be proposed:

1. Occupation of receptors on acinar cell membranes by agonists (muscarinic, α-adrenergic, various peptides) triggers a breakdown of phosphatidylinositol.
2. On cleavage of phosphatidylinositol, membrane Ca channels are changed from an inactive to an active state or Ca is released from binding sites.
3. Ca moves into the cell passively down its electrochemical gradient.
4. Intracellular Ca activates channels (possibly distinct) for K and Na by an unknown mechanism.

5. The passive fluxes of K and Na down their respective electrochemical gradients lead to a decrease in intracellular K concentration and an increase in intracellular Na concentration.
6. The elevated intracellular Na concentration stimulates the Na,K-pump.

These events may be involved in receptor-mediated exocrine gland activity universally. The only aspect not totally ubiquitous is the K permeability change, which appears absent in the pancreas. This may suggest that it is the Na permeability and increased pump activity that are more relevant to driving transepithelial water flow. This could easily be accomplished if Na permeability were enhanced preferentially at the basal membrane, while being pumped into the acinar lumen at the apical surface. Such a scheme is admittedly highly speculative. It is at least consistent with observations that the primary secretion, obtained by micropuncture close to the acinar lumen, is high in Na and low in K. This has been found to be the case in a number of exocrine glands despite the fact that the final secretory products may differ markedly in these glands (247).

C. Calliphora Salivary Gland

The story of the blowfly salivary gland, similar to the mammalian gland in many ways and quite different in others, represents an excellent example of what can be accomplished by careful study of a simple model system. The structure of the fly gland, a closed epithelial tube of a single cell type (199), makes this preparation ideal for simultaneous study of ion transport and water movement and their control by membrane receptors (26).

Using a combination of sucrose gap and microelectrode techniques, Berridge and coworkers have studied the effects of serotonin (the physiological agonist, 24) on the electrical properties of the glandular epithelium (24, 26, 28, 29, 31, 216, 217). Serotonin decreases transepithelial potential and resistance and this effect is abolished (in fact, reversed) if the impermeant anion, isethionate, is substituted for Cl. The depolarization also fails (or is only transient) if Ca is removed from the medium. Either Sr or Ba can substitute for Ca in producing this effect. Finally the ionophore, A-23187, can also produce the depolarization and decrease in resistance. These results indicate that serotonin probably acts to stimulate Ca influx, which, in turn, activates membrane Cl channels. In support of this, serotonin significantly increased the uptake of ^{45}Ca by the salivary glands (217). Intracellular recording indicated that the conductance change occurred primarily at the apical membrane (26).

In the absence of Ca or Cl, serotonin is not without effect on the transepithelial potential. Under these conditions, a sizeable hyperpolarization occurs. This hyperpolarization is not associated with changes in membrane resistance and is relatively insensitive to resting membrane potential. Intracellular recording located the hyperpolarizing current at the apical membrane. Potassium, the major cation in the salivary secretion of *Calliphora*, best supports this hyperpolarizing current; Na can substitute less efficiently in the absence of K. These observations are consistent with an action of serotonin whereby an electrogenic apical membrane K pump is activated by a mechanism independent of external Ca (26).

It is likely that the actions of serotonin in stimulating a K pump are mediated by cyclic AMP. Serotonin increases the gland levels of cyclic AMP (217). Also, the stimulation of the K pump and fluid secretion can be potentiated by phosphodiesterase inhibitors and mimicked by exogeneous cyclic AMP (24).

This simultaneous activation of ion permeability and ion pumping provides the necessary ingredients to generate transepithelial water flow. The system is therefore similar to that in the mammalian exocrine glands, differing in the nature of the ionic permeability and pump affected.

The salivary gland of *Calliphora* also differs from mammalian glands in utilizing cyclic AMP as a coupling factor for activat-

ing ionic flux (not strictly a permeability change, however). This difference may relate only to the mode of Ca control rather than to the actual nature of the secretory process. Berridge (26) has called attention to three relevant observations: 1) that cyclic AMP stimulates release of ^{45}Ca from fly glands; 2) that A-23187 can stimulate fluid secretion in the absence of changes in cyclic AMP; and 3) despite normal synthesis of cyclic AMP in the absence of external Ca, serotonin can stimulate secretion only temporarily under these conditions. These observations, Berridge reasons (26), could indicate that cyclic AMP acts by releasing Ca from intracellular stores and the released Ca may activate the K movement. Such a scheme requires that Ca released from intracellular pools and Ca entering through the surface membrane can produce different effects. This could arise because of the different spatial distribution of the Ca, as well as to different sensitivities of the different processes to Ca. This proposal is strikingly similar to that suggested for the rat parotid gland (232). Here it has been suggested that Ca influx regulates membrane permeability to K, while cyclic AMP releases Ca from intracellular stores, and the released Ca in turn activates exocytosis. The evidence for this system was similar to that invoked for the fly gland. Influx of Ca can support exocytosis in the absence of changes in cyclic AMP (58, 169); cyclic AMP derivatives or isoproterenol stimulate release of ^{45}Ca (224, 232), and depletion of intracellular Ca inhibits the cyclic AMP mediated exocytosis (224, 232).

Recent experiments by Fain and Berridge indicate that phosphatidylinositol hydrolysis may be important in the Ca-gating actions of serotonin in *Calliphora* salivary glands (27, 89). In fact, this preparation turns out to be uniquely well suited for such studies. Conveniently, on incubation of the fly glands with ^{32}P-phosphate or ^{3}H-inositol, a small fraction of total phosphatidylinositol labels to what appears to be a relatively constant specific activity in about 2 hours. When serotonin is added to preparations labelled with ^{3}H-inositol, phosphatidylinositol breakdown and ^{3}H-inositol release are well correlated (89). This release, therefore, serves as a convenient marker for phosphatidylinositol breakdown. With this system, the following observations were made: 1) whereas fluid secretion due to serotonin was potentiated by a phosphodiesterase inhibitor, inositol release was not; 2) fluid secretion gradually disappeared in the absence of external Ca but inositol release remained constant; and 3) the ionophore A-23187 efficiently stimulated Ca influx and secretion, but did not provoke inositol release (89). These observations suggest that, as for the mammalian glands, breakdown of phosphatidylinositol may be a reaction intrinsic to the mechanism of Ca channel activation by the serotonin receptor. The concentration-effect relationship for inositol release was found to lie at least an order of magnitude to the right of that for Ca flux, suggesting that submaximal rates of phosphatidylinositol breakdown may occur when all Ca channels are fully activated.

Berridge and Fain (27) also found that serotonin inhibited the synthesis of phosphatidylinositol but that this effect was Ca-dependent. They subsequently found that prolonged preincubation in the presence of serotonin and Ca prevented a stimulation of secretion or Ca flux by a subsequent test challenge with serotonin. The response could be restored by incubating with 2 mM inositol (27).

These latter observations raise a basic question concerning the role of phosphatidylinositol hydrolysis in Ca gating: Does the breakdown represent opening or closing of the channel? Clearly it is not unreasonable to suppose that breakdown of phosphatidylinositol opens the channel and resynthesis closes it. Berridge and Fain (27) suggest two possibilities. First, phosphatidylinositol breakdown may be involved in opening the gate, which relaxes spontaneously. Secondly, a phosphatidylinositol-gate complex may become activated by serotonin and this involves exposure of phos-

phatidylinositol to phospholipases. The breakdown of phosphatidylinositol collapses the channel, which must reassociate with membrane phosphatidylinositol (priming) to regain sensitivity to serotonin.

Definitive evidence distinguishing these two possibilities is not presently available. A third possibility comes to mind based on arguments raised previously for the mammalian parotid gland. For the fly gland, as for the mammalian gland, there is the suggestion that phosphatidylinositol breakdown may be more closely related to receptor occupation than to Ca gating. Thus it seems conceivable that agonist binding to receptor activates phosphatidylinositol breakdown, which results in conversion of the receptor (or some other intermediate) from a resting to an activated state. The activated receptor may either activate a Ca channel, or relax spontaneously. A few such activated receptors may suffice to keep all of the Ca gates open, but higher concentrations of agonist would break down more phosphatidylinositol and produce an excess of activated receptors. Admittedly, this scheme is no less speculative than any others but is put forth simply as an alternative that encompasses the spare receptor phenomenon.

D. Liver

The *Calliphora* salivary gland provides for a convenient transition from the mammalian exocrine glands to the liver. In the liver, as for the fly gland, both Ca and cyclic AMP may modulate membrane permeability, cyclic AMP perhaps through intracellular Ca release.

A variety of agonists increase liver membrane permeability to K following receptor activation. These may be subdivided into two categories: those which activate adenylate cyclase, and those which do not. For neither category is the role of Ca as obvious as in the exocrine glands or the red cell. Nonetheless, the indirect evidence strongly suggests that Ca is involved in these responses. For the sake of continuity with previous material, the agents that do not affect cyclic AMP levels will be considered first, though the cyclic AMP mechanism may be more important historically. Also, for both categories, use will be made of data on control of glucose metabolism where necessary to strengthen conclusions on Ca fluxes or distribution.

Haylett and Jenkinson (117) were the first to show that catecholamines hyperpolarize guinea-pig liver cells. They also showed that the catecholamines stimulated K efflux from guinea-pig or rabbit liver slices, or dispersed guinea-pig liver cells, and that both of these effects were mediated by alpha-adrenergic receptors (116–120, 144, 145). More recent studies show that a similar effect can be produced by adenine nucleotides (146) and the peptides angiotensin II (278) and vasopressin (author's unpublished observation). Beta-agonists do not stimulate K efflux from liver of rabbit or guinea pig normally, but will do so if the tissue is pretreated with an alpha-agonist (146, and see below). Unlike the situation in the exocrine glands, the response in the guinea-pig liver slices is transient and monophasic [i.e., no sustained phase is detected (278)]. Also, removal of extracellular Ca does not inhibit the response (278). The metabolic effects (glucose release, phosphorylase activation) of alpha-agonists, angiotensin II, and vasopressin require Ca, however, and these agents can stimulate uptake of ^{45}Ca (14, 81, 160). The stimulation of ^{45}Ca influx persists for at least 60 minutes (160) while the transient K efflux is complete in 6 minutes (278).

These observations suggest that the transient release of K caused by alpha-agonists or peptides is not mediated by an influx of Ca ions. It is likely, however, that the K permeability response is Ca-mediated but that the Ca comes from the transient release of bound Ca as described for the transient phase of K efflux in the exocrine glands. The evidence for this hypothesis is the following:

1. Alpha-adrenergic agonists stimulate a transient efflux of ^{45}Ca (116, 278), which slightly outlasts the K efflux. As for the

pancreas (see above), the release of Ca exceeds Ca influx so that a net loss of exchangeable Ca occurs (36, 68).

2. For the alpha-adrenergic and angiotensin II responses, cross-receptor inactivation occurs (278); that is, to obtain a response to phenylephrine or angiotensin II after prior exposure to either agonist, Ca must be present in the medium.

3. The release of ^{45}Ca is also greatly diminished by prior treatment with agonist (278). The Ca release that persists is more sustained in nature and may reflect the increase in membrane permeability to Ca that also results from these agonists. This would also explain why the ^{45}Ca release outlasts the K efflux, since the sustained Ca permeability effect would tend to prolong the apparent time course of the ^{45}Ca release (278).

These findings not only suggest that a transient release of Ca mediates the K efflux, but that the alpha-adrenergic and peptide receptors regulate the release of a common pool. Speculating further, if the analogy with the exocrine glands is complete, this would suggest that the release of this Ca may be associated with Ca channel activation, and that the alpha-adrenergic receptors and peptide receptors also regulate the same Ca channels. What would be helpful in evaluating such an hypothesis is a comparison of dose-response relationships for Ca influx and Ca release (or phosphorylase activation and K release), and a study to determine whether these agonists are additive in stimulating Ca influx or responses dependent on Ca influx (such as glucose release or phosphorylase activation).

The similarity of action of the "non-cyclic AMP" agonists (phenylephrine, vasopressin, angiotensin II) to actions of similar agents in the exocrine glands practically demands that alpha-agonists, angiotensin II, and vasopressin cause a phospholipid effect in the liver. In fact they do, and the properties of the response are also similar to those in the exocrine glands. Thus, Kirk et al. (163) demonstrated that epinephrine and vasopressin, but not glucagon, stimulated the incorporation of ^{32}P into phosphatidylinositol. The effects of epinephrine, but not of vasopressin, were blocked by the alpha-adrenergic antagonist, dihydroergotamine (163). Billah and Michell (34) have shown that angiotensin II produces similar effects, although rather high concentrations are required for the effect. They also found that ionophore A-23187 did not consistently affect phosphatidylinositol labelling (34). Vasopressin, in addition to its ability to enhance labelling, also stimulated a net breakdown of phosphatidylinositol (34). Omission of Ca lessened, but did not prevent the phospholipid labelling effect. It seems unlikely that Ca influx acts as a major stimulus for phospholipid turnover in the liver, however, since A-23187 was without consistent effect. The effect of Ca-withdrawal could be due to nonspecific deleterious effects of low Ca, but experimental confirmation of this conjecture is lacking.

On the basis of these observations, and by analogy with the exocrine glands, which appear quite similar in many respects, a working hypothesis can be constructed for agonists acting independent of cyclic AMP. Agonists such as phenylephrine, angiotensin II, or vasopressin would occupy or activate membrane receptors. The result is phosphatidylinositol breakdown, which leads to activation of membrane Ca channels. Associated with Ca channel activation is a release of bound Ca. This Ca release elevates intracellular Ca to an extent sufficient to activate K channels. Ca influx also occurs and elevates intracellular Ca to an extent sufficient to activate glycogen phosphorylase.

The reason that the sustained influx of Ca fails to activate K efflux is unknown. Two possibilities are evident. One is that the Ca concentration required is very high, a supposition consistent with observations in the red cell (see above) and parotid gland (232). An alternative explanation would be that the K channels desensitize rapidly. An experimental result that favors the former alternative is the failure of A-23187 to stim-

ulate K efflux (278). This ionophore causes phosphorylase activation of a magnitude similar to phenylephrine (14) and thus would presumably stimulate Ca influx to the same extent. The ionophore had no discernible effect on K permeability and, as expected, also did not cause a transient release of ^{45}Ca from liver slices (278). Thus it appears that the extent to which internal Ca is elevated by Ca influx may not be sufficient to activate liver membrane K channels, at least not in guinea-pig liver slices.*

Calcium may also be involved in the actions of agents that stimulate cyclic AMP synthesis. It is an old observation that epinephrine, glucagon, and cyclic AMP can stimulate efflux of K from the liver of certain species (87, 94, 274). Friedmann and Park (97) showed that the action of these substances to stimulate K release was preceded by an enhanced release of Ca, which could amount to 20% of tissue Ca stores. Subsequently, it was shown that cyclic AMP hyperpolarized the parenchymal cell membrane (98, 265) and this response was not blocked by ouabain (76). These data are consistent with a mechanism in which cyclic AMP releases Ca intracellularly and the elevated intracellular Ca activates K permeability. This is a comfortable hypothesis based on the analogy with the alpha-receptor mechanism and with the other systems discussed thus far. However, it is one for which direct supportive evidence is notably lacking. For example, it is not known whether the effects of glucagon or cyclic AMP on K efflux require external Ca. Net loss of Ca has been demonstrated only in low Ca media. It is not known, therefore, whether cyclic AMP can also stimulate Ca influx, which might contribute to the K permeability effect. Glucagon, believed to act primarily by activation of adenylate cyclase, does stimulate uptake of ^{45}Ca (160), but unlike vasopressin, its effect on metabolism does not require Ca (81, 271).

If Ca is released by cyclic AMP, the source of that Ca is unknown. Probably, either the endoplasmic reticulum or the mitochondria contain a sufficient amount of Ca to account for the quantity released by cyclic AMP (69). Mitochondria, isolated from liver exposed to glucagon, show an enhanced initial rate of Ca uptake (12). A liver microsomal fraction shows a similar effect (276). The significance of these observations to the effects of glucagon or cyclic AMP on Ca movements in the intact liver is still unknown.

At one time, considerable interest was generated by reports that cyclic AMP could release Ca from isolated mitochondria (43, 44). Subsequent investigations failed to reproduce these findings (45, 246). The latter reports, however, do not constitute sufficient evidence to discard the Ca-release theory of cyclic AMP action. Rather they may reflect technical inadequacies in the broken cell preparations as models for processes occurring in intact tissue. Continued exploration and innovative experimental approaches are sorely needed to resolve this central controversy so relevant to Ca metabolism in a number of tissues.

Evidence that Ca release may mediate the effects of alpha-agonists and beta-agonists on K flux comes from experiments with guinea-pig liver slices recently reported by Jenkinson et al. (144). Some caution is necessary in generalizing these results, since the guinea pig shows little or no permeability response to beta-agonists under normal conditions. Nonetheless, the evidence is rather straightforward. As stated previously, beta-agonists will stimulate K release from guinea-pig liver slices if preceded by an alpha-agonist. When an alpha-agonist and a beta-agonist are presented in series while monitoring efflux of ^{45}Ca, the beta-agonist causes a sizable stimulation of ^{45}Ca efflux. When the beta-agonist is added first, no stimulation of ^{45}Ca release is ob-

* Actually, A-23187 will stimulate K efflux in dispersed hepatocytes (57a). Perhaps with more rapid diffusion of ionophore to the liver cell membrane, intracellular Ca rises rapidly enough to activate K channels. Alternatively, this difference may reflect an improved metabolic condition of the cells compared to the slices.

served. At least two possible explanations may be applied to these findings. First, it may be that a pool of Ca (and thus ^{45}Ca) is available for beta-adrenergic release (via cyclic AMP), but an unknown effect of alpha-stimulation permits coupling of beta-receptors (or cyclic AMP) to release the pool. On the other hand, since alpha-agonists are suggested to release Ca internally, intracellular sites that can buffer Ca may act to sequester the Ca released by alpha-agonists. These sites may thus temporarily contain unusually large quantities of Ca. If these sites are the same as those discharged by beta-agonists (or cyclic AMP), a much higher cytosolic Ca concentration may result as compared to that occurring with untreated tissue, with the result that the threshold for K release may be reached. Perhaps experiments of a similar type, if employed with the perfused rat liver where the beta-effect may be more physiological, will strengthen the hypothesis that Ca mediates the hyperpolarization and K flux response due to cyclic AMP.

E. Smooth Muscle

There is probably no other category of tissue that has been treated with a greater variety of neurotransmitter and peptide agonists and antagonists than smooth muscle. Virtually all such agents have been suggested to act, at least in part, by affecting membrane permeability to ions in one way or another. Nonetheless, by comparison with the exocrine glands, we know embarrassingly little about mechanisms and coupling factors involved in these permeability responses. This is probably due to the extreme confusion generated in trying to interpret fluxes and permeability changes produced by a drug in a tissue that shows excitable behavior. In addition, the ions affected by acetylcholine or catecholamines may vary greatly in different smooth muscles (41, 77, 178).

One smooth muscle rather extensively characterized (although hardly typical) is the guinea-pig taenia coli. Epinephrine, through stimulation of alpha-receptors, hyperpolarizes the guinea-pig taenia coli and reduces the frequency of spontaneous action potentials. Although the primary cause of these phenomena was thought to be an increase in K permeability, an increase in K flux could not be demonstrated, since the electrical effects tended to override subtle changes in resting flux. Jenkinson and Morton (147–149) depolarized the muscle by replacing Na with K. Under these conditions, the electrical behavior of the membrane is linear and inexcitable, and no driving force exists for K movement and membrane potential to affect one another. With this preparation, enhanced influx and efflux of ^{42}K due to catecholamines were readily apparent. Several investigators using the double sucrose gap technique have confirmed that K conductance (as well as Cl) is increased in the taenia coli by catecholamines (39, 51, 53, 54, 178, 197; but also see 156, 157, 166).

Recent studies by Bülbring and Tomita (54) provided strong evidence that the increased K conductance produced in taenia coli by alpha-adrenergic agonists was mediated by Ca influx. Earlier investigations had revealed that omission of Ca inhibited the alpha-action on the taenia coli (50–52, 131). However, Ca removal per se produced depolarization and a reduction in membrane resistance, making it difficult to interpret the effects of agonists. Therefore, high concentrations of Mg were used to prevent the effects of decreasing external Ca on the basal permeabilities. Under these conditions, the response to epinephrine (hyperpolarization and decreased resistance) disappeared in media containing no added Ca and 0.5 mM EGTA, and returned upon reintroduction of 2.5 mM Ca to the medium. When Ca was added to Ca-free media, an increase in membrane resistance was observed. If epinephrine was present, however, the addition of Ca decreased membrane resistance and produced hyperpolarization. These observations were interpreted to indicate that Ca is an essential ingredient for the alpha-action of catecholamines on the taenia coli (54). It is reason-

able to speculate from this that alpha-adrenoceptor activation causes Ca influx, which in turn activates K channels. To support such conjecture it would be useful to know whether alpha-agonists stimulate Ca influx in taenia coli and whether the cationophore, A-23187, can enhance K permeability in this tissue.

The taenia coli of the guinea pig is, however, atypical in that it is inhibited by alpha-adrenoceptor activation. The general response of smooth muscle is to increase activity or contractility upon alpha-adrenergic or muscarinic receptor activation and to relax, or show a decreased activity, with beta-adrenergic agonists. It is quite interesting that in the taenia coli, Ca influx (which would normally be considered to facilitate contractility) may act to produce an inhibitory response. Presumably, this is accomplished by a localized effect near the surface membrane. Thus, the taenia coli provides another example of how the actions of intracellular Ca may be somewhat restricted to areas of the cell where Ca is specifically elevated.

The inhibitory actions of catecholamines in the uterus are mediated by beta-adrenergic receptors and are due partly to an increase in K permeability and partly to a stimulation of the Na,K pump (178). The latter effect is probably mediated by cyclic AMP. The enhancement of K permeability may be mediated by Ca influx since it is blocked by Ca removal or by La.

The stimulatory actions of alpha-adrenergic and muscarinic agonists generally involve an increase in membrane conductance and depolarization (41, 178). The conductance change involves Na or Cl to varying degrees (178, 225), although an increase in K permeability also invariably occurs (41, 144). In the ileum, the dose-response relationship for the K permeability response more closely approximates the ligand binding curves for muscarinic agonists than does the relationship for contractility (41, 57). Thus, strictly speaking, as Bolton (41) argues, the maximal contractile effect of carbachol occurs when depolarization occurs to the membrane equilibrium potential. This effect apparently requires but a few occupied receptors, although even greater conductance changes are possible.

These observations illustrate how difficult it is to make meaningful comparisons between dose-effect curves in smooth muscle. Possibly, the parameter that limits tension development in smooth muscle is the number of voltage-dependent (rather than receptor regulated) Ca channels that are activated. Since these may become maximally activated at some set value of membrane potential, tension development may be maximal at very low receptor occupancy. The occurrence of a "low affinity" response, K efflux, suggests that this response may be more closely related to early events following receptor activation. Consistent with this is the observation that the K efflux is relatively voltage-insensitive (57) and, in many cases, shows dose-response characteristics similar to ligand binding data [although exceptions are numerous (35)]. In many smooth muscles, the response is somewhat insensitive to external Ca, which may suggest an analogy with the transient K release believed to reflect Ca channel activation in the exocrine glands and liver (see above). In this regard, it would be helpful to know whether the K release response in smooth muscle can be rendered Ca-dependent by prior treatment with agonist under Ca-free conditions.

Despite such conjecture, it has to be admitted that there is no decisive evidence to implicate Ca as an obligatory intermediate in the excitatory actions of agonists on smooth muscle membranes (apart from the obvious role in activating contraction). Some rather intriguing speculation has appeared, however, based on recent studies on phospholipid metabolism in iris smooth muscle (1–5). Abdel-Latif et al. found, as did Jafferji and Michell (143) with guinea-pig ileum, that alpha-adrenergic or muscarinic stimuli increased the labelling of phosphatidylinositol in the iris smooth muscle. This effect was independent of the presence of Ca and was not produced by the iono-

phore, A-23187. In addition, if ATP levels were decreased by incubation with 2-deoxyglucose, a net breakdown of triphosphoinositide (but not diphosphoinositide) was also observed. In contrast to phosphatidylinositol labelling, triphosphoinositide breakdown required Ca in the medium and could be stimulated by the ionophore, A-23187. Strontium, but not barium or manganese, could substitute for Ca in producing this effect.

These observations have led Abdel-Latif to propose that the Ca-induced breakdown of triphosphoinositide is associated with the enhanced permeabilities to Na and K that occur upon alpha-adrenergic or muscarinic stimulation (3, 5). The hypothesis is an attractive one but, unfortunately, one for which there is little direct supportive evidence. The evidence against this possibility in the red cell has already been discussed. It is somewhat ironic that it is smooth muscle for which an effect of Ca on membrane biochemistry can be most clearly shown when it is also smooth muscle for which the role of Ca in regulating permeability is most questionable. What is urgently needed is the demonstration of a triphosphoinositide effect in other noncontracting tissues and a clearer delineation of the possible role of Ca as a mediator of the effects of excitatory transmitters on membrane permeability in smooth muscle.*

Cross-receptor inactivation, similar to that seen with the exocrine glands and liver, has also been demonstrated for vascular smooth muscle but with contraction as the end response (79, 80, 275). In these experiments, contractions could be obtained with rabbit aorta in response to norepinephrine, histamine, or angiotensin. In the absence of Ca, or in the presence of La, a single transient contraction occurred in response to any of the agonists, but a second response could not be obtained (or was greatly diminished), regardless of the agonist employed. Addition of norepinephrine to media during ^{45}Ca efflux caused a transient release of ^{45}Ca that could not be duplicated upon readdition of agonist at a later time. The similarity in these results to those in the exocrine glands and liver is apparent. It seems likely, as Deth and van Breemen (79, 80) suggest, that these receptors regulate a common pool of membrane-bound Ca that is released upon activation and dependent upon extracellular Ca for replenishment. As for the exocrine glands, it is also tempting to speculate that the release of this Ca and activation of Ca channels are associated events, although evidence in this regard is notably lacking.

Summarizing, there is circumstantial evidence to suggest that agonists may control membrane permeability in smooth muscle (at least partially) by a mechanism similar to that in nonexcitable tissues such as the exocrine glands and liver. Definitive conclusions in this area are complicated by the excitable behavior of the smooth muscle membrane, which may add an additional set of permeabilities with quite different properties. The early steps—receptor occupation, phosphatidylinositol breakdown, Ca influx—have considerable experimental support. The actions of Ca on membrane permeability, however, have thus far been substantiated only for the taenia coli.

F. Miscellaneous Nonexcitable Tissues

Included in this section are brief discussions of tissues where some evidence exists that receptor-mediated control of ion permeability may involve Ca. The brevity of treatment is not meant to imply a lack of functional or historical significance, but rather an inability to conclusively resolve

* In a recent report, Rosenberger and Triggle (238) observed that A-23187 probably stimulates intestinal smooth muscle contraction by first enhancing Na entry, which in turn activates the voltage-sensitive Ca channels. They suggest that the effect of A-23187 on Na influx may be a direct one, resulting from a certain degree of nonselectivity in the ionophoric action of this compound. An alternative interpretation would be that A-23187 first mediates Ca entry, which activates Ca-dependent Na channels, as Abdel-Latif et al. (3, 5) have proposed. Of course, this latter explanation is highly speculative, and at present there seems to be no firm basis for choosing between these two alternatives.

the mechanisms involved or to relate them to the general pattern already discussed.

1. Gastrointestinal and Other Epithelia. In addition to the salivary glands and pancreas, as discussed above, almost all levels of the gastrointestinal tract can be stimulated to secrete or absorb electrolytes and water by neurotransmitters or peptide hormones. Several recent reviews have dealt with various aspects of the control of these processes in the gastrointestinal tract and other model epithelial systems (13, 91–93, 162, 242, 243, 268). Largely, the emphasis of prior work and speculation has concerned the role of cyclic nucleotides (primarily cyclic AMP) in mediating these responses. Indeed, the role of adenylate cyclase in the actions of cholera enterotoxin served as a valuable tool not only in studies of cyclic AMP and intestinal secretion but in studies of adenylate cyclase in other tissues as well (23, 92, 107). The generally accepted role for Ca in these epithelia is in maintaining the integrity of tight junctions and possibly as an inhibitory regulator of Na channels. This requirement for Ca for junctional patency has hindered studies of the role of Ca as a second messenger in such systems.

Some fragments of evidence suggest that Ca could be involved in the actions of agents that stimulate adenylate cyclase. In gastric mucosa, cyclic AMP or theophylline can stimulate acid secretion but, under certain conditions, Ca is required for this effect. These agonists also stimulate release of ^{45}Ca from isolated oxyntic cells, suggesting that cyclic AMP may release Ca from internal stores (243). The similarity of this scheme to that in the fly salivary gland (see above) is apparent.

Similar observations have been made for the stimulation of Na transport in the toad bladder epithelium by vasopressin. Thus, vasopressin, believed to act through stimulation of adenylate cyclase in the toad bladder, stimulated efflux of ^{45}Ca from preloaded bladders (75, 273). Theophylline and prostaglandin E_1 (but not exogenous cyclic AMP) mimicked this effect. Vasopressin also caused a decrease in mitochondrial Ca in toad bladder, which is consistent with a mechanism whereby cyclic AMP may act to release Ca from this intracellular pool (25, 32, 264). In support of this hypothesis, Snart (263) has reported that cyclic AMP can release Ca from isolated kidney mitochondria. The controversy surrounding these results has been discussed above for the liver. The most condemning evidence against the relevance of such a scheme comes from a recent study on the effects of ionophore A-23187 on sodium transport in the toad bladder (282). The ionophore (1 μM) was found to inhibit both the base-line and vasopressin-stimulated Na transport, and in both cases, the effects depended on extracellular Ca. The effects on the vasopressin-stimulated transport may in part be due to inhibition of adenylate cyclase by Ca. Thus, vasopressin did not significantly increase cyclic AMP levels in the presence of ionophore plus 2.5 mM Ca, but increased cyclic AMP levels normally when Ca was reduced to 0.2 mM. These results suggest that intracellular Ca may act to inhibit the response to vasopressin by inhibition of adenylate cyclase. The possibility exists that Ca could be normally released by cyclic AMP and in turn feeds back to inhibit adenylate cyclase. In any event, it seems unlikely that Ca acts as a direct link in stimulating Na transport in the toad bladder since the ionophore was also inhibitory in the absence of vasopressin.

One instance where Ca almost assuredly acts in stimulus-permeability coupling (actually, "stimulus-transport" coupling) is in the activation of Cl transport in the rabbit intestine. Two simultaneous reports appearing recently provide strong evidence for such a mechanism (40, 99). Frizzell (99) found that in rabbit colon the Ca ionophore A-23187 reversed the active Cl absorption to active Cl secretion without affecting Na transport. Similar effects on ion transport were obtained in response to cyclic AMP. Decreasing the external Ca concentration prevented the activation of Cl secretion due to ionophore but not that due to exogenous

cyclic AMP. Ionophore did not alter levels of cyclic AMP in colonic mucosa, whereas exogenous cyclic AMP stimulated release of ^{45}Ca from prelabelled tissue (99). These results strongly support Frizzell's conclusion that the secretory response (Cl transport) due to cyclic AMP results at least in part from a release of Ca internally and a subsequent activation of Cl transport by the elevated cytosolic Ca.

Bolton and Field (4) similarly observed that in rabbit ileal mucosa, stimulation of NaCl secretion by ionophore required Ca, but stimulation by theophylline did not. In addition, they found that carbachol and serotonin, agonists that do not stimulate adenylate cyclase in intestinal mucosa, required Ca to stimulate secretion. Vasoactive intestinal peptide and prostaglandin E_1, agonists that raise the mucosal levels of cyclic AMP, did not require Ca. Taken with the results of Frizzell (99), these observations suggest that intestinal secretion of electrolytes may be mediated by internal Ca and that agonists may act either to stimulate Ca influx or Ca release through a cyclic AMP mechanism.

A recent report suggests that a similar mechanism may control Cl transport across the corneal epithelium (62). This raises the possibility of Ca-controlled Cl transport in epithelia generally and suggests a possible direction for future studies of transport regulation in other epithelial tissues.

2. Adipocytes. A number of observations, mostly quite recent, suggest that alpha-adrenoceptors may regulate ion permeability of the adipocyte by mechanisms similar to that in the exocrine glands and liver. Stimulation of adenylate cyclase by peptide hormones or beta-adrenergic agonists activates lipolysis (88). Alpha-adrenergic antagonists, under various conditions, can stimulate or potentiate the effects of catecholamines or peptides on lipolysis, but the specificity of these effects with regard to alpha-adrenoceptor activation has been questioned (88).

Perry and Hales (207) found that epinephrine, adrenocorticotrophic hormone, dibutyryl cyclic AMP, and theophylline all stimulated ^{42}K efflux from rat adipocytes. The effects of epinephrine could be partially blocked by phentolamine and by propranolol. The Ca-dependency of this response was not determined. Phentolamine did not inhibit the lipolytic effect of epinephrine (207). Thus, if Ca release or influx occurs upon alpha-adrenoceptor activation, Ca apparently does not act on the machinery involved in triglyceride breakdown. In support of this, ionophore A-23187 was found to inhibit rather than to stimulate lipolysis (259).

Evidence that alpha-stimuli affect membrane permeability in brown adipocytes was obtained in a recent study by Fink and Williams (95). These investigators found that norepinephrine, phenylephrine, and isoproterenol all produced a depolarization of brown adipocytes of about 25 mV. The effects of phenylephrine were blocked by phentolamine but not by propranolol; for the effects of isoproterenol, the reverse was true. The ionic dependency of this response was not reported.

Similarity between the alpha-adrenoceptor mechanism in the adipocyte and that in the liver is suggested by studies on the effects of adrenergic drugs on phosphorylase and glycogen synthase in rat adipocytes (167). Epinephrine, norepinephrine, and isoproterenol stimulated cyclic AMP formation, phosphorylase *a* activity, and decreased glycogen synthase I activity. Alpha-adrenergic antagonists inhibited the effects of epinephrine and norepinephrine by about 25% but potentiated the effects on cyclic AMP. Phenylephrine similarly increased phosphorylase *a* activity and decreased glycogen synthase I activity but did not affect the levels of cyclic AMP. These observations indicate the presence of alpha-adrenoceptors on adipocytes that stimulate phosphorylase activation by a mechanism independent of cyclic AMP. By analogy with the mechanism in the liver, it would seem likely that Ca may function as a second messenger for this effect and thus also for the K flux observed by Perry and Hales

(207). As yet, however, a requirement for Ca in producing any of these responses has not been demonstrated, nor have effects of alpha-adrenergic stimuli on Ca movements been demonstrated.

There is some evidence that alpha-adrenoceptor activation can cause a phospholipid effect in adipocytes (267). Epinephrine was also shown to increase ^{32}P incorporation into total adipocyte phospholipids. The effect was blocked by phenoxybenzamine, but not by phentolamine or by beta-adrenergic antagonists. This paradoxical insensitivity to phentolamine must obviously be resolved before the role of phospholipid turnover in alpha-adrenoceptor mechanisms in the adipocyte can be evaluated.

The sum of these studies suggests that alpha-adrenoceptor activation may cause increased K permeability of the adipocyte membrane by a mechanism similar to the exocrine glands and liver. Obviously, the Ca-dependency and relevant Ca movements must be determined before such a suggestion can be considered tenable.

3. Neutrophils. Many of the various functions of leukocytes are stimulated by receptor activation and in some cases associated ion movements have been documented. One extensively studied system is the secretion of lysosomal enzymes by neutrophils (112, 132, 195, 258, 262). Secretion can be stimulated by complement (or zymosan-treated serum), acetylcholine, formylmethionyl leucyl phenylalanine (F-met-leu-phe, a chemotactic peptide), valinomycin, and A-23187. All of these agents depend at least quantitatively upon the presence of Ca.

The secretory effects of F-met-leu-phe are greatly potentiated by cytochalasin B (258). Both F-met-leu-phe (with cytochalasin B) and ionophore A-23187 stimulate influx of ^{45}Ca and ^{22}Na and efflux of ^{42}K (195). The effects of F-met-leu-phe plus cytochalasin B on Na influx did not require Ca in the medium, but the effect on K efflux did requre Ca. On the other hand, A-23187 only stimulated Na influx when Ca was present (195). It has recently been suggested that F-met-leu-phe can elicit a phosphatidylinositol effect in lymphocytes (cf. 188). These results are consistent with a mechanism by which receptor activation leads to a stimulation of Ca and Na influx. The elevated Ca catalyzes secretion and K efflux, and perhaps some Na influx.

Chemotactic agents also act to stimulate chemotaxis by alterations in ion movements, although the mechanism appears quite different (22, 257). Optimal chemotaxis requires Ca and Mg (21). However, cytochalasin B does not potentiate the effects of F-met-leu-phe on chemotaxis but greatly potentiates the effect on Ca influx. Also, the chemotactic response is inhibited by ouabain while secretory responses were affected only occasionally (258). In recent studies, a direct stimulation by F-met-leu-phe of Na,K-ATPase activity in a neutrophil plasmalemma fraction was observed (22). This effect was blocked by carbobenzoxy-methionyl-phenylalanine, a competitive antagonist to the chemotactic actions of F-met-leu-phe. The significance of these effects in the mechanism of chemotaxis is not yet fully understood.

Activation of lymphocytes by mitogens may also involve alterations in ion movements, although the details here are far from clear. Several investigators have observed a stimulation of ^{45}Ca influx in lymphocytes by phytohemagglutinin A (10, 279, 280) and Ca has been suggested as the critical signal for initiating the mitotic response (25). Ouabain-sensitive K influx is stimulated (233), but so is K efflux (253). It is, therefore, conceivable that Ca influx activates both mitosis and K permeability, although the ionic requirements for the latter effect have not been systematically studied.

IV. Conclusions

The purpose of this review has been to summarize the advances in our understanding of the role of calcium in stimulus-permeability coupling. As is often the case, new information generates new enigmas. It now seems clear that in many tissues, where hormones and neurotransmitters affect membrane permeability, Ca appears to

function as a second messenger in these responses. The most common response is an increase in K permeability (salivary glands, lacrimal gland, liver, taenia coli, adipocytes). In many cases, fluxes of Na (salivary glands, lacrimal gland, pancreas) and Cl (fly salivary gland, intestine, cornea) may be activated as well, although evidence for a role of Ca in these responses is less complete. The mechanism by which Ca produces effects on membrane permeability is currently unknown.

Apparently, the permeabilities are controlled by the level of ionized cytoplasmic Ca. The source of Ca may be either the extracellular space (Ca influx) or cellular binding sites (Ca release). Possibly, occupation of membrane receptors may activate both mechanisms simultaneously (salivary glands, lacrimal gland, pancreas, liver), but in some cases this point remains a source of controversy.

The theory of Michell may provide a clue to the mechanism of coupling of membrane receptors to Ca channels or binding sites. Indirect evidence strongly suggests that, subsequent to receptor occupation, breakdown of surface membrane phosphatidylinositol occurs. The exact role of the phosphatidylinositol breakdown in receptor activation, receptor-channel coupling, or Ca mobilization is unknown. The occurrence of many similarities in the stimulus-permeability coupling mechanism in such diverse tissues as exocrine glands, liver, smooth muscle, epithelia, adipocytes, and neutrophils suggests that aspects of this scheme may be applicable to other, less well understood systems.

Acknowledgments. Critical evaluation by B. H. Marks and G. E. Dambach and assistance in proofreading by B. A. Leslie greatly facilitated the preparation of this review. I am also grateful to R. J. Parod, S. J. Weiss, C. M. Van De Walle, C. A. Landis, and R. A. Haddas for many valuable discussions.

REFERENCES

1. ABDEL-LATIF, A. A., AKHTAR, R. A., AND HAWTHORNE, J. N.: Acetylcholine increases the breakdown of triphosphoinositide of rabbit iris muscle prelabelled with [^{32}P]phosphate. Biochem. J. **162**: 61–73, 1977.
2. ABDEL-LATIF, A. A., AKHTAR, R. A., AND SMITH, J. P.: Studies on the role of triphosphoinositide on cholinergic muscarinic and α-adrenergic receptor function of iris smooth muscle. *In* International Conference on the Cyclitols and Phosphoinositides, ed. by F. Eisenberg and W. W. Wells, Academic Press, New York, in press.
3. ABDEL-LATIF, A. A., GREEN, K., SMITH, J. P., MCPHERSON, J. C., JR., AND MATHENY, J. L.: Norepinephrine-stimulated breakdown of triphosphoinositide of rabbit iris smooth muscle: Effects of surgical sympathetic denervation and *in vivo* electrical stimulation of the sympathetic nerve of the eye. J. Neurochem. **30**: 517–525, 1978.
4. ABDEL-LATIF, A. A., OWEN, M. P., AND MATHENY, J. L.: Adrenergic and cholinergic stimulation of ^{32}P-labelling of phospholipids in rabbit iris muscle. Biochem. Pharmacol. **25**: 461–469, 1976.
5. AKHTAR, R. A., AND ABDEL-LATIF, A. A.: Calcium ion requirement for acetylcholine-stimulated breakdown of triphosphoinositide in rabbit iris smooth muscle. J. Pharmacol. Exp. Ther. **204**: 655–668, 1978.
6. ALEXANDER, J. H., VANLENNEP, E. W., AND YOUNG, J. A.: Water and electrolyte secretion by the exorbital lacrimal gland of the rat studied by micropuncture and catheterization techniques. Pflügers Arch. Gesamte Physiol. Menschen Tiere **337**: 299–309, 1972.
7. ALLAN, D., AND MICHELL, R. H.: Calcium ion-dependent diacylglycerol accumulation in erythrocytes is associated with microvesiculation but not with efflux of potassium ions. Biochem. J. **166**: 495–499, 1977.
8. ALLAN, D., AND MICHELL, R. H.: A calcium-activated polyphosphoinositide phosphodiesterase in the plasma membrane of human and rabbit erythrocytes. Biochem. Biophys. Acta **508**: 277–286, 1978.
9. ALLAN, D., WATTS, R., AND MICHELL, R. H.: Production of 1,2-diacylglycerol and phosphatidate in human erythrocytes treated with calcium ions and ionophore A23187. Biochem. J. **156**: 225–232, 1976.
10. ALLWOOD, G., ASHERSON, G. L., DANEY, M. J., AND GOODFORD, P. J.: The early uptake of radioactive calcium by human lymphocytes treated with phytohemagglutinin. Immunology **21**: 509, 1971.
11. AMSTERDAM, A., AND JAMIESON, J. D.: Structural and functional characterization of isolated pancreatic exocrine cells. Proc. Nat. Acad. Sci. U.S.A. **69**: 2028–2032, 1972.
12. ANDIA-WALTENBAUGH, A. M., KIMURA, S., DIVAKARAN, P., AND LAM, A.: Changes in calcium uptake in mitochondria prepared from perfused livers following glucagon and insulin administration (Abstract). Fed. Proc. **37**: 1300, 1978.
13. ANDREOLI, T. E., AND SCHAFER, J. A.: Mass transport across cell membranes: The effects of antidiuretic hormone on water and solute flows in epithelia. Annu. Rev. Physiol. **38**: 451–500, 1976.
14. ASSIMACOPOULOS-JEANNET, F. D., BLACKMORE, P. F., AND EXTON, J. H.: Studies on α-adrenergic activation of hepatic glucose output. Studies on role of calcium in α-adrenergic activation of phosphorylase. J. Biol. Chem. **252**: 2662–2669, 1977.
15. ASTRUP, J.: Na and K in human red cells. Variations among centrifuged cells. Scand. J. Lab. Clin. Invest. **33**: 231–237, 1974.
16. BARLOW, T. E., GREENWELL, J. R., HARPER, A. A., AND SCRATCHERD, T.: The effect of adrenaline and noradrenaline on the blood flow, electrical conductance and external secretion of the pancreas. J. Physiol. (London) **217**: 665–678, 1971.
17. BASSINGTHWAITE, J. B., FRY, C. H., AND MCGUIGAN, J. A. S.: Relationship between internal calcium and outward current in mammalian ventricular muscle; a mechanism for the control of the action potential duration? J. Physiol. (London) **262**: 15–37, 1976.
18. BATZRI, S., AMSTERDAM, A., SELINGER, Z., OHAD, I., AND SCHRAMM, M.: Epinephrine-induced vacuole formation in parotid gland cells and its independence of the secretory process. Proc. Nat. Acad. Sci. U.S.A. **68**: 121–123, 1971.
19. BATZRI, S., SELINGER, Z., AND SCHRAMM, M.: Potassium

ion release and enzyme secretion: Adrenergic regulation of α- and β-receptors. Science 174: 1029–1031, 1971.
20. BATZRI, S., SELINGER, Z., SCHRAMM, M., AND ROBINOVITCH, M. R.: Potassium release mediated by the epinephrine α-receptor in rat parotid slices. Properties and relation to enzyme secretion. J. Biol. Chem. 248: 361–368, 1973.
21. BECKER, E. L.: Stimulated neutrophil locomotion. Arch. Pathol. Lab. Med. 101: 509–513, 1977.
22. BECKER, E. L., TALLEY, J. V., SHOWELL, H. J., NACCACHE, P. H., AND SHA'AFI, R. I.: Activation of the rabbit polymorphonuclear leukocyte membrane "Na^+, K^+" ATPase by chemotactic factor. J. Cell Biol. 77: 329–333, 1978.
23. BENNET, V., AND CUATRECASAS, P.: Mechanism of activation of adenylate cyclase by *Vibrio cholerae* enterotoxin. J. Membrane Biol. 22: 29–52, 1975.
24. BERRIDGE, M. J.: The role of 5-hydroxytryptamine and cyclic AMP in the control of fluid secretion by isolated salivary glands. J. Exp. Biol. 53: 171–186, 1970.
25. BERRIDGE, M. J.: The interaction of cyclic nucleotides and calcium in the control of cellular activity. *In* Advances in Cyclic Nucleotide Research, vol. 6, ed. by P. Greengard and G. A. Robison, pp. 1–98, Raven Press, New York, 1975.
26. BERRIDGE, M. J.: Cyclic AMP, calcium and fluid secretion. *In* Transport of Ions and Water in Animals, ed. by B. L. Gupta, R. B. Moreton, J. L. Oschman, and B. J. Wall, pp. 225–238, Academic Press, New York, 1977.
27. BERRIDGE, M. J., AND FAIN, J. N.: Inhibition of phosphatidylinositol synthesis and the inactivation of calcium entry after prolonged exposure of the blowfly salivary gland to 5-hydroxytryptamine. Biochem. J. 178: 59–69, 1979.
28. BERRIDGE, M. J., LINDLEY, B. D., AND PRINCE, W. T.: Membrane permeability changes during stimulation of isolated salivary glands of *Calliphora* by 5-hydroxytryptamine. J. Physiol. (London) 244: 549–567, 1975.
29. BERRIDGE, M. J., LINDLEY, B. D., AND PRINCE, W. T.: Studies on the mechanism of fluid secretion by isolated salivary glands of *Calliphora*. J. Exp. Biol. 64: 311–322, 1976.
30. BERRIDGE, M. J., AND OSCHMAN, J. L.: Transporting Epithelia. Academic Press, New York, 1972.
31. BERRIDGE, M. J., AND PRINCE, W. T.: Transepithelial potential changes during stimulation of isolated salivary glands with 5-hydroxytryptamine and cyclic AMP. J. Exp. Biol. 56: 139–153, 1972.
32. BESLEY, G. T. N., AND SNART, R. N.: Effect of vasopressin on the uptake of calcium ions by kidney mitochrondria and on the concentration of adenosine 3′,5′-cyclic monophosphate in toad bladder. Biochem. J. 125: 60P, 1971.
33. BIANCHI, C. P.: Cell Calcium. Butterworths, London, 1968.
34. BILLAH, M. M., AND MICHELL, R. H.: Stimulation of the breakdown and resynthesis of phosphatidylinositol in rat hepatocytes by angiotensin, vasopressin and adrenaline. Biochem. Soc. Trans., in press.
35. BIRDSALL, N. J. M., AND HULME, E. C.: Biochemical studies on muscarinic acetylcholine receptors. J. Neurochem. 27: 7–16, 1976.
36. BLACKMORE, P. F., BRUMLEY, F. T., MARKS, J. L., AND EXTON, J. H.: Studies on α-adrenergic activation of hepatic glucose output. Relationship between α-adrenergic stimulation of calcium efflux and activation of phosphorylase in isolated rat liver parenchymal cells. J. Biol. Chem. 253: 4851–4858, 1978.
37. BLAUSTEIN, M. P.: The regulation of intracellular calcium in nerve terminals; Lessons from the synaptosome. Neurosci. Res. Program Bull. 15: 582–590, 1977.
38. BLUM, R. M., AND HOFFMAN, J. F.: The membrane locus of Ca-stimulated K transport in energy-depleted human red blood cells. J. Membrane Biol. 6: 315–328, 1971.
39. BOGACH, P. G., AND KLEVETS, M. Y.: Effect of noradrenaline and adrenaline on the membrane potential and membrane resistance of the cells of smooth muscles to direct electric current in solutions devoid of sodium and chloride ions. Biophysics 12: 1140–1146, 1967.
40. BOLTON, J. E., AND FIELD, M.: Ca ionophore-stimulated ion secretion in rabbit ileal mucosa: Relation to actions of cyclic 3′,5′-AMP and carbamylcholine. J. Membrane Biol. 35: 159–173, 1977.
41. BOLTON, T. B.: The permeability change produced by acetylcholine in smooth muscle. *In* Drug Receptors, ed. by H. P. Rang, pp. 87–102, University Park Press, Baltimore, 1973.
42. BONTING, S. L.: Sodium-potassium activated adenosinetriphosphatase and cation transport. *In* Membranes and Ion Transport, vol. 1, ed. by E. E. Bittar, pp. 257–363, Wiley-Interscience, London, 1970.
43. BORLE, A. B.: Cyclic AMP stimulation of calcium efflux from kidney, liver and heart mitchondria. J. Membrane Biol. 16: 221–236, 1974.
44. BORLE, A. B.: Modulation of mitochondrial control of cytoplasmic calcium activity. *In* Calcium Transport in Contraction and Secretion, ed. by E. Carafoli, F. Clementi, W. Drabikowski, and A. Margreth, pp. 77–86, Elsevier/North-Holland, Amsterdam, 1975.
45. BORLE, A. B.: Cyclic AMP stimulation of calcium efflux from isolated mitochondria: A negative report. J. Membrane Biol. 29: 209–210, 1976.
46. BOTELHO, S. Y.: Tears and the lacrimal gland. Sci. Amer. 211: 78–86, 1964.
47. BOTELHO, S. Y., GOLDSTEIN, A. M., AND HISADA, M.: The effects of autonomic nerve impulses and autonomic drugs on secretion by the lacrimal gland. *In* The Exocrine Glands, Procedings of the 24th Satellite Symposium of the International Congress of the Physiological Sciences, pp. 227–239, 1969.
48. BRUNS, R. R., AND PALADE, G. E.: Studies on blood capillaries. I. General organization of blood capillaries in muscle. J. Cell Biol. 37: 244–299, 1968.
49. BUCKLEY, J. T., AND HAWTHORNE, J. N.: Erythrocyte membrane polyphosphoinositide metabolism and the regulation of calcium binding. J. Biol. Chem. 247: 7218–7223, 1972.
50. BÜLBRING, E., AND KURIYAMA, H.: Effects of changes in ionic environment on the action of acetylcholine and adrenaline on the smooth muscle cells of guinea-pig taenia coli. J. Physiol. (London) 166: 59–74, 1963.
51. BÜLBRING, E., AND TOMITA, T.: Increase of membrane conductance by adrenaline in the smooth muscle of guinea-pig taenia coli. Proc. Roy. Soc. Ser. Biol. Sci. 172: 89–102, 1969.
52. BÜLBRING, E., AND TOMITA, T.: Effect of calcium, barium and manganese on the action of adrenaline in the smooth muscle of the guinea-pig taenia coli. Proc. Roy. Soc. Ser. Biol. Sci. 172: 121–136, 1969.
53. BÜLBRING, E. AND TOMITA, T.: The α-action of catecholamines on the guinea-pig taenia coli in K-free and Na-free solution and in the presence of ouabain. Proc. Roy. Soc. Ser. Biol. Sci. 197: 255–269, 1977.
54. BÜLBRING, E., AND TOMITA, T.: Calcium requirement for the α-action of catecholamines on guinea-pig taenia coli. Proc. Roy. Soc. Ser. Biol. Sci. 197: 271–284, 1977.
55. BURGEN, A. S. V.: The secretion of potassium in saliva. J. Physiol. (London) 132: 20–39, 1956.
56. BURGEN, A. S. V., AND EMMELIN, N. G.: Physiology of the Salivary Glands. Edward Arnold, London, 1961.
57. BURGEN, A. S. V., AND SPERO, L.: The action of acetylcholine and other drugs on the efflux of potassium and rubidium from smooth muscle of the guinea-pig intestine. Brit. J. Pharmacol. 34: 99–115, 1968.
57a. BURGESS, G. M., CLARET, M., AND JENKINSON, D. H.: Effects of catecholamines, ATP and the ionophore A 23187 on potassium and calcium movements in isolated hepatocytes. Nature, in press.
58. BUTCHER, F. R.: The role of calcium and cyclic nucleotides in α-amylase release from slices of rat parotid:

Studies with the divalent cation ionophore A-23187. Metabolism **24**: 409–418, 1975.

59. BUTCHER, F. R.: Calcium and cyclic nucleotides in the regulation of secretion from the rat parotid by autonomic agonists. *In* Advances in Cyclic Nucleotide Research, ed. by W. J. George and L. J. Ignarro, vol. 9, pp. 707–721, Raven Press, New York, 1978.
60. BUTCHER, F. R., MCBRIDE, P. A., AND RUDICH, L.: Cholinergic regulation of cyclic nucleotide levels, amylase release and K^+ efflux from rat parotid glands. Mol. Cell. Endocrinol. **5**: 243–254, 1976.
61. BUTCHER, F. R., RUDICH, L., EMLER, C., AND NEMEROVSKI, M.: Adrenergic regulation of cyclic nucleotide levels, amylase release and potassium efflux in rat parotid gland. Mol. Pharmacol. **12**: 862–870, 1976.
62. CANDIA, O. A., MONTOREANO, R., AND PODOS, S. M.: Effect of the ionophore A23187 on chloride transport across isolated frog cornea. Amer. J. Physiol. **233**: F94–F101, 1977.
63. CASE, R. M.: The role of calcium and of cyclic AMP in pancreatic secretory processes. *In* Secretory Mechanisms of Exocrine Glands, ed. by N. A. Thorn and O. H. Petersen, pp. 344–354, Munksgaard, Copenhagen, 1974.
64. CASE, R. M.: Synthesis, intracellular transport and discharge of exportable proteins in the pancreatic acinar cell and other cells. Biol. Rev. **53**: 211–354, 1978.
65. CASE, R. M., CLAUSEN, T., AND SCOTT-WILSON, C. J.: Extracellular sodium and stimulus-secretion coupling in the rat exocrine pancreas. J. Physiol. (London) **284**: 47p–48p, 1978.
66. CHAMBAUT-GUÉRIN, A.-M., MULLER, P., AND ROSSIGNOL, B.: Microtubules and protein secretion in rat lacrimal glands. Relationship between colchicine binding and its inhibitory effect on the intracellular transport of proteins. J. Biol. Chem. **253**: 3870–3876, 1978.
67. CHANDLER, D. E., AND WILLIAMS, J. A.: Pancreatic acinar cells: Effects of lanthanum ions on amylase release and calcium ion fluxes. J. Physiol. (London) **243**: 831–846, 1974.
68. CHEN, J.-L. J., BABCOCK, D. F., AND LARDY, H. A.: Norepinephrine, vasopressin, glucagon, and A23187 induce efflux of calcium from an exchangeable pool in isolated rat hepatocytes. Proc. Nat. Acad. Sci. U.S.A. **75**: 2234–2238, 1978.
69. CLARET-BERTHON, B., CLARET, M., AND MAZET, J. L.: Fluxes and distribution of calcium in rat liver cells: Kinetic analysis and identification of pools. J. Physiol. (London) **272**: 529–552, 1977.
70. CURRAN, P. F.: Na, Cl, and water transport by rat ileum *in vitro*. J. Gen. Physiol. **43**: 1137–1148, 1960.
71. CURRAN, P. F.: Ion transport in intestine and its coupling to other transport processes. Fed. Proc. **24**: 993–999, 1965.
72. CURRAN, P. F., AND MCINTOSH, J. R.: A model system for biological water transport. Nature (London) **193**: 347–348, 1962.
73. CUTHBERT, A. W.: Characteristics of sodium channels in transporting epithelia. *In* Drugs and Transport Processes, ed. by B. A. Callingham, pp. 173–184, University Park Press, Baltimore, 1974.
74. CUTHBERT, A. W.: Aspects of the pharmacology of passive ion transfer across cell membranes. *In* Progress in Medicinal Chemistry, ed. by G. P. Ellis and G. B. West, vol. 14, pp. 1–49, Elsevier/North Holland, Amsterdam, 1977.
75. CUTHBERT, A. W., AND WONG, P. Y. D.: Calcium release in relation to permeability changes in toad bladder epithelium following antidiuretic hormone. J. Physiol. (London) **241**: 407–422, 1974.
76. DAMBACH, G., AND FRIEDMANN, N.: Substrate-induced membrane potential changes in the perfused rat liver. Biochim. Biophys. Acta **367**: 366–370, 1974.
77. DANIEL, E. E., PATON, D. M., TAYLOR, G. S., AND HODGSON, B. J.: Adrenergic receptors for catecholamine effects on tissue electrolytes. Fed. Proc. **29**: 1410–1425, 1970.
78. DAVSON, H.: The effect of some metabolic poisons on the permeability of the rabbit erythrocyte to potassium. J. Cell. Comp. Physiol. **18**: 173–185, 1941.
79. DETH, R., AND VAN BREEMEN, C.: Relative contributions of Ca^{2+} influx and cellular Ca^{2+} release during drug induced activation of the rabbit aorta. Pflügers Arch. Gesamte Physiol. Menschen Tiere **348**: 13–32, 1974.
80. DETH, R., AND VAN BREEMEN, C.: Agonist induced release of intracellular Ca^{2+} in the rabbit aorta. J. Membrane Biol. **30**: 363–380, 1977.
81. DEWULF, H., AND KEPPENS, S.: Is calcium the second messenger in liver for cyclic AMP-independent glycogenolytic hormones? Arch. Int. Physiol. Biochem. **84**: 159–160, 1976.
82. DIAMOND, J. M., AND BOSSERT, W. H.: Standing-gradient osmotic flow. A mechanism for coupling of water and solute flow in epithelia. J. Gen. Physiol. **50**: 2061–2083, 1967.
83. DIAMOND, J. M., AND BOSSERT, W. H.: Functional consequences of ultrastructural geometry in "backwards" fluid-transporting epithelia. J. Cell Biol. **37**: 694–702, 1968.
84. DIAMOND, J. M., AND TORMEY, J. MCD.: Role of long extracellular channels in fluid transport across epithelia. Nature (London) **210**: 817–820, 1966.
85. DIAMOND, J. M., AND TORMEY, J. MCD.: Studies on the structural basis of water transport across epithelial membranes. Fed. Proc. **25**: 1458–1463, 1966.
86. DOUGLAS, W. W., AND POISNER, A. M.: The influence of calcium on the secretory response of the submaxillary gland to acetylcholine or to noradrenaline. J. Physiol. (London) **165**: 528–541, 1963.
87. D'SILVA, J. L.: The action of adrenaline on serum potassium. J. Physiol. (London) **82**: 393–398, 1934.
88. FAIN, J. N.: Biochemical aspects of drug and hormone action on adipose tissue. Pharmacol. Rev. **25**: 67–118, 1973.
89. FAIN, J. N., AND BERRIDGE, M. J.: Relationship between hormonal activation of phosphatidylinositol hydrolysis, fluid secretion and calcium flux in the blowfly salivary gland. Biochem. J. **178**: 45–58, 1979.
90. FERREIRA, H. G., AND LEW, V. L.: Use of ionophore A23187 to measure cytoplasmic Ca buffering and activation of the Ca pump by internal Ca. Nature (London) **259**: 47–49, 1976.
91. FIELD, M.: Intestinal secretions. Gastroenterology **66**: 1063–1084, 1974.
92. FIELD, M.: Cholera toxin, adenylate cyclase, and the process of active secretion in the small intestine: The pathogenesis of diarrhea in cholera. *In* Physiology of Membrane Disorders, ed. by T. E. Andreoli, J. F. Hoffman and D. D. Fanestil, Plenum Press, New York, in press.
93. FIELD, M.: Some speculations on the coupling between sodium and chloride transport processes in mammalian and teleost intestine. *In* Membrane Transport Processes, ed. by J. F. Hoffman, vol. 1, pp. 277–292, Raven Press, New York, 1978.
94. FINDER, A. G., BOYME, T., AND SHOEMAKER, W. G.: Relationship of hepatic potassium efflux to phosphorylase activation induced by glucagon. Amer. J. Physiol. **206**: 738–742, 1964.
95. FINK, S. A., AND WILLIAMS, J. A.: Adrenergic receptors mediating depolarization in brown adipose tissue. Amer. J. Physiol. **231**: 700–706, 1976.
96. FLECKSTEIN, A.: Specific pharmacology of calcium in myocardium, cardiac pacemakers, and vascular smooth muscle. Annu. Rev. Pharmacol. Toxicol. **17**: 149–166, 1977.

96a. FRIEDMAN, Z. Y., AND SELINGER, Z.: A transient release of potassium mediated by the action of substance P on rat parotid slices. J. Physiol. (London) **278**: 461–469, 1978.

97. FRIEDMANN, N., AND PARK, C. R.: Early effects of 3′,5′-adenosine monophosphate on the fluxes of calcium

and potassium in the perfused liver of normal and adrenalectomized rats. Proc. Nat. Acad. Sci. U.S.A. 61: 501–508, 1968.

98. FRIEDMANN, N., SOMLYO, A. V., AND SOMLYO, A. P.: Cyclic AMP, cyclic GMP and glucagon: Effect on rat liver cell membrane potential. Science 171: 400–402, 1971.

99. FRIZZELL, R. A.: Active chloride secretion by rabbit colon: Calcium-dependent stimulation by ionophore A23187. J. Membrane Biol. 35: 175–187, 1977.

100. FURCHGOTT, R. F.: Pharmacological characterization of receptors: Its relation to radioligand-binding studies. Fed. Proc. 37: 115–120, 1978.

101. GARDNER, J. D., CHRISTOPHE, J. P., CONLON, T. P., AND FRANDSEN, E. K.: Initial steps in the action of cholecystokinin and cholinergic agents on pancreatic acinar cells. *In* First International Symposium on Hormonal Receptors in Digestive Tract Physiology, INSERM Symposium No. 3, ed. by S. Bonfils, P. Fromageot, and G. Rosselin, pp. 237–246, Elsevier/North-Holland, Amsterdam, 1977.

102. GARDNER, J. D., CONLON, T. P., KLAVEMAN, H. L., ADAMS, T. D., AND ONDETTI, M. A.: Action of cholecystokinin and cholinergic agents on calcium transport in isolated pancreatic acinar cells. J. Clin. Invest. 56: 366–375, 1975.

103. GARDNER, J. D., AND HAHNE, W. F.: Calcium transport in dispersed acinar cells from rat pancreas. Biochim. Biophys. Acta 471: 466–476, 1977.

104. GARDOS, G.: Effect of ethylendiaminetetraacetate on the permeability of human erythrocytes. Acta Physiol. Acad. Sci. Hung. 14: 1–5, 1958.

105. GARDOS, G.: The function of calcium in the potassium permeability of human erythrocytes. Biochim. Biophys. Acta 30: 653–654, 1958.

106. GARDOS, G.: The role of calcium in the potassium permeability of human erythrocytes. Acta Physiol. Acad. Sci. Hung. 15: 121–125, 1959.

107. GILL, D. M.: Mechanism of action of cholera toxin. *In* Advances in Cyclic Nucleotide Research, ed. by P. Greengard and G. A. Robison, vol. 8, pp. 85–118, Raven Press, New York, 1977.

108. GODFRAIND, J. M., KAMAMURA, H., KRNJEVIC, K., AND PUMAIN, R.: Actions of dinitrophenol and some other metabolic inhibitors on cortical neurones. J. Physiol. (London) 215: 199–222, 1971.

109. GODFRAIND, J. M., KRNJEVIC, K., AND PUMAIN, R.: Unexpected features of the action of dinitrophenol on cortical neurones. Nature (London) 228: 562–564, 1970.

110. GOLDBERG, N. D., AND HADDOX, M. K.: Cyclic GMP metabolism and involvement in biological regulation. Annu. Rev. Biochem. 46: 823–896, 1977.

111. GOLDBERG, N. D., HADDOX, M. K., NICOL, S. E., GLASS, D. B., SANFORD, C. H., KUEHL, F. A., JR., AND ESTENSEN, R.: Biological regulation through opposing influences of cyclic GMP and cyclic AMP: The Yin Yang hypothesis. *In* Advances in Cyclic Nucleotide Research, ed. by G. I. Drummond, P. Greengard, and G. A. Robison, vol. 5, pp. 307–330, Raven Press, New York, 1975.

112. GOLDSTEIN, I. M., WEISSMAN, G., DUNHAM, P. B., AND SOBERMAN, R.: The role of calcium in secretion of enzymes by human polymorphonuclear leukocytes. *In* Calcium Transport in Contraction and Secretion, ed. by E. Carafoli, F. Clementi, W. Drabikowski, and A. Margreth, pp. 185–193, Elsevier/North Holland, Amsterdam, 1975.

112a. HADDAS, R. A., LANDIS, C. A., AND PUTNEY, J. W., JR.: Relationship between calcium release and potassium release in rat parotid gland. J. Physiol. (London), in press.

113. HAND, A. R., AND OLIVER, C.: Relationship between the Golgi apparatus, GERL, and the secretory granules in acinar cells of the rat exorbital lacrimal gland. J. Cell Biol. 74: 399–413, 1977.

114. HARPER, J. F., AND BROOKER, G.: Refractoriness to muscarinic and adrenergic agonists in the rat parotid: Responses of adenosine and guanosine cyclic 3′,5′-monophosphates. Mol. Pharmacol. 13: 1048–1059, 1977.

115. HARPER, J. F., AND BROOKER, G.: Amylase secretion from the rat parotid: Refractoriness to muscarinic and adrenergic agonists. Mol. Pharmacol. 14: 1031–1045, 1978.

116. HAYLETT, D. G.: Effects of sympathomimetic amines on ^{45}Ca efflux from liver slices. Brit. J. Pharmacol. 57: 158–160, 1976.

117. HAYLETT, D. G., AND JENKINSON, D. H.: Effects of noradrenaline on the membrane potential and ionic permeability of parenchymal cells in the liver of the guinea-pig. Nature (London) 224: 80–81, 1969.

118. HAYLETT, D. G., AND JENKINSON, D. H.: Effects of noradrenaline on potassium efflux, membrane potential and electrolyte levels in tissue slices prepared from guinea-pig liver. J. Physiol. (London) 225: 721–750, 1972.

119. HAYLETT, D. G., AND JENKINSON, D. H.: The receptors concerned in the actions of catecholamines on glucose release, membrane potential and ion movements in guinea-pig liver. J. Physiol. (London) 225: 751–772, 1972.

120. HAYLETT, D. G., AND JENKINSON, D. H.: Actions of catecholamines on the membrane properties of liver cells. *In* Drug Receptors, ed. by H. P. Rang, pp. 15–25, University Park Press, Baltimore, 1973.

121. HEISLER, S., AND GRONDIN, G.: Effect of lanthanum on ^{45}Ca flux and secretion of protein from rat exocrine pancreas. Life Sci. 13: 783–794, 1973.

122. HENRIQUES, V., AND ORSKOV, S. L.: Untersuchungen über die Schwankungen des Kationengehalts der roten Blutkörperchen. II. Änderung des Kaliumgehalts der Blutkörperchen bei Bleivergiftung. Skand. Arch. Physiol. 74: 78–85, 1936.

123. HERMAN, G., BUSSON, S., OVTRACHT, L., MAURS, C., AND ROSSIGNOL, B.: Regulation of protein discharge in two exocrine glands: rat parotid and exorbital lacrimal glands. Analogies between cholinergic (muscarinic) and α-adrenergic stimulation and importance of extracellular calcium. Biol. Cellulaire 31: 255–264, 1978.

124. HERZOG, V., SIES, H., AND MILLER, F.: Exocytosis in secretory cells of rat lacrimal gland. Peroxidase release from lobules and isolated cells upon cholinergic stimulation. J. Cell Biol. 70: 692–706, 1976.

125. HEYER, C. B., AND LUX, H. D.: Control of the delayed outward potassium currents in bursting pacemaker neurones of the snail, *Helix pomatia*. J. Physiol. (London) 262: 349–382, 1976.

126. HOFFMAN, J. F., AND BLUM, R. M.: On the nature of the transport pathway used for Ca-dependent K movement in human red blood cells. *In* Membrane Toxicity, ed. by M. W. Miller and A. E. Shamoo, pp. 381–406, Plenum Press, New York, 1977.

127. HOFFMAN, J. F., AND KNAUF, P. A.: The mechanism of the increased K transport induced by Ca in human red blood cells. *In* Erythrocytes, Thrombocytes and Leucocytes, ed. by E. Gerlach, K. Moser, E. Deutsch, and W. Wilmanns, pp. 66–70, Georg Thieme, Stuttgart, 1973.

128. HOKIN, L. E.: Effects of calcium omission on acetylcholine-stimulated amylase secretion and phospholipid synthesis in pigeon pancreas slices. Biochim. Biophys. Acta 115: 219–221, 1966.

129. HOKIN, M. R.: Breakdown of phosphatidylinositol in the pancreas in response to pancreozymin and acetylcholine. *In* Secretory Mechanisms of Exocrine Glands, ed. by N. A. Thorn and O. H. Petersen, pp. 101–112, Munksgaard, Copenhagen, 1974.

130. HOKIN, M. R., AND HOKIN, L. E.: Enzyme secretion and the incorporation of P^{32} into phospholipids of pancreas slices. J. Biol. Chem. **203**: 967-977, 1953.

131. HOTTA, Y., AND TSUKUI, R.: Effect on the guinea-pig taenia coli of the substitution of strontium or barium ions for calcium ions. Nature (London) **217**: 867-869, 1968.

132. IGNARRO, L. J., AND CECH, S. Y.: Bidirectional regulation of lysosomal enzyme secretion and phagocytosis in human neutrophils by guanosine 3',5'-monophosphate and adenosine 3',5'-monophosphate. Proc. Soc. Exp. Biol. Med. **151**: 448-452, 1975.

133. ISENBERG, G.: Is potassium conductance of cardiac Purkinje fibres controlled by $[Ca^{2+}]_i$? Nature (London) **253**: 273-274, 1975.

134. ISENBERG, G.: Cardiac Purkinje fibres. Resting, action, and pacemaker potential under the influence of $[Ca^{2+}]_i$ as modified by intracellular injection technique. Pflügers Arch. Gesamte Physiol. Menschen Tiere **371**: 51-59, 1977.

135. ISENBERG, G.: Cardiac Purkinje fibres. The slow inward current component under the influence of modified $[Ca^{2+}]_i$. Pflügers Arch. Gesamte Physiol. Menschen Tiere **371**: 61-69, 1977.

136. ISENBERG, G.: Cardiac Purkinje fibres. $[Ca^{2+}]_i$ controls steady state potassium conductance. Pflügers Arch. Gesamte Physiol. Menschen Tiere **371**: 71-76, 1977.

137. ISENBERG, G.: Cardiac Purkinje fibres. $[Ca^{2+}]_i$ controls the potassium permeability via the conductance components g_{K1} and $\bar{g}_{K2}$. Pflügers Arch. Gesamte Physiol. Menschen Tiere **371**: 77-85, 1977.

138. IWATSUKI, H., AND PETERSON, O. H.: Pancreatic acinar cells: The acetylcholine equilibrium potential and its ionic dependency. J. Physiol. (London) **269**: 735-751, 1977.

139. IWATSUKI, N., AND PETERSEN, O. H.: Acetylcholine-like effects of intracellular calcuim application in pancreatic acinar cells. Nature (London) **268**: 147-149, 1977.

140. IWATSUKI, N., AND PETERSEN, O. H.: Pancreatic acinar cells: Acetylcholine-evoked electrical uncoupling and its ionic dependency. J. Physiol. (London) **274**: 81-96, 1978.

141. IWATSUKI, N., AND PETERSEN, O. H.: In vitro action of bombesin on amylase secretion, membrane potential, and membrane resistance in rat and mouse pancreatic acinar cells. A comparison with other secretagogues. J. Clin. Invest. **61**: 41-46, 1978.

142. IWATSUKI, N., AND PETERSEN, O. H.: Membrane potential, resistance, and intercellular communication in the lacrimal gland: Effects of acetylcholine and adrenaline. J. Physiol. (London) **275**: 507-520, 1978.

143. JAFFERJI, S. S., AND MICHELL, R. H.: Muscarinic cholinergic stimulation of phosphatidylinositol turnover in the longitudinal smooth muscle of guinea-pig ileum. Biochem. J. **154**: 653-657, 1976.

144. JENKINSON, D. H., HAYLETT, D. G., AND KOLLER, K.: Effects of catecholamines on the ionic permeability of cell membranes. *In* Cell Membrane Receptors for Drugs and Hormones: A Multidisciplinary Approach, ed. by L. Bolis and R. W. Straub, pp. 89-105, Raven Press, New York, 1978.

145. JENKINSON, D. H., HAYLETT, D. G., KOLLER, K., AND BURGESS, G.: Classification and actions of liver cell adrenoceptors. *In* Recent Advances in the Pharmacology of Adrenoceptors, Elsevier/North Holland, Amsterdam, in press.

146. JENKINSON, D. H., AND KOLLER, K.: Interactions between the effects of α- and β-adrenoceptor agonists and adenine nucleotides on the membrane potential of cells in guinea-pig liver slices. Brit. J. Pharmacol. **59**: 163-175, 1977.

147. JENKINSON, D. H., AND MORTON, I. K. M.: The effect of noradrenaline on the permeability of depolarized intestinal smooth muscle to inorganic ions. J. Physiol. (London) **188**: 373-386, 1967.

148. JENKINSON, D. H., AND MORTON, I. K. M.: The role of α- and β-adrenergic receptors in some actions of catecholamines on intestinal smooth muscle. J. Physiol. (London) **188**: 387-402, 1967.

149. JENKINSON, D. H., AND MORTON, I. K. M.: Adrenergic blocking drugs as tools in the study of the action of catecholamines on the smooth muscle membrane. Ann. N.Y. Acad. Sci. **139**: 762-771, 1967.

150. JONES, L. M., AND MICHELL, R. H.: Breakdown of phosphatidylinositol provoked by muscarinic cholinergic stimulation of rat parotid-gland fragments. Biochem. J. **142**: 583-590, 1974.

151. JONES, L. M., AND MICHELL, R. H.: Cholinergically stimulated phosphatidylinositol breakdown in parotid-gland fragments is independent of the ionic environment. Bichem. J. **158**: 505-507, 1976.

152. JONES, L. M., AND MICHELL, R. H.: Enhanced phosphatidylinositol breakdown as a calcium-independent response of rat parotid fragments to substance P. Biochem. Soc. Trans., in press.

153. KANAGASUNTHERAM, P., AND RANDLE, P. J.: Calcium metabolism and amylase release in rat parotid acinar cells. Biochem. J. **160**: 547-564, 1976.

154. KANNO, T., AND NISHIMURA, O.: Stimulus-secretion coupling in pancreatic acinar cells: Inhibitory effects of calcium removal and manganese addition on pancreozymin-induced amylase release. J. Physiol. (London) **257**: 309-324, 1976.

155. KANNO, T., AND YAMAMOTO, M.: Differentiation between the calcium-dependent effects of cholecystokinin-pancreozymin and the bicarbonate-dependent effects of secretin in exocrine secretion of the rat pancreas. J. Physiol. (London) **264**: 787-799, 1977.

156. KAO, C. Y.: Some new leads into the physiology of mammalian smooth muscles. *In* Research in Physiology, ed. by F. F. Kao, K. Koizumi, and M. Vassale, pp. 365-372, Aulo Gaggi, Bologna, 1971.

157. KAO, C. Y., INOMATA, H., MCCULLOUGH, J. R., AND YUAN, J. C.: Voltage clamp studies of the actions of catecholamines and adrenergic blocking agents on mammalian smooth muscles. *In* Smooth Muscle Pharmacology and Physiology, ed. by M. Worcel and G. Vassort, pp. 165-176, INSERM, 1976.

158. KAYE, G. I.: Studies on the cornea. III. The fine structure of the frog cornea and the uptake and transport of colloidal particles by the cornea *in vitro*. J. Cell Biol. **15**: 241-258, 1962.

159. KAYE, G. I., WHEELER, H. O., WHITLOCK, R. T., AND LANE, N.: Fluid transport in the rabbit gallbladder. A combined physiological and electron microscopic study. J. Cell Biol. **30**: 237-268, 1966.

160. KEPPENS, S., VANDENHEEDE, J. R., AND DEWULF, H.: On the role of calcium as second messenger in liver for the hormonally induced activation of glycogen phosphorylase. Biochim. Biophys. Acta **496**: 448-457, 1977.

161. KERYER, G., AND ROSSIGNOL, B.: Effect of carbachol on ^{45}Ca uptake and protein secretion in rat lacrimal glands. Amer. J. Physiol. **230**: 99-104, 1976.

162. KIMBERG, D. V.: Cyclic nucleotides and their roles in gastrointestinal secretion. Gastroenterology **67**: 1023-1064, 1974.

163. KIRK, C. J., VERRINDER, T. R., AND HEMS, D. A.: Rapid stimulation, by vasopressin and adrenaline, of inorganic phosphate incorporation into phosphatidylinositol in isolated hepatocytes. Fed. Eur. Biochem. Soc. Lett. **83**: 267-271, 1977.

164. KONDO, S., AND SCHULZ, I.: Calcium ion uptake in isolated pancreas cells induced by secretagogues. Biochim. Biophys. Acta **419**: 76-92, 1976.

165. KRNJEVIC, K., AND LISIEWIECZ, A.: Injections of calcium ions into spinal motoneurones. J. Physiol. (London) **225**: 363-390, 1972.

166. KURIYAMA, H., OSA, T., SAKAMOTO, Y., AND ITO, Y.: Cellular pharmacology of autonomic nerve-effector junction and smooth muscle membranes. *In* Cellular Pharmacology of Excitable Tissues, ed. by T. Narahashi, pp. 262–359, Charles C Thomas, Publisher, Springfield, Ill., 1975.

167. LAWRENCE, J. C., JR., AND LARNER, J.: Evidence for *alpha* adrenergic activation of phosphorylase and inactivation of glycogen synthase in rat adipocytes. Effects of *alpha* and *beta* adrenergic activation of phosphorylase and inactivation of glycogen synthase. Mol. Pharmacol. **13**: 1060–1075, 1977.

168. LEGG, P. G.: Fluorescence studies on neural structures and endocrine cells in the pancreas of the cat. Z. Zellforsch. Mikrosk. Anat. **88**: 487–495, 1968.

169. LESLIE, B. A., PUTNEY, J. W., JR., AND SHERMAN, J. M.: α-Adrenergic, β-adrenergic and cholinergic mechanisms for amylase secretion by rat parotid gland *in vitro*. J. Physiol. (London) **260**: 351–370, 1976.

170. LEW, V. L.: On the ATP dependence of the Ca^{2+}-induced increase in K^+ permeability observed in human red cells. Biochim. Biophys. Acta **233**: 827–830, 1971.

171. LEW, V. L.: On the mechanism of the Ca-induced increase in K permeability observed in human red cell membranes. *In* Comparative Biochemistry and Physiology of Transport, ed. by L. Bolis, K. Bloch, S. E. Luria, and F. Lynen, pp. 310–316, Elsevier/North Holland, Amsterdam, 1974.

172. LEW, V. L., AND BEAUGÉ, L.: Passive cation fluxes in red cell membranes. *In* Transport Across Biological Membranes, ed. by G. Giebisch, D. C. Tosteson, and H. H. Ussing, vol. II, Springer-Verlag, Berlin, in press.

173. LEW, V. L., AND FERREIRA, H. G.: Variable Ca sensitivity of a K-selective channel in intact red-cell membranes. Nature (London) **263**: 336–338, 1976.

174. LEW, V. L., AND FERREIRA, H. G.: Ca transport and the properties of a Ca-sensitive K channel in red cell membranes. Curr. Top. Membranes and Transport **10**: 217–277, 1978.

175. LOEWENSTEIN, W. R.: Permeability of membrane junctions. Ann. N.Y. Acad. Sci. **137**: 441–472, 1966.

176. LOEWENSTEIN, W. R.: Permeable junctions. Cold Spring Harbor Symp. Quant. Biol. **40**: 49–63, 1976.

177. MARIER, S. H., PUTNEY, J. W., JR., AND VAN DE WALLE, C. M.: Control of calcium channels by membrane receptors in the rat parotid gland. J. Physiol. (London) **279**: 141–151, 1978.

178. MARSHALL, J. M.: Modulation of smooth muscle activity by catecholamines. Fed. Proc. **36**: 2450–2455, 1977.

179. MARTINEZ, J. R., AND QUISSEL, D. O.: Potassium release from the rat submaxillary gland *in vitro*. II. Induction by parasympathomimetic secretagogues. J. Pharmacol. Exp. Ther. **199**: 518–525, 1976.

180. MARTINEZ, J. R., QUISSEL, D. O., AND GILES, M.: Potassium release from the rat submaxillary gland *in vitro*. I. Induction by catecholamines. J. Pharmacol. Exp. Ther. **198**: 385–394, 1976.

181. MEECH, R. W.: Intracellular calcium injection causes increased potassium conductance in *Aplysia* nerve cells. Comp. Biochem. Physiol. **42A**: 493–499, 1972.

182. MEECH, R. W.: Intracellular calcium and the control of membrane permeability. *In* Calcium in Biological Systems, pp. 161–191, Cambridge University Press, Cambridge, 1976.

183. MEECH, R. W.: Calcium-dependent potassium activation in nervous tissues. Annu. Rev. Biophys. Bioeng. **7**: 1–18, 1978.

184. MEECH, R. W., AND STRUMWASSER, F.: Intracellular calcium injection activates potassium conductance in *Aplysia* nerve cells (Abstract). Fed. Proc. **29**: 834, 1970.

185. MELDOLESI, J.: Regulation of pancreatic exocrine secretion. Pharmacol. Res. Commun. **8**: 1–24, 1976.

186. MELDOLESI, J., AND MACCHI, G.: Effect of adrenaline on the *in vitro* enzyme secretion in the guinea-pig pancreas. Experientia (Basel) **28**: 50–51, 1971.

187. MICHELL, R. H.: Inositol phospholipids and cell surface receptor function. Biochim. Biophys. Acta **415**: 81–147, 1975.

188. MICHELL, R. H.: Inositol phospholipids in membrane function. Trends in Biochemical Sciences, in press.

189. MICHELL, R. H., JAFFERJI, S. S., AND JONES, L. M.: Receptor occupancy dose-response curve suggests that phosphatidylinositol breakdown may be intrinsic to the mechanism of the muscarinic cholinergic receptor. Fed. Eur. Biochem. Soc. Lett. **69**: 1–5, 1976.

190. MICHELL, R. H., JAFFERJI, S. S., AND JONES, L. M.: The possible involvement of phosphatidylinositol breakdown in the mechanism of stimulus-response coupling at receptors which control cell-surface calcium gates. Advan. Exp. Biol. Med. **83**: 447–464, 1977.

191. MICHELL, R. H., JONES, L. M., AND JAFFERJI, S. S.: The relationship between agonist-stimulated phosphatidylinositol metabolism and the mechanisms of receptor systems. *In* Stimulus-Secretion Coupling in the Gastrointestinal Tract, ed. by R. M. Case and H. Goebell, pp. 89–103, University Park Press, Baltimore, 1976.

192. MILLER, B. E., AND NELSON, D. L.: Calcium fluxes in isolated acinar cells from rat parotid: The effect of adrenergic and cholinergic stimulation. J. Biol. Chem. **252**: 3629–3636, 1977.

193. MILLER, D. J., AND MÖRCHEN, A.: On the effects of divalent cations and ethylene glycol-bis-(β-aminoethyl ether) N,N,N',N'-tetraacetate on action potential duration in frog heart. J. Gen. Physiol. **71**: 47–67, 1978.

194. MORISSET, J., AND BEAUDOIN, A. R.: Biochemical reactions involved in pancreatic enzyme secretion. 2. Inhibitory effects of tetracaine and atropine. Can. J. Physiol. Pharmacol. **55**: 639–643, 1977.

195. NACCACHE, P. H., SHOWELL, H. J., BECKER, E. L., AND SHA'AFI, R. I.: Changes in ionic movements across rabbit polymorphonuclear leukocyte membranes during lysosomal enzyme release. Possible ionic basis for lysosomal enzyme release. J. Cell Biol. **75**: 635–649, 1977.

196. OGILVIE, J. T., MCINTOSH, J. R., AND CURRAN, P. F.: Volume flow in a series—membrane system. Biochim. Biophys. Acta **66**: 441–444, 1963.

197. OHASHI, H.: The relative contribution of K and Cl to the total increase of membrane conductance produced by adrenaline on the smooth muscle of guinea-pig taenia coli. J. Physiol. (London) **212**: 561–575, 1971.

198. ORON, Y., LOWE, M., AND SELINGER, Z.: Incorporation of inorganic [^{32}P] phosphate into rat parotid phosphatidylinositol. Induction through activation of *alpha* adrenergic and cholinergic receptors and relation to K^+ release. Mol. Pharmacol. **11**: 79–86, 1975.

199. OSCHMAN, J. L., AND BERRIDGE, M. J.: Structural and functional aspects of salivary fluid secretion in Calliphora. Tissue and Cell **2**: 281–310, 1970.

200. PALADE, G.: Intracellular aspects of the process of protein synthesis. Science **189**: 347–358, 1975.

201. PAROD, R. J.: Stimulus-permeability Coupling in the Rat Lacrimal Gland, Ph.D. Dissertation, Wayne State University, 1979.

202. PAROD, R. J., AND PUTNEY, J. W., JR.: An alpha-adrenergic receptor mechanism controlling potassium permeability in the rat lacrimal gland acinar cell. J. Physiol. (London) **281**: 359–369, 1978.

203. PAROD, R. J., AND PUTNEY, J. W., JR.: Role of calcium in the receptor-mediated control of potassium permeability in the rat lacrimal gland. J. Physiol. (London) **281**: 371–381, 1978.

204. PARSONS, D. S.: Multimembrane systems: Membrane function and energetics in intestinal mucosal epithelium. *In* Biological Membranes, ed. by D. S. Parsons, pp. 172–192, Oxford University Press, London, 1975.

205. PASSOW, H.: Ion and water permeability of the red blood cell. *In* The Red Blood Cell, ed. by C. Bishop and D.

M. Surgenor, pp. 77–145, Academic Press, New York, 1964.

206. PEDERSEN, G. L., AND PETERSEN, O. H.: Membrane potential measurement in parotid acinar cells. J. Physiol. (London) **234**: 217–227, 1973.
207. PERRY, M. C., AND HALES, C. N.: Factors affecting the permeability of isolated fat-cells from the rat to [^{42}K] potassium and [^{36}Cl] chloride ions. Biochem. J. **117**: 615–621, 1970.
208. PETERSEN, O. H.: Initiation of salt and water transport in mammalian salivary glands by acetylcholine. Phil. Trans. Roy. Soc. London Ser. B Biol. Sci. **262**: 307–314, 1971.
209. PETERSEN, O. H.: Electrophysiology of mammalian gland cells. Physiol. Rev. **56**: 535–577, 1976.
210. PETERSEN, O. H., AND PEDERSEN, G. L.: Membrane effects mediated by alpha- and beta-adrenoceptors in mouse parotid acinar cells. J. Membrane Biol. **16**: 353–362, 1974.
211. PETERSEN, O. H., AND UEDA, N.: Pancreatic acinar cells: The role of calcium in stimulus-secretion coupling. J. Physiol. (London) **254**: 583–606, 1976.
212. PETERSEN, O. H., AND UEDA, N.: Secretion of fluid and amylase in the perfused rat pancreas. J. Physiol. (London) **264**: 819–835, 1977.
213. PORZIG, H.: Comparative study of the effects of propranolol and tetracaine on cation movements in resealed human red cell ghosts. J. Physiol. (London) **249**: 27–49, 1975.
214. POULSEN, J. H.: Acetylcholine-induced transport of Na^+ and K^+ in the perfused cat submandibular gland. Pflügers Arch. Gesamte Physiol. Menschen Tiere **349**: 215–220, 1974.
215. POULSEN, J. H., AND WILLIAMS, J. A.: Effects of the calcium ionophore A23187 on pancreatic acinar cell membrane potentials and amylase release. J. Physiol. (London) **264**: 323–339, 1977.
216. PRINCE, W. T., AND BERRIDGE, M. J.: The role of calcium in the action of 5-hydroxytryptamine and cyclic AMP on salivary glands. J. Exp. Biol. **58**: 367–384, 1973.
217. PRINCE, W. T., BERRIDGE, M. J., AND RASMUSSEN, H.: Role of calcium and adenosine-3′:5′-cyclic monophosphate in controlling fly salivary gland secretion. Proc. Nat. Acad. Sci. U.S.A. **69**: 553–557, 1972.
218. PUTNEY, J. W., JR.: Biphasic modulation of potassium release in the rat parotid gland by carbachol and phenylephrine. J. Pharmacol. Exp. Ther. **198**: 375–384, 1976.
219. PUTNEY, J. W., JR.: Stimulation of ^{45}Ca influx in rat parotid gland by carbachol. J. Pharmacol. Exp. Ther. **199**: 526–537, 1976.
220. PUTNEY, J. W., JR.: Muscarinic, α-adrenergic and peptide receptors regulate the same calcium influx sites in the parotid gland. J. Physiol. (London) **268**: 139–149, 1977.
221. PUTNEY, J. W., JR.: Role of calcium in the actions of agents affecting membrane permeability. *In* Calcium in Drug Action, ed. by G. B. Weiss, pp. 173–194, Plenum Press, New York, 1978.
222. PUTNEY, J. W., JR.: Ionic milieu and control of K permeability in the rat parotid gland. Amer. J. Physiol. **235**: c180–c187, 1978.
223. PUTNEY, J. W., JR.: Role of calcium in the fade of the potassium release response in the rat parotid gland. J. Physiol. (London) **281**: 383–394, 1978.
224. PUTNEY, J. W., JR.: On the role of cellular calcium in the response of the parotid to dibutyryl and monobutyryl cyclic AMP. Life Sci. **22**: 631–638, 1978.
225. PUTNEY, J. W., JR., AND ASKARI, A.: Modification of membrane function by drugs. *In* Physiology of Membrane Disorders, ed. by T. E. Andreoli, J. F. Hoffman, and D. D. Fanestil, pp. 417–445, Plenum Press, New York, 1978.
226. PUTNEY, J. W., JR., LESLIE, B. A., AND MARIER, S. H.: Calcium and the control of potassium permeability in the sublingual gland. Amer. J. Physiol. **235**: c128–c135, 1978.
227. PUTNEY, J. W., JR., AND PAROD, R. J.: Calcium-mediated effects of carbachol on cation pumping and Na uptake in rat parotid gland slices. J. Pharmacol. Exp. Ther. **205**: 449–458, 1978.
228. PUTNEY, J. W., JR., PAROD, R. J., LESLIE, B. A., WEISS, S. J., VAN DE WALLE, C. M., AND MARIER, S. H.: Adrenoceptors on exocrine gland cells. *In* Recent Advances in the Pharmacology of Adrenoceptors, ed. by E. Szabadi, C. M. Bradshaw, and P. Bevan, pp. 35–43, Elsevier/North Holland, Amsterdam, 1978.
229. PUTNEY, J. W., JR., PAROD, R. J., AND MARIER, S. H.: Control by calcium of exocytosis and membrane permeability to potassium in the rat lacrimal gland. Life Sci. **20**: 1905–1912, 1977.
229a. PUTNEY, J. W., JR., AND VAN DE WALLE, C. M.: Effect of carbachol on ouabain-sensitive uptake of ^{86}Rb by dispersed lacrimal gland cells. Life Sci. **24**: 1119–1124, 1979.
230. PUTNEY, J. W., JR., VAN DE WALLE, C. M., AND LESLIE, B. A.: Receptor control of calcium influx in parotid acinar cells. Mol. Pharmacol. **14**: 1046–1053, 1978.
231. PUTNEY, J. W., JR., VAN DE WALLE, C. M., AND LESLIE, B. A.: Stimulus-secretion coupling in the rat lacrimal gland. Amer. J. Physiol. **235**: c188–c198, 1978.
232. PUTNEY, J. W., JR., WEISS, S. J., LESLIE, B. A., AND MARIER, S. H.: Is calcium the final mediator of exocytosis in the rat parotid gland? J. Pharmacol. Exp. Ther. **203**: 144–155, 1977.
233. QUASTEL, M. R., AND KAPLAN, J. G.: Early stimulation of potassium uptake in lymphocytes treated with PHA. Exp. Cell Res. **63**: 230–233, 1970.
234. RASMUSSEN, H., AND GOODMAN, D. B. P.: Relationships between calcium and cyclic nucleotides in cell activation. Physiol. Rev. **57**: 421–509, 1977.
235. RASMUSSEN, H., JENSEN, P., LAKE, W., FRIEDMANN, N., AND GOODMAN, D. B. P.: Cyclic nucleotides and cellular calcium metabolism. *In* Advances in Cyclic Nucleotide Research, Second International Conference on Cyclic AMP, ed. by G. I. Drummond, P. Greengard, and G. A. Robison, vol. 5, pp. 375–394, Raven Press, New York, 1975.
236. ROBERTS, M. L., AND PETERSEN, O. H.: Membrane potential and resistance changes induced in salivary gland acinar cells by microiontophoretic application of acetylcholine and adrenergic agonists. J. Membrane Biol. **39**: 297–312, 1978.
237. ROSE, B., AND LOEWENSTEIN, W. R.: Permeability of a cell junction and the local cytoplasmic free ionized calcium concentration: A study with aequorin. J. Membrane Biol. **28**: 87–119, 1976.
238. ROSENBERGER, L. B., AND TRIGGLE, D. J.: The mechanism of action of ionophore A 23187 on guinea-pig intestinal smooth muscle. Can. J. Physiol. Pharmacol. **57**: 348–358, 1979.
239. RUBIN, R. P.: The role of calcium in the release of neurotransmitter substances and hormones. Pharmacol. Rev. **22**: 389–428, 1970.
240. RUBIN, R. P.: Calcium and the Secretory Process, Plenum Press, New York, 1974.
241. RUDICH, L., AND BUTCHER, F. R.: Effect of substance P and eledoisin on K^+ efflux, amylase release and cyclic nucleotide levels in slices of rat parotid gland. Biochim. Biophys. Acta **444**: 704–711, 1976.
242. SACHS, G.: Ion transport by gastric mucosa. *In* Physiology of Membrane Disorders, ed. by T. E. Andreoli, J. F. Hoffman, and D. D. Fanestil, pp. 563–576, Plenum Press, New York, 1978.
243. SACHS, G., SPENNEY, J. G., AND REHM, W. S.: Gastric secretion. *In* International Review of Physiology, Gastrointestinal Physiology II, ed. by R. K. Crane, vol. 12, pp. 127–170, University Park Press, Baltimore, 1977.
244. SANDOW, A.: Excitation-contraction coupling in skeletal

muscle. Pharmacol. Rev. 17: 265–320, 1965.
245. SARLES, H.: The exocrine pancreas. *In* International Review of Physiology, Gastrointestinal Physiology II, ed. by R. K. Crane, vol. 12, pp. 173–221, University Park Press, Baltimore, 1977.
246. SCARPA, A., MALMSTROM, K., CHIESTI, M., AND CARAFOLI, E.: On the problem of the release of mitochondrial calcium by cyclic AMP. J. Membrane Biol. 29: 205–208, 1976.
247. SCHNEYER, L. H., YOUNG, J. A., AND SCHNEYER, C. A.: Salivary secretion of electrolytes. Physiol. Rev. 52: 720–777, 1972.
248. SCHRAMM, M., AND SELINGER, Z.: The function of α- and β-adrenergic receptors and a cholinergic receptor in the secretory cell of rat parotid gland. *In* Advances in Cytopharmacology, ed. by B. Ceccarelli, F. Clementi, and J. Meldolesi, vol. 2, pp. 29–32, Raven Press, New York, 1974.
249. SCHRAMM, M., AND SELINGER, Z.: The functions of cyclic AMP and calcium as alternative second messengers in parotid gland and pancreas. J. Cyclic Nucleotide Res. 1: 181–192, 1975.
250. SCHULZ, I., AND HEIL, K.: Ca^{++} control of electrolyte permeability: Effect on Na^+ fluxes in isolated membrane vesicles from the cat pancreas. Proceedings of the Symposium on Gastric Ion Transport, Uppsala. Acta Physiol. Scand., Special Supplement, pp. 381–387, 1977.
251. SCHULTZ, S. G.: Mechanisms of absorption. *In* Biological Membranes, ed. by R. M. Dowben, pp. 59–108, Little, Brown and Co., Boston, 1969.
252. SCHULTZ, S. G.: Ion coupled transport across biological membranes. *In* Physiology of Membrane Disorders, ed. by T. E. Andreoli, J. F. Hoffman, and D. D. Fanestil, pp. 273–286, Plenum Press, New York, 1978.
253. SEGEL, G. B., GORDON, B. R., LICHTMAN, M. A., HOLLANDER, M. M., AND KLEMPERER, M. R.: Exodus of $^{42}K^+$ and $^{86}Rb^+$ from rat thymic and human blood lymphocytes exposed to phytohemagglutinin. J. Cell. Physiol. 87: 337–344, 1976.
254. SELINGER, Z.: Diverse functions of calcium in the rat parotid acinar cell. *In* Calcium Transport in Contraction and Secretion, ed. by E. Carafoli, F. Clementi, W. Drabikowski, and A. Margreth, pp. 139–146, Elsevier/North Holland, Amsterdam, 1975.
255. SELINGER, Z., BATZRI, S., EIMERL, S., AND SCHRAMM, M.: Calcium and energy requirements for K^+ release mediated by the epinephrine α-receptor in rat parotid slices. J. Biol. Chem. 248: 369–372, 1973.
256. SELINGER, Z., EIMERL, S., AND SCHRAMM, M.: A calcium ionophore simulating the action of epinephrine on the α-adrenergic receptor. Prod. Nat. Acad. Sci. U.S.A. 71: 128–131, 1974.
257. SHOWELL, H. J., AND BECKER, E. L.: The effects of external K^+ and Na^+ on the chemotaxis of rabbit peritoneal neutrophils. J. Immunol. 116: 99–105, 1976.
258. SHOWELL, H. J., NACCACHE, P. H., SHA'AFI, R. I., AND BECKER, E. L.: The effects of extracellular K^+, Na^+ and Ca^{++} on lysosomal enzyme secretion from polymorphonuclear leukocytes. J. Immunol. 119: 804–811, 1977.
259. SIDDLE, K., AND HALES, C. N.: Calcium and lipolysis in adipose tissue: Effects of ethanedioxybis(ethylamine)tetra-acetate (EGTA) and ionophore A23187. Biochem. Soc. Trans. 5: 959–962, 1977.
260. SIMONS, T. J. B.: The preparation of human red cell ghosts containing calcium buffers. J. Physiol. (London) 256: 209–225, 1976.
261. SIMONS, T. J. B.: Calcium-dependent potassium exchange in human red cell ghosts. J. Physiol. (London) 256: 227–244, 1976.
262. SMITH, R. J., AND IGNARRO, L. J.: Bioregulation of lysosomal enzyme secretion from human neutrophils: Roles of guanosine 3′:5′-monophosphate and calcium in stimulus-secretion coupling. Proc. Nat. Acad. Sci. U.S.A. 72: 108–112, 1975.
263. SNART, R. S.: Role of mitochondrial Ca^{2+} in antidiuretic hormone action. Curr. Probl. Clin. Biochem. 6: 238–248, 1976.
264. SNART, R. S., AND DALTON, T.: Thermal activation of the cyclic AMP stimulated sodium transport across isolated toad bladder and Na^+/K^+ ATPase in toad bladder homogenates. Experientia (Basel) 28: 1028–1029, 1972.
265. SOMLYO, A. P., SOMLYO, A. V., AND FRIEDMANN, N.: Cyclic adenosine monophosphate, cyclic guanosine monophosphate and glucagon: Effects on membrane potential and ion fluxes in the liver. Ann. N.Y. Acad. Sci. 185: 108–114, 1971.
266. SPEARMAN, T. N., AND PRITCHARD, E. T.: Potassium release from submandibular salivary gland *in vitro*. Biochim. Biophys. Acta 466: 198–207, 1977.
267. STEIN, J. M., AND HALES, C. N.: The effect of adrenaline on ^{32}P incorporation into rat fat-cell phospholipids. Biochem. J. 128: 531–541, 1972.
268. STREWLER, G. J., AND ORLOFF, J.: Role of cyclic nucleotides in the transport of water and electrolytes. *In* Advances in Cyclic Nucleotide Research, ed. by P. Greengard and G. A. Robison, vol. 8, pp. 311–362, Raven Press, New York, 1977.
269. STRITTMATTER, W. J., DAVIS, J. N., AND LEFKOWITZ, R. J.: α-Adrenergic receptors in rat parotid cells. I. Correlation of [^{3}H]-dihydroergocryptine binding and catecholamine-stimulated potassium efflux. J. Biol. Chem. 252: 5472–5477, 1977.
270. STRITTMATTER, W. J., DAVIS, J. N., AND LEFKOWITZ, R. J.: α-Adrenergic receptors in rat parotid cells. II. Desensitization of receptor binding sites and potassium release. J. Biol. Chem. 252: 5478–5482, 1977.
271. STUBBS, M., KIRK, C. J., AND HEMS, D. A.: Role of extracellular calcium in the action of vasopressin on hepatic glycogenolysis. Fed. Eur. Biochem. Soc. Lett. 69: 199–202, 1976.
272. THAYSEN, J. H., AND THORN, N. A.: Excretion of urea, sodium, potassium and chloride in human tears. Amer. J. Physiol. 178: 160–164, 1954.
273. THORN, N. A., AND SCHWARTZ, I. L.: Effect of antidiuretic hormone on washout curves of radiocalcium from isolated toad bladder tissue. Gen. Comp. Endocrinol. 5: 710, 1965.
274. TSUJIMOTO, A., TANINO, S., NISHIUE, T., AND KUROGOCHI, Y.: Potassium mobilizing action of 3′,5′-cyclic adenosine monophosphate. Jap. J. Pharmacol. 15: 441–443, 1965.
275. VAN BREEMEN, C., AND DETH, R.: La^{+++} and excitation contraction coupling in vascular smooth muscle. *In* Ionic Actions on Vascular Smooth Muscle, ed. by E. Betz, pp. 26–31, Springer-Verlag, Berlin, 1976.
276. WALTENBAUGH, A. -M. A., AND FRIEDMANN, N.: Hormone sensitive calcium uptake by liver microsomes. Biochem. Biophys. Res. Commun. 82: 603–608, 1978.
277. WEBSTER, P. D., III, BLACK, O., JR., MAINZ, D. L., AND SINGH, M.: Pancreatic acinar cell metabolism and function. Gastroenterology 73: 1434–1449, 1977.
278. WEISS, S. J., AND PUTNEY, J. W., JR.: Does calcium mediate the increase in potassium permeability due to phenylephrine or angiotensin II in the liver? J. Pharmacol. Exp. Ther. 207: 669–676, 1978.
279. WHITNEY, R. B., AND SUTHERLAND, R. M.: Requirement for calcium ions in lymphocyte transformation stimulated by phytohemagglutinin. J. Cell. Physiol. 80: 329–338, 1972.
280. WHITNEY, R. B., AND SUTHERLAND, R. M.: Enhanced uptake of calcium by transforming lymphocytes. Cell. Immunol. 5: 137–147, 1972.
281. WHITTAM, R.: Control of membrane permeability to potassium in red blood cells. Nature (London) 219: 610, 1968.

282. WIESMANN, W., SINHA, S., AND KLAHR, S.: Effects of ionophore A23187 on baseline and vasopressin-stimulated sodium transport in the toad bladder. J. Clin. Invest. **59**: 418–425, 1977.

283. WILBRANDT, W.: A relation between the permeability of the red cell and its metabolism. Trans. Faraday Soc. **33**: 956–959, 1937.

284. WILBRANDT, W.: Die Abhängigkeit der Ionenpermeabilitat der Erythrocyten vom glycolytischen Stoffwechsel. Pflügers Archiv. Gesamte Physiol. Menschen Tiere **243**: 519–536, 1940.

285. WILLIAMS, J. A.: Intracellular control mechanisms regulating secretion by exocrine and endocrine glands. *In* Secretory Mechanisms of Exocrine Glands, ed. by N. A. Thorn and O. H. Petersen, pp. 389–399, Munksgaard, Copenhagen, 1974.

286. WILLIAMS, J. A.: An *in vitro* evaluation of possible cholinergic and adrenergic receptors affecting pancreatic amylase secretion. Proc. Soc. Exp. Biol. Med. **150**: 513–516, 1975.