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THE ROLE OF CALCIUM IN THE RELEASE OF NEUROTRANSMITTER SUBSTANCES AND HORMONES

R. P. RUBIN

Department of Pharmacology, State University of New York Downstate Medical Center, Brooklyn, New York

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I. INTRODUCTION

In the last two or three decades much experimental work has been focused on the role of calcium in the secretory process. As a result, there is now a vast amount of evidence obtained from a wide variety of secretory systems to support the theory that calcium somehow acts as a link in "stimulus-secretion coupling." The present review will attempt to present a coherent picture of what is known concerning the action of calcium (and other ions) on secretory systems, and then will use this picture to discuss possible explanations for the general involvement of calcium in the mechanism whereby substances of diverse nature are extruded from cells. Discussion will focus primarily on the direct effects of calcium on the secretory process, rather than on indirect effects that calcium might have on secretion as the result of its manifold actions on cell processes. For example, by actions at the cell surface calcium controls cohesiveness (223) and permeability of cells (217, 273) and excitability of nervous tissues (32, 38, 294). By actions within the cell, calcium affects metabolism (32, 205) and alters physicochemical properties of the cytoplasm (144). Many of the

biological effects of calcium have been ascribed to the ability of this cation to form stable complexes with various ligands, as evidenced by the tenacity of calcium binding to membrane fragments, lipid monolayers, and adenine nucleotides (1, 48, 113).

Although it is not possible at our present state of knowledge to give a definitive answer as to the site of calcium action, the author hopes that this account will direct attention to unanswered problems in this field and will help to spur further research which will eventually lead to the elucidation of the function of calcium in the basic physiologic process of secretion.

Recent reviews have covered varying aspects of this general subject (71–73, 121, 298), and in order to avoid repetition these aspects will be alluded to only briefly.

II. ACTIONS OF CALCIUM AND OTHER CATIONS IN THE SECRETORY PROCESS

A. Release of neurotransmitter substances

1. Cholinergic synapses. The importance of calcium in synaptic transmission has long been recognized, and the effects of calcium on the excitability of the presynaptic nerve, on the release of transmitter, and on the postsynaptic elements have all been demonstrated (for review see Desmedt, 62). Although the author is well aware of the diverse actions of calcium at the synapse, the scope of this discussion will be limited to the role of this cation in transmitter release. Reports of the effects of calcium on synaptic transmission are too numerous to recount individually, and only certain studies will be specifically cited.

During the latter part of the last century, Locke (214) showed that a muscle would no longer respond to nerve stimulation after 1 or 2 hr in a calcium-deprived medium, although it would still contract upon direct stimulation. A few years later, Mines (238) found that both strontium and barium, but not magnesium, could replace calcium in maintaining synaptic transmission, and he suggested that it is not their electric charge, but some special chemical property which enables these cations to enter into combination with certain tissue constituents in a manner in which magnesium cannot. In 1912, Hagan and Ormond (135) observed that the inhibitory control of the vagus nerve to the amphibian heart was depressed and then restored by the removal and subsequent restoration of calcium. These authors suggested that "the relation of calcium to vagus inhibition should not be stated in the form that calcium is necessary to the process of inhibition in the heart, but rather that calcium is necessary in the nerve apparatus for the conveyance of the vagus impulses to the heart muscle." About 25 years later, Feng (108) carried out his studies on the frog neuromuscular junction, in which he demonstrated an antagonism between calcium and curare, and he concluded that the observed facilitatory action of calcium was somewhere at the synapse and not directly on the muscle.

Although studies such as these suggested that calcium had a presynaptic action on transmitter release, it was not until a method for quantitatively measuring acetylcholine output had been developed that the action of calcium on transmitter release could be more clearly elucidated. In 1940, Harvey and

MacIntosh (141) used the eserinized isolated perfused cervical sympathetic ganglion of the cat to study directly the output of acetylcholine. This classical study, which was the forerunner of most of the work cited in this review, showed that in the absence of calcium there is no release of acetylcholine from preganglionic nerve endings, either during electrical stimulation of the sympathetic trunk or after the injection of potassium. These workers ascribed the effects of calcium deprivation to an action directly on transmitter release and not to an effect on nerve conduction, since they noted that calcium lack produced hyperexcitability of the nervous elements although synaptic transmission was obtunded (141). Hutter and Kostial (169) further showed that acetylcholine release elicited by nerve stimulation was doubled by high calcium (8 to 10 mM), and that sodium ions were not required for acetylcholine release as long as enough sodium (30% of normal) was present to maintain nerve conduction. When acetylcholine release was induced by excess potassium, which presumably acts by local depolarization of the preganglionic nerve terminals, transmitter output was completely independent of the sodium concentration (170). On the other hand, Birks (15) showed that when sodium is reduced to 50 mM there is about a 40% reduction in acetylcholine release during prolonged nerve stimulation of the perfused ganglion; when excess potassium was employed to produce prolonged stimulation, the inhibition during sodium deprivation was observed only after an initial delay of about 3 min. These findings suggest that sodium has some effect on synthesis or mobilization of transmitter, or both (15, 16), although there is also evidence that sodium ions more directly affect the release process by competing with calcium ions (*vide infra*). Magnesium (15 to 25 mM), which antagonizes the actions of calcium in many biological systems (101), depresses acetylcholine output, and high calcium (4 to 10 mM) relieves the magnesium block (169). Barium can replace calcium in maintaining acetylcholine output from the cervical sympathetic ganglion during nerve stimulation (79).

The neuromuscular junction has provided a test system for studying transmitter release despite the fact that acetylcholine is not measured directly. It has been well established from the work of Katz and his associates (57, 58, 103) that when the concentration of calcium is low enough, or the magnesium concentration high, the end-plate potential is reduced to a minimal amplitude and becomes identical in size and shape to the spontaneously occurring miniature end-plate potentials. It was proposed that the miniature end-plate potentials represent basic units (quanta) of transmitter activity, and that the end-plate potential consists of a summation of such quanta (57, 58). The quanta of transmitter, which participate in both spontaneous and evoked release, are uniform in size, and it is this uniformity that enables quantification of transmitter release in terms of a change in the frequency of the miniature end-plate potentials or the quantal content of the end-plate potential. The discovery that acetylcholine was released in discrete quantal packages was made about the same time that electronmicroscopists were discovering the presence of the so-called synaptic vesicles in the cholinergic nerve terminals (60), and now most workers in the field of cholinergic mechanisms feel that the quantal release of transmitter is

related to acetylcholine present in these vesicles (165, 181). The quantal nature of synaptic transmission has been covered in great detail in previous reviews (58, 180, 181, 226) and will not be considered here.

The early work of Feng (108), Cowan (45), and Kuffler (199) suggested that, among its varied effects, calcium was also important for transmitter release at the frog neuromuscular junction. Kuffler (199) showed that during calcium deprivation there was a decrease in the end-plate potential (recorded extracellularly), while the nerves could still conduct impulses and the sensitivity of the end-plate to applied acetylcholine was not diminished but even enhanced. He concluded that the major effect of calcium deprivation was due to a decrease in the transmitter release. Kuffler's findings were initially confirmed by Fatt and Katz (104) and del Castillo and Stark (59) with intracellular recording, and extended to the mammalian neuromuscular junction (31, 99, 209, 220). The effects of calcium were ascribed to the ability of this cation to increase the probability of release of quanta of acetylcholine during depolarization by nerve impulses. Quantitative analysis of the dependence of acetylcholine release on the external calcium concentration suggests that 3 to 4 calcium ions are necessary for the release of each quantal packet of transmitter by the nerve impulse (69, 202). It should be emphasized that the effects of calcium on transmitter release as measured by the increase in quantal content of the end-plate potential cannot be due to a calcium-mediated change in the sensitivity of the end-plate to acetylcholine, since high calcium does not enhance, but even depresses, the end-plate sensitivity to acetylcholine (59, 222, 307).

For a number of years the possibility was entertained that the passage of the impulse into the nerve terminals might be blocked during calcium deprivation. This idea was based upon the fact that complete withdrawal of calcium can render nerve inexcitable after a period of hyperexcitability (112). However, there is indirect evidence that this explanation is not tenable. Magnesium, which can mimic many of the effects of calcium on electrical properties of excitable tissues (112, 294), antagonizes the effects of calcium on evoked acetylcholine release (31, 55, 164, 176). Moreover, in a series of elegant experiments, Katz and Miledi (182–184) provided more direct evidence that the action of calcium is on release and is not mediated by alterations in impulse conduction through the most distal nerve terminals. Electrophoretic application of calcium to the nerve terminal increased the quantal components of the end-plate potential even when the action potential of the nerve terminal diminished in size (182). Furthermore, pulses of calcium increased transmitter release only when immediately preceded by a depolarizing pulse (183, 184); magnesium pulses inhibited release (184). These data indicate that calcium ions are involved in an essential step between membrane depolarization and acetylcholine release that is not related to effects on the electrical properties of the nerve terminal. Strontium and barium are the only cations that can substitute for calcium in maintaining acetylcholine release at the neuromuscular junction (96, 98, 233), although strontium has much less effect than calcium in equimolar concentrations (68).

Acetylcholine release from nerve-muscle preparations can also be augmented

by excess potassium, as evidenced by an increase in miniature end-plate potential frequency (98, 115, 162, 190, 210, 254). The calcium-magnesium antagonism is also observed during potassium stimulation. Calcium-deprivation and high magnesium both depress miniature end-plate potential frequency (30, 98, 162, 210). Calcium concentrations above 2 mM reduce the augmented miniature end-plate potential frequency (98, 117). It does not appear as if the potentiating effect of potassium can be ascribed merely to its depolarizing action, since the progressive increase in miniature end-plate potential frequency continues to develop even after maximal depolarization is attained (116, 254). Thus, potassium ions appear to have a potentiating action on transmitter release in addition to its depolarizing action. This potentiating action may result from potassium mobilizing more quanta into the readily releasable store (254).

The effects of sodium deprivation on transmitter release from motor nerve terminals has been previously considered (298); however, further discussion is needed at this time to elucidate the role of sodium in transmitter release. Evaluating the importance of sodium for transmitter release is fraught with the danger that a reduction in sodium concentration may diminish the amplitude of the action potentials in the nerve terminals. However, with nerve impulse release when low calcium concentrations are employed, a sodium-calcium antagonism can be observed at the neuromuscular junction (17, 42, 115, 189, 190). When the release is evoked by excess potassium, this competition can be observed even with higher calcium concentrations (117). Thus, when the extracellular calcium concentration is low, a decrease in the sodium concentration produces an increase in the quantal content, but when the calcium content is normal, the same decrease in sodium concentration produces smaller changes. The effect of sodium deprivation appears to arise from a competitive interaction between calcium and sodium on the postulated sites necessary for release. The antagonism of calcium by sodium appears to be nonspecific, since other monovalent cations, such as lithium, will also compete with calcium (190). It has been suggested that calcium combines with a hypothetical receptor (X) on the membrane surface that somehow effects acetylcholine release (56, 69, 115, 117, 176), and the suggestion has been advanced that both magnesium and sodium may compete with calcium for entry by their affinity for X (17). On the other hand, the kinetics of the sodium-calcium competition suggest that sodium and magnesium do not compete with calcium at the same site (117). The competition among cations, which depends upon their respective concentrations in the bathing medium, suggests that magnesium and sodium interfere with calcium entry during depolarization. Indeed, electrical stimulation or high potassium enhances calcium uptake into isolated nerve preparations, and this augmented uptake is reduced by excess magnesium (152, 212). In the squid axon, calcium influx is increased 5- to 40-fold when the external sodium is removed (3). Evidence from other calcium-dependent secretory systems will also show a sodium-magnesium-calcium competition and an increased calcium uptake and exchangeability into secretory cells during stimulation. The enhanced calcium fluxes may be depressed by certain inhibitors of secretion, such as magnesium (see section II B).

From evidence obtained by Birks and his associates (15–19), it appears as if *intracellular* sodium also has some effect on acetylcholine release. Their conclusions were made from indirect evidence, and were based mainly upon findings with the cardiac glycoside, digoxin. Cardiac glycosides increase the spontaneous and evoked release of transmitter from motor nerve terminals (15, 18, 97), but the facilitative action is eventually followed by blockade of synaptic transmission (18). The fact that these effects of digoxin are slowed by sodium deprivation (16, 19) and are mimicked by potassium deprivation (18), suggested to these investigators that the effects produced by the cardiac glycosides on motor terminals are generated by an inhibition of the sodium pump and a resulting accumulation of intracellular sodium. Birks and his associates concluded that in order to explain fully the effects of sodium deprivation on acetylcholine release, one must also take into account an intracellular action of sodium. In harmony with this suggestion is the finding that the increase in miniature end-plate potential frequency usually seen with hypertonic solutions was not observed in nerve-muscle preparations depleted of sodium (245). It has been suggested that the carrier responsible for calcium entry is dependent on the intracellular, as well as the extracellular, sodium concentration (17), and this suggestion is supported by the recent observation of Baker *et al.* (3) that calcium influx into squid axons is augmented by increasing the intra-axonal sodium concentration. It will be seen that cardiac glycosides have stimulant actions in a number of other calcium-dependent secretory systems (7, 67, 89, 137, 193). In some of these preparations the effects of the glycosides seem to result from inhibition of the sodium pump, because they are not seen in the absence of external sodium (137) and can be mimicked by potassium-free solutions (18, 277). Thus, the effects of the glycosides in these preparations could be explained by a rise in the internal sodium concentration, which would promote calcium influx (3).

On the other hand, one must be aware of the limitations in over-emphasizing the possible importance of sodium in acetylcholine release. Simpson (298) has correctly pointed out that sodium-deprivation has many effects at the synapse, including a role in acetylcholine synthesis or mobilization (15, 16), in conduction of the nerve impulse, and in the sensitivity of the postsynaptic membrane to the transmitter (104, 305). Furthermore, cardiac glycosides, including ouabain, also appear to have other actions in addition to inhibiting the sodium pump, such as mobilizing calcium (97) and increasing the sensitivity of the postsynaptic elements to acetylcholine (114). Finally, at the neuromuscular junction the release of transmitter seems to depend primarily on the presence of external calcium rather than external sodium. Katz and Miledi (185, 187) have shown that in the complete absence of sodium or in the presence of tetrodotoxin, which should eliminate the regenerative influx of sodium (179), the release of acetylcholine still occurs in response to local depolarization, provided that calcium is present. These data of Katz and Miledi provide further evidence that the inward movement of calcium is the critical step in the process of "stimulus-secretion coupling." Thus, any proposed role of sodium in transmitter release must be related to effects on calcium entry.

In considering the role of calcium in release of transmitter, the question arises

as to whether quantal release by nerve impulses is an acceleration of spontaneous release, or whether spontaneous and evoked acetylcholine release occur through two independent mechanisms. The persistence of miniature end-plate potentials at the frog neuromuscular junction, either in the absence of calcium or in the presence of excess magnesium, originally led del Castillo and Katz (56) to postulate "a calcium independent" quantal release of acetylcholine. However, data on most mammalian preparations suggest that spontaneous release of acetylcholine is calcium-mediated, as evidenced by the increase in miniature end-plate potential frequency in high calcium, or decrease in miniature end-plate potential frequency in high magnesium (30, 96, 162, 163). When mammalian preparations are bathed in calcium-free solutions in the presence of calcium-chelating agents (ethylenediaminetetraacetic acid), the spontaneous release of acetylcholine, as measured by the frequency of miniature end-plate potentials, is greatly reduced or abolished (96, 162, 163); on the other hand, Hubbard *et al.* (163) postulated that there is a small fraction of the spontaneous release that is independent of the calcium concentration. In amphibian preparations, it has recently been shown that in the presence of ethanol concentrations that enhance miniature end-plate potential frequency, increasing the calcium concentration further augments and high magnesium depresses the miniature end-plate potential frequency (250). Thus, it appears that the calcium-dependent mechanism that governs acetylcholine release from cholinergic nerve terminals evoked by nerve stimulation also controls the mechanism of spontaneous release.

At other cholinergic synapses, the effect of low calcium or high magnesium is also to reduce the amount of transmitter release. Thus, in the chick ciliary ganglion, lowering the calcium concentration or elevating the magnesium concentration decreases both the mean quantum content of transmitter and the excitatory postsynaptic potential after nerve stimulation (227). Transmitter release from postganglionic parasympathetic fibers is also proportional to the calcium concentration (119, 324), and calcium and magnesium exert antagonistic effects on potassium-induced acetylcholine release from the isolated guinea-pig intestine (119). In this latter investigation, synthesis as well as release of acetylcholine was measured, and evidence was also obtained that both sodium and magnesium can affect synthesis (118, 119). Sodium deprivation inhibited acetylcholine production (119) and excess magnesium enhanced synthesis (118). The enhanced synthesis observed with excess magnesium was thought to be responsible for an increased spontaneous liberation of acetylcholine after an initial depression of output (118). The ability of magnesium to augment acetylcholine synthesis (see also 63), provides an explanation for the seemingly anomalous reports that magnesium is able to facilitate acetylcholine release (147, 163).

When evaluating the actions of cations on transmitter release, one should always consider the possibility that the effects of a given cation might be not on release *per se*, but on some other process that precedes the extrusion process, such as transmitter synthesis or mobilization. In peripheral nerve acetylcholine appears to exist in at least two pools: a readily releasable and a nonreleasable store. Although the prime effect of calcium is to increase that fraction of the readily releasable store which is released by the nerve impulse, this store can

be easily depleted, expecially when the preparation is bathed in a saline medium deprived of many critical substrates (20, 99). Thus, evidence presented here shows that ionic effects on synthesis and mobilization of acetylcholine can and do have important consequences in determining the amount of transmitter actually released.

The actions of calcium on acetylcholine release from mammalian brain were found to be similar to its actions on acetylcholine release from peripheral cholinergic nerve terminals. Thus, both the spontaneous and the evoked release by electrical stimulation were reduced by calcium deprivation (147, 268) and increased by about 20% when the calcium concentration was doubled (147). It was further found that a high concentration of magnesium lowered release, but the presence of a certain low concentration of magnesium was essential for optimal acetylcholine release (147), possibly due to the aforementioned importance of magnesium in acetylcholine synthesis.

2. Adrenergic synapses. The release of neurotransmitter from adrenergic synapses, as from cholinergic synapses, can be studied either by measuring the response of the end organ after electrical or chemical stimulation of the nerve or by perfusing the organ with a saline medium and assaying the effluent after stimulation. The former procedure has the disadvantage that a change in response could be due to an action of calcium and other cations on either presynaptic or postsynaptic sites. The latter procedure is obviously preferable, since the transmitter output can be determined directly. Studies on different adrenergic effector organs have been based on both of these approaches, and all have disclosed the critical role of calcium in the release of catecholamines from adrenergic nerves. First, Hukovic and Muscholl (166) found that lowering the calcium concentration of the fluid perfusing the isolated rabbit heart to 10 to 20% of normal decreased norepinephrine output resulting from nerve stimulation by 50 to 70%. On the other hand, release of tritiated norepinephrine from rat atria induced by electrical field stimulation is dependent upon calcium only at lower strengths of stimulation (188). More detailed studies on the role of cations in norepinephrine release from adrenergic nerve terminals were carried out in separate studies by Kirpekar *et al.* (192, 194) on the cat spleen perfused *in situ*, and by Boullin (29) on the cat colon perfused *in vitro*. Removal of calcium from the perfusion fluid abolished the release of noradrenaline in response to nerve stimulation (29, 192); furthermore, the norepinephrine output varied directly with the calcium concentration up to 7.5 mM (192). Magnesium antagonized evoked release, and barium and strontium could substitute for calcium in sustaining the release process (29, 192). Norepinephrine release induced by chemical agents such as acetylcholine, dimethylphenylpiperazinium, and excess potassium is also dependent on the calcium concentration of the bathing medium (134, 194, 211, 215). On the other hand, norepinephrine release from adrenergic nerves by the indirectly-acting sympathomimetic amine, tyramine, does not appear to depend on the presence of calcium (211); and this contrasts with certain findings in the adrenal medulla (see section II B). In fact, the indirect phase of tyramine action is inhibited by high calcium (102).

Kirpekar and coworkers (192, 194) have also studied in some detail the role

of monovalent cations in norepinephrine release from the cat spleen preparation. The removal of potassium from the perfusion fluid has no adverse effect on the secretory response to nerve stimulation (192). Lowering the sodium concentration below 50 mM blocks the electrically induced release of noradrenaline. This inhibitory effect of sodium-deprivation must be attributed to blockade of nerve conduction, for, when output of adrenergic transmitter from effector organs was elicited either by the injection of potassium chloride (194) or by acetylcholine (215), a lowering of the sodium concentration, even to a point of its complete absence, not only did not depress output but even potentiated it.

The spontaneous output of norepinephrine from adrenergic effector organs is very difficult to analyze accurately with the present techniques available, because of the relatively small amounts released. However, incubating tissues with radioactive (^{3}H) norepinephrine and measuring the efflux of the labeled material has enabled some workers to assay the resting rate of secretion (29, 309). Spontaneous release of radioactivity increases with calcium-free solutions (29, 309), or with solutions in which the calcium and potassium have been replaced by sucrose (309). Bioassay procedures have also detected an increase in the spontaneous release of norepinephrine from the spleen during perfusion with sodium-deprived solutions (194). The increase in the spontaneous release of norepinephrine during calcium deprivation might be ascribed to an increase in spontaneous firing of the sympathetic nerves, produced by calcium deprivation. However, if this were the case, one would have to assume that the calcium deprivation was not severe enough to affect significantly the release of transmitter. Alternatively, the observed output of norepinephrine from adrenergic nerve endings is the amount of amine initially released, minus the amount that was taken back up by a retrieval mechanism, which in adrenergic neurons conserves and terminates the action of catecholamines. Calcium (122) as well as sodium and potassium (26, 173, 195) are required for the optimal functioning of this uptake mechanism at least in certain tissues. Thus, a block of the re-uptake system could account for the increased amounts of norepinephrine appearing in the perfusate. Finally, the enhanced secretion during sodium deprivation can also be explained by the well known sodium-calcium antagonism, which was discussed in the previous section.

The more indirect method of measuring release of transmitter substance from adrenergic nerve endings by the response of effector organs has also underscored the importance of calcium in transmitter release (25, 35, 36, 102, 200, 283). Kuriyama (200) used electrophysiological techniques on the hypogastric nerve-vas deferens (smooth muscle) preparation to uncover a junction potential with characteristics of the end-plate potential. Calcium and magnesium manifest antagonistic effects on the amplitude of the junction potential similar to those observed on the endplate potential. Thus, low calcium and high magnesium decrease the amplitude of the junction potential, and produce stepwise, though inconsistent, fluctuations in its size. This finding suggests that the adrenergic transmitter release from the prejunctional catecholamine-containing vesicles takes place in "quanta," as has been demonstrated for acetylcholine release at the neuromuscular junc-

tion. Burn and Gibbons (35) have also shown that the response of adrenergic effector organs to stimulating postganglionic fibers, as well as to acetylcholine, varies with the external calcium concentration, at a time when there is little or no change in the response of the smooth muscle to challenges with norepinephrine. These latter workers have drawn parallels between the ionic requirements for the release of norepinephrine from sympathetic fibers and the ionic requirements for the release of catecholamines from the adrenal medulla by acetylcholine (see section II B), which they feel fortify the theory of a cholinergic link in the release of norepinephrine from sympathetic postganglionic fibers (34).

Norepinephrine is present in the brain and has been proposed as a neurotransmitter in the central nervous system. Electrical stimulation of rat brain slices previously incubated with tritiated (^{3}H) norepinephrine releases the radioactive amine into the Krebs-Ringer incubation medium, and calcium-free Ringer medium produces a significant inhibition of the norepinephrine output (4). Thus, calcium is also required for the release of norepinephrine from neurons of the central nervous system, as well as from peripheral nerves.

3. Other synapses. The inward movement of calcium appears to be a critical link between depolarization of the nerve terminal and the release of transmitter at peripheral cholinergic and adrenergic synapses. A similar sequence of events seems to occur at other synapses. Although the identity of the transmitter substance at the axo-axonal synapse of the squid stellate ganglion has not been elucidated, synaptic transmission across this synapse also depends upon a reciprocal relation between the calcium and magnesium concentrations (33, 186, 306) but does not depend upon sodium or potassium currents (115, 186). Furthermore, Miledi and Slater (234) have shown that in low calcium solutions transmission can be restored by *extracellular* ionophoretic application of calcium to a localized area of the squid giant synapse. It is of interest that calcium was ineffective when injected intracellularly, and Miledi and Slater suggest that transmitter release involves the combination of calcium with a membrane component which is accessible only from the outside of the membrane.

Transmission across other invertebrate synapses is also dependent upon calcium and inhibited by magnesium (13, 161). Recent studies have provided evidence that the transmitter at the insect neuromuscular junction is L-glutamate, and the recovery of glutamate from the bathing medium after nerve stimulation is increased by high calcium and depressed by high magnesium (319). In the absence of calcium, action potentials are still propagated in the isolated crab pericardial organ although the release of neurosecretory material is inhibited (13). The secretory product of this neurohemal organ has not been clearly identified, although it is peptide in nature and is assayed by virtue of its cardiotonic effects.

Calcium also may regulate the release of transmitter substances from inhibitory synapses in the central nervous system. Perfusion with a high calcium solution in the perilymph of the inner ear results in a characteristic alteration of the response to electrical stimulation of the olivo-cochlear fibers as recorded from the scala media (109). The transmitter substance of the olivo-cochlear inhibitory

efferent fibers has not been identified, and the conclusion that calcium participates in release of transmitter substance at this synapse was based upon the indirect evidence of a recorded response. Also, it was recently shown that the release of tritiated *gamma*-aminobutyric acid from rat brain slices is significantly inhibited by calcium deprivation when secretion is elicited by 40 mM potassium, but not when it is elicited by electrical stimulation (239). This finding suggests either that this system contains enough residual calcium to bring about *gamma*-aminobutyric acid release induced by nerve stimulation despite the absence of calcium in the incubation medium, or that the physiological release of this proposed inhibitory transmitter is brought about by an unconventional type of secretory mechanism. The employment of calcium chelating agents, such as ethylenediaminetetraacetic acid, in this system may help to resolve this disparity.

B. Release of hormones from endocrine glands

1. Catecholamines from the adrenal medulla. The role of calcium and other ions in medullary secretion has been studied from three main perspectives. First, the ionic requirements for acetylcholine-evoked catecholamine secretion from isolated perfused glands have been investigated (85, 86, 260, 288, 291). Second, the calcium fluxes associated with stimulation of the adrenal gland by acetylcholine have been studied (28, 80, 278). Third, the electrophysiological correlates of acetylcholine stimulation have been studied on cultures of isolated chromaffin cells (77, 78). In addition, the ionic influences on isolated catecholamine-containing medullary granules have also been investigated in some detail, and this approach will be discussed in section III.

Many years ago Houssay and Molinelli (160), with a venous cross-perfusion technique, found that lowering of the plasma calcium concentration by the injection of citrate (or oxalate) blocked the pressor response of a recipient dog after splanchnic stimulation of a donor dog. The question, of course, is whether the inhibition of catecholamine secretion observed by these investigators during hypocalcemia was the result of an inhibition of acetylcholine release from the splanchnic nerve endings or a block of the response of the medullary chromaffin cells to acetylcholine. Thus, in order to elucidate clearly the mechanism of medullary secretion evoked by the normal physiological neurotransmitter, acetylcholine must be presented to the chromaffin cells by exogenous administration rather than through splanchnic nerve stimulation. Calcium-deprived perfusion solutions depress or abolish the secretory response of the adrenal medulla to acetylcholine (or carbachol) in isolated cat adrenal glands perfused *in situ* (85), in bovine adrenal glands perfused retrograde *in vitro* (260, 261, 288, 291), and in bovine adrenal slices (248). In addition, the secretory response to acetylcholine varies directly with calcium concentration of the perfusion medium up to at least 17.6 mM (85). Whereas calcium deprivation depresses secretion of the adrenal medulla in response to acetylcholine, it enhances impulse generation of the homologous sympathetic ganglion cells in response to acetylcholine (32). This contrast emphasizes the distinction between the actions of calcium on the secretory process and on tissue excitability. The adrenal medulla secretes cate-

cholamines in response to a variety of substances including excess potassium, nicotinic and muscarinic agents, histamine, serotonin, sympathomimetic amines, polypeptides, and ouabain; and in the cat adrenal preparation, the stimulating action of all of these substances depends upon the presence of calcium (84, 89, 263, 279). Only barium releases medullary catecholamines in the absence of calcium (88). Calcium depletion also markedly depresses the response of the isolated canine adrenal to angiotensin (272), and the response of the bovine adrenal to phenylethylamine (288) and to ouabain (7).

There are a few reports in the literature concerning the ability of certain sympathomimetic amines, as well as reserpine, to release catecholamines from perfused glands (260, 261) or from adrenal slices (248) in the absence of calcium. It is the author's opinion that responses to excess potassium are most sensitive to calcium deprivation, responses to acetylcholine and other nicotine-like agents somewhat less sensitive, and responses to certain of the sympathomimetic amines least sensitive. However, in all cases, calcium deprivation will eventually depress or completely abolish the secretory response to all of the agents so far tested. A discussion of whether such disparities in the sensitivity to calcium deprivation indicate differences in the mode of action of acetylcholine and sympathomimetic amines on the adrenal medulla is beyond the scope of this review.

The comprehensive investigation by Douglas and co-workers on the mode of action of acetylcholine on the adrenal medulla has been thoroughly reviewed (71–73), and any detailed discussion of these studies at this time would be superfluous. In brief, the ionic requirements for acetylcholine-evoked secretion closely parallel those observed at adrenergic synapses. Sodium and potassium are not required for acetylcholine action (85, 86), magnesium inhibits secretion (86), and barium and strontium can replace calcium (87). Prolonged sodium deprivation (>1 hr) has an adverse effect on carbachol stimulation of the bovine adrenal gland (8); and the suggestion has been advanced that sodium has some regulatory effect on the entry of calcium into the chromaffin cells (8), in a manner similar to that described for cholinergic nerves (see section II A). However, the rather poor secretory responses obtained with carbachol in glands perfused with Locke's solution after prolonged perfusion with sodium-deprived solutions may indicate a rather nonspecific deleterious effect on prolonged sodium deprivation, rather than some specific effect on calcium entry. In any event, the role for sodium in adrenomedullary secretion is, at best, a secondary one.

An increase in radiocalcium uptake into the chromaffin cells is associated with acetylcholine stimulation (80), and the local anesthetic, tetracaine, which blocks acetylcholine-evoked secretion in adrenal glands, depresses this enhanced calcium influx in perfused adrenal glands (278) and specifically blocks the inward calcium current in isolated chromaffin cells (76). Magnesium, an inhibitor of medullary secretion (86, 142), markedly depresses calcium exchange between the gland and the perfusate (278). Thus, it appears that acetylcholine stimulation enhances the entry of calcium into the medullary chromaffin cells, and it is the entrance of

calcium that somehow triggers the secretory process. This sequence of events has been termed "stimulus-secretion" coupling, with calcium viewed as the link between these two processes (85–87).

The effect of acetylcholine on the permeability of chromaffin cells is thought to occur through a depolarization brought about by a nonspecific increase in ionic permeability, in a manner similar to its action at other sites of synaptic transmission (73); however, the findings that acetylcholine can still initiate catecholamine secretion in the complete absence of sodium, or in the presence of isotonic potassium sulfate, indicate that depolarization is not a *sine qua non* for acetylcholine action as long as calcium is present in the perfusion medium (73, 86). On the other hand, under normal conditions excess calcium is not sufficient to augment secretion in the absence of a depolarizing agent; only barium is able to elicit secretion in the absence of acetylcholine or excess potassium (88). The depolarization of isolated chromaffin cells and the secretion of catecholamines from the perfused cat adrenal gland both vary directly with the potassium concentration of the medium in the presence of a constant calcium concentration (78, 281), and this suggests that the amount of calcium entering the chromaffin cell can depend upon the extent of the cell depolarization, as well as the calcium concentration of the medium. The means whereby calcium enters the medullary chromaffin cell after acetylcholine depolarization has not yet been clearly elucidated. However, the secretory response elicited by acetylcholine or potassium varies directly with the calcium concentration of the extracellular medium, which suggests that calcium entry into the chromaffin cell is a passive diffusion process down a concentration gradient, although it does not rule out a facilitative diffusion process. The notion of a passive diffusion process is valid only if the free intracellular calcium concentration of the chromaffin cell is very low, as has been reported, for example, in invertebrate nerve (191).

Although there is an increase in calcium influx during adrenal stimulation with acetylcholine, chemical determination of medullary calcium indicates that there is no net increase in the calcium concentration of the medulla during secretion induced by the acetylcholine analogue, carbachol (28). The lack of a net increase in the calcium content during stimulation is consistent with the finding of a large increase in calcium exchangeability during high secretory activity of the perfused adrenal gland (278). The radioactive calcium entering the chromaffin cell appears to penetrate readily the catecholamine-containing granules (28), and the concentration of calcium within the granular fraction might suggest a possible mechanism for the role of calcium in the secretory process. Alternatively, the granules may act as a "pump" to maintain the low level of free calcium in cytoplasm, and thereby help to terminate the stimulant activity of calcium in a manner analogous to that by which the sarcoplasmic reticulum terminates the stimulant activity of calcium in muscle. Indeed, the chromaffin granules do contain an ATP-magnesium-dependent pump which concentrates catecholamines, and Taugner and Hasselbach (308) have observed that this granular pump has many of the characteristics of the calcium pump in

muscle. Studies on the calcium-pumping activity of the medullary granular fraction may contribute valuable information as to the fate of calcium within the interior of the chromaffin cell.

Finally, a brief mention should be made of the role of ions in spontaneous release of catecholamines. In the absence of calcium, spontaneous output of catecholamines is very low (7, 85, 272), but sodium-deficient (8, 85, 86) and potassium-deficient solutions (8, 277) cause an increase in spontaneous medullary secretion. The spontaneous output engendered by sodium deprivation was directly related to the degree of sodium deprivation (274), was independent of the osmotic substituent (274), and was not abolished by calcium deprivation (85). These stimulant effects of monovalent cation deprivation might be ascribed to a competition between monovalent cations and calcium, which is observed in other tissues. The findings that both ouabain (7, 89) and the block of energy metabolism (275) increase spontaneous release of medullary catecholamines are also of interest, since all of these results indicate the presence of an active sodium-potassium pump within the chromaffin cell membrane. A detailed discussion of the intimate role of calcium in secretion will be reserved for the concluding sections; however, it should be stated that calcium has little stimulant effect on isolated chromaffin granules (6, 148, 265), but adenosine triphosphate (ATP) causes a dose-dependent release of catecholamines from these granules *in vitro* (249, 265). These findings, together with the fact that in the intact gland the secretory action of calcium requires the presence of the normal energy metabolism of the chromaffin cell (196, 275, 276), suggest that an interaction of calcium and high-energy phosphates might be a critical step in the secretory process.

2. Corticosteriods from the adrenal cortex. In most secretory organs, the secretory products are stored in granules or vesicular structures, and the release of the secretory product is thought to occur by exocytosis (*vide infra*). However, in the adrenal cortex, electronmicroscopic studies have so far failed to provide evidence for the existence of intracellular organelles that might act as a vehicle for the storage or extrusion of corticosteroids (24, 216). It is thought that during stimulation of the cortex by pituitary corticotropin (ACTH) the steroids are synthesized *de novo* through the action of cyclic 3′,5′-adenosine monophosphate (3′,5′-AMP) and then diffuse out to the cell exterior (143, 159). Studies on quartered glands have shown that calcium is an important factor in increased steroid production in response to ACTH (or cyclic 3′,5′-AMP) (21, 317). Further studies *in vitro* have indicated that the site of action of calcium is within the mitochondria, where many of the steps of the biosynthetic pathway from cholesterol to corticosterone (or hydrocortisone) are carried out (256).

Recently, studies have been conducted on isolated cat adrenal glands perfused *in situ* to investigate the importance of calcium and other cations on ACTH-induced steroidogenesis and release (174, 280). With such a preparation, both synthesis and release can be simultaneously determined by assaying both the gland and the perfusate. In the absence of calcium, ACTH increases steroidogenesis within the gland (174), but there is little or no increase in corticosteroid output (174, 280). The output varies with the extracellular calcium concentra-

tion up to 0.5 mM (174); a further increase in the calcium concentration produces no additional augmentation in corticosteroid release. Magnesium inhibits secretion *in situ* (174), and strontium can replace calcium (174, 280).

It thus appears as if calcium has at least two effects on cortical cells—one on synthesis and one on release, although these two processes may be interrelated. The locus of the calcium and its mode of action remain to be elucidated; however, there is evidence to suggest that calcium might control the level of cyclic 3′,5′-AMP within cortical cells by regulating the activity of adenyl cyclase (9). Sodium and potassium deprivation do not produce a marked depression of ACTH-induced corticosteroid release (174). Excess potassium, which depolarizes cortical cells, causes a small, transient increase in steroid output (174). However, high potassium does not affect either steroid production *in vitro* (228) or steroid output *in situ* in response to ACTH (174). Although ACTH can produce depolarization—even action potentials—of cortical tissue in potassium-deprived media (229), there appears to be no correlation between the steroidogenic effect of ACTH and its ability to cause cell depolarization (174, 228). Despite the obvious importance of calcium in the mechanism of ACTH action, it remains to be determined whether the action of ACTH primarily involves alterations in ionic fluxes across cortical cell membranes.

3. Vasopressin and oxytocin from the neurohypophysis. The neural lobe of the mammalian pituitary gland is the site of storage and release of vasopressin and oxytocin. From 50 to 80 % of these hormones is found within the so-called granular fraction closely associated with binding protein (neurophysin) (53)—the remainder is presumed to be localized in the cytoplasm. Since the initial work by Douglas and Poisner (70, 82, 83), the importance of calcium for the release of vasopressin and oxytocin from isolated rat hypophyses *in vitro* (67, 171, 172), as well as *in vivo* (312), has been clearly demonstrated. *In vitro*, studies have generally involved excess potassium (56 mM) as the secretogogue, although electrical stimulation gives similar results (232). Thus, evoked release of vasopressin and oxytocin is strongly dependent on the presence of calcium (70, 171, 172) and is inhibited by sodium (82, 171), or excess magnesium (82, 172). Barium can substitute for calcium (172). Output increases with increasing calcium concentrations up to 4 mM calcium (82); but further increases in the calcium concentration depress secretion. The depression of secretion observed with high calcium concentrations may be ascribed to the stabilizing effects of calcium on the cell membrane (294). It is of interest that the addition of barium to normal Locke's solution greatly potentiates vasopressin output elicited by electrical stimulation (139), just as it potentiates acetylcholine and norepinephrine release from nerve endings (79, 192). Barium also increases the spontaneous release of vasopressin (139) just as it enhances spontaneous catecholamine secretion from medullary chromaffin cells (88) and adrenergic nerve endings (192), and insulin secretion from the pancreas (137). This facilitatory action of barium emphasizes that the effects of barium on membrane properties of tissue are in some ways opposite from those of calcium, although the effects of these two alkali earths on the secretory process are quite similar.

Ouabain also manifests a calcium-dependent release of oxytocin and vasopressin from isolated neurohypophyses of adult rats (67). This stimulant effect of ouabain is accompanied by an extrusion of potassium and an influx of sodium which suggest some action on a sodium-potassium activated ATPase. Enhanced secretion produced by sulfhydryl inhibitors (75) or cooling (74) not only can occur in the absence of calcium, but also appears to be unaffected by a variety of changes in the ionic milieu, such as sodium deprivation or excess magnesium or potassium. It is difficult to explain the stimulant actions of cooling or the sulfhydryl inhibitors within the scope of physiological mechanisms; however, these studies are of interest since they do show that under certain unusual conditions, the release of pituitary hormone, like release of medullary catecholamine (see above), can occur in the absence of extracellular calcium.

Studies on isolated neurohypophyses with radiocalcium ($calcium^{45}$) have shown that calcium influx and efflux are greatly augmented (5-fold) during exposure to excess potassium (83). Although the uptake of calcium rises with the calcium concentration, calcium uptake and hormonal secretion fail to parallel one another. Thus, maximal uptake was obtained with 8.8 mM calcium, whereas maximal secretion occurred with 2 to 4 mM calcium; high magnesium inhibited calcium uptake by only 30% but blocked secretion by 70 to 80%; sodium deprivation enhanced secretion but produced no enhancement of calcium uptake. Tetrodotoxin has been shown by Ishida (171) to depress potassium-induced secretion and calcium uptake by isolated neurohypophyses; however, the toxin depressed secretion by 80% and calcium influx by only 30%. This blocking effect of tetrodotoxin, which is observed even in sodium-deprived media (171, 172), might seem rather unexpected in light of the fact that the toxin appears to have a selective blocking action on the inward sodium current, or on ions that have the major responsibility for carrying the inward current (179). On the other hand, tetrodotoxin depresses the increase in miniature end-plate potential frequency induced by excess potassium at mammalian motor nerve terminals (202) and blocks the action potentials of squid giant axons when they occur in a sodium-free medium containing isotonic calcium chloride (326). Such data indicate that tetrodotoxin can also block calcium currents in certain tissues. Although the inability to correlate precisely calcium entry and neurohypophyseal secretion does not refute the importance of calcium in the mechanism of pituitary secretion, it does suggest that at least some of the ions might produce their effects not only by affecting the uptake of calcium into the cell but also by interacting with calcium in the interior of the cell. In summary, the evidence cited appears consistent with the view that the release of vasopressin and oxytocin from neurosecretory terminals is due to the entry of calcium ions after depolarization. Douglas and Poisner (82, 83) have drawn attention to the similarities in the processes of "stimulus-secretion coupling" in neurosecretory cells and medullary chromaffin cells and suggested that a common calcium-dependent mechanism is responsible for the release of their respective secretory products.

Although most of the general discussion concerning the intimate role of calcium in the secretory process will be reserved for the final section of this review,

some brief mention should be made about the approaches being used by investigators of pituitary secretion. This problem has been comprehensively reviewed by Ginsburg (123, 124), so only a brief discussion is needed at this time. Two hypotheses have been advanced concerning the nature of the cellular mechanisms involved in the release of vasopressin and oxytocin that follows depolarization of the nerve endings in the posterior-pituitary. According to one theory, calcium enters the nerve ending and frees extragranular hormone from a binding protein (neurophysin) and the free hormone diffuses out of the cell. One important piece of evidence to support this theory is the finding that, *in vitro*, calcium interferes with the binding of vasopressin and oxytocin to neurophysin (126, 302, 311); magnesium and sodium have no effect on this binding (126, 311). The other theory, originally suggested by Douglas and Poisner (82), states that the neurohypophyseal secretory granules are the source of the readily releasable hormone and that release occurs by the process of reverse pinocytosis (exocytosis). One basic piece of experimental evidence required to support the theory of exocytosis and make questionable the idea that calcium acts by dissociating extragranular-neurophysin complexes is that one or more of the other components of the granule must also be released along with the hormone. The neurosecretory particles (granular fraction) contain more neurophysin than the other cell fractions (125), so if the theory of exocytosis is tenable then neurophysin should appear in the incubation medium with the hormone. Indeed, after labeling of protein (both hormone and neurophysin) *in vivo*, glands subsequently incubated *in vitro* and challenged with excess potassium simultaneously released both vasopressin and neurophysin (105). This supports the idea of secretion by exocytosis. Studies on the release of medullary catecholamines, as well as other components of the catecholamine-containing medullary granules, have added further support to the view of secretion by exocytosis (73).

4. Thyrotropin, luteinizing hormone, adrenocorticotropin, and prolactin from the adenohypophysis. The anterior pituitary, a non-nervous tissue, contains at least six major hormones, each of which is sequestered in a characteristic type of cell. Experiments *in vitro* have shown so far that four of these hormones also require calcium for their release (121). The incubation of isolated adenohypophyses in calcium-free media greatly diminishes the release of thyrotropin (322, 323), luteinizing hormone (285), adrenocorticotropin (ACTH) (198), and prolactin (253). Excess potassium or the respective hypothalamic releasing factor was generally employed to evoke secretion in these investigations. Prolactin secretion was not augmented by an exogenous stimulus, although "stimulating" conditions were assumed to exist in the preparations *in vitro*, which implied removal of a hypothalamic inhibitory factor (253). Although the absence of calcium inhibits release of hormone, it does not inhibit its synthesis, as determined by the incorporation of ^{14}C-leucine into luteinizing hormone (285). Extracellular calcium appears to be a critical pool for adenohypophyseal secretion. The amount of prolactin secreted is related to the calcium concentration in the medium (253), and a block of potassium-induced ACTH release can be readily produced by a rapid rinsing of the tissue in a calcium-free medium before

incubation in the high-potassium solution (198). Parallels drawn between these findings and those obtained on the neurohypophysis and adrenal medulla provide further support for the model of stimulus-secretion coupling as a basis for describing the process of hormone release.

5. Insulin from the pancreas. Evidence for the importance of calcium for insulin secretion from β-cells has been obtained *in vitro* from the isolated perfused rat pancreas (46, 47), pieces of rabbit pancreas (138, 235), organ cultures of fetal rat pancreas (201), and *in vivo* from the bovine pancreas (213). Various stimuli enhance the output of insulin, including excess glucose, potassium, ouabain, tobutamide, and glucagon; and the actions of all of these agents as secretogogues are reversibly inhibited by the absence of calcium (46, 138, 235). The key role of calcium in the secretory process of the β-cell is illustrated by the finding that when a pancreas is perfused with a calcium-free solution containing excess glucose there is a very low level of spontaneous release, which is rapidly augmented by the addition of calcium to the medium (46). This rapid effect of calcium observed by Curry *et al.* (46) is analogous to the prompt enhancement of medullary catecholamine secretion when calcium is restored to the perfusion medium after calcium deprivation (85). During prolonged exposures to excess glucose (>1 hr) insulin release occurs in two phases—an early phase, which subsides within 2 minutes, followed by a late phase, with continually increasing release (47). Puromycin treatment affects only the late phase; this indicates that the second phase is intimately associated with insulinogenesis. Both phases of secretion are calcium-dependent and both exhibit a similar quantitative dependence upon the calcium concentration of the perfusate (maximal effect with 2.0 mM). On the other hand, the fact that calcium, unlike puromycin, has no effect on the incorporation of radioactive amino acids into pancreatic protein, indicates that while calcium is required for insulin secretion it plays no critical role in the biosynthesis of the hormone (47). The amount of insulin released in response to glucose is directly related to the calcium concentration of the perfusion medium up to 2 mM (46); however, with higher calcium concentrations the response either remains constant (46) or is diminished (138). Calcium is also required for glucose-evoked insulin-secretion from the pancreas of a 27-day-old rabbit fetus, whose β-cells, in contrast to those of the adult rabbit pancreas, do not contain secretory granules as visualized by light microscopy (235). Milner and Hales (235) concluded from this finding that the action of calcium is not on the secretory granule.

Just as in many other secretory organs, magnesium cannot substitute for calcium (132, 138) and inhibits insulin secretion when added to the perfusion solution containing calcium (12, 138, 235). The effects of barium on insulin secretion are in some ways quite similar to its effects on medullary catecholamine release. Thus, barium as a stimulant of insulin secretion does not require the presence of calcium but, in fact, excess calcium depresses barium-induced insulin release (138). Excess magnesium inhibits barium-stimulated insulin secretion (138) just as it depresses barium-induced catecholamine secretion (87). On the other hand, barium appears to be a stimulus of the pancreas *in vitro* only in the presence of extracellular sodium (138, 236). This finding is in marked contrast to that on the

adrenal medulla, where, in the absence of sodium, barium continues to act as a powerful secretogogue (88) and can still depolarize isolated chromaffin cells (78).

Hales and Milner (137, 138, 236), by using pieces of rabbit pancreas, concluded that the entry of sodium into the β-cell may be important in the mechanism of insulin release. Both ouabain and potassium deprivation, which inhibit the active transport of sodium and cause an intracellular accumulation of sodium, stimulate secretion (137). The augmentation of insulin secretion by these agents is inhibited by sodium deprivation (137). Furthermore, the stimulant actions of glucose and tolbutamide are reversibly depressed by the replacement of sodium by choline, potassium, or lithium (137). It should be noted, however, that the secretory response to potassium, which varies directly with the potassium concentration up to 55 mM, is not affected by the reduction in the sodium concentration of the medium that is required to maintain isotonicity (137, 201). It is only when pieces of pancreas are incubated for 2 hr in a sodium-deprived medium that high potassium is ineffective as a secretogogue (137). Excess glucose and tolbutamide induce changes in the electrical activity of cell membranes of islet cells which lead to the production of action potentials (54). Since action potentials would be associated with an inward sodium current, these electrophysiological findings are consistent with the conclusion of Hales and Milner. On the other hand, both ACTH (229) and acetylcholine (77) are able to induce electrical changes in cortical and chromaffin cells respectively, yet the secretory processes in the adrenal gland can proceed independently of the membrane potential. Furthermore, tetrodotoxin (1 μM) has no effect on insulin release stimulated by glucose, ouabain, potassium, or barium (237); however, resistance to tetrodotoxin is not by itself an adequate basis for excluding sodium as an important factor in insulin release (179).

Thus, the actual importance of sodium in the mechanism of insulin secretion is difficult to assess. An increase in the spontaneous output occurs during periods of sodium deprivation in the pancreas (138) as well as in other secretory organs (85, 86, 194). This effect might either mask stimulation by the secretogogue or deplete the "readily-releasable pool" of secretory product. Sodium is the major cation in physiological solutions, and prolonged periods of perfusion with sodium-deprived solutions appear to diminish the general viability of preparations. This phenomenon may be the direct result of prolonged sodium-deprivation or the toxic effect of the osmotic substituent, *e.g.*, lithium. A study of the effects of sodium-deprivation on the dynamics of insulin secretion, similar to the one with calcium carried out by Curry *et al.* (47), may help to clarify the role of sodium in the secretory process.

6. Parathyroid hormone and thyrocalcitonin. In order to complete the survey of the effects of calcium on endocrine secretion, one should also mention the importance of calcium in the release of parathyroid hormone and thyrocalcitonin, two hormones involved with maintaining serum calcium within rigid physiological limits. Many reviews concerning these hormones have been published (for references, see 43, 150, 296), so only a brief summary will be presented here. The control of secretion of parathormone and thyrocalcitonin *in vivo* seems to be a complicated affair, with many factors playing important roles; *e.g.*, interactions of

parathormone and thyrocalcitonin, phosphate levels, *etc.* However, when either the parathyroid or thyroid gland is perfused in isolation from other factors, the calcium concentration of the perfusion medium exerts a direct control over the amount of hormone secreted. Thus, perfusion of isolated parathyroid glands with calcium-deprived solutions *augments* parathormone release (44, 255), and the amount of parathormone secreted bears a simple linear inverse relation to the plasma calcium concentration (297). In isolated perfused thyroid glands of pigs, the thyrocalcitonin secretion rate varies with the calcium concentration over the range of 3 to 5 mM (37). Two important differences are apparent in the effects of calcium on parathormone and thyrocalcitonin secretion as compared to other endocrine organs. These two glands respond to changes in the calcium concentration without the apparent need of a stimulating agent, which contrasts with other endocrine glands in which some prior stimulation is required to initiate the activity of calcium on the secretory process. In addition, unlike other secretory systems, parathormone secretion varies *inversely* with the calcium concentration. This finding suggests that the calcium-sensitive receptor within the parathyroid cells may be entirely different from those in other endocrine organs. One must also consider the possibility that calcium can influence the release of these hormones by modulating their rates of synthesis (140).

C. Release of other secretory substances

1. Water and protein from the salivary gland. The salivary glands secrete water, electrolytes, and protein in response to stimulation of either parasympathetic or sympathetic nerves (100). Amylase is one of the proteins secreted by these exocrine glands, and more than half of it is found in the zymogen granule fraction, the remainder in the supernatant (290). The mechanism of salivary secretion is not well understood; however, nerve stimulation appears to produce a hyperpolarization of the acinar cell (the secretory potential), which is followed by the release of salt, water, and protein (219). In the duct system, secretion seems to be modified by reabsorption of sodium chloride and secretion of potassium (339). After chemical stimulation by parasympathomimetic or sympathomimetic agents, potassium and calcium levels within salivary glands may fall (93, 289), and sodium levels either increase or decrease (289).

In an attempt to obtain evidence that salivary secretion is initiated by the entry of calcium after stimulation, Douglas and Poisner (81) used the isolated perfused submaxillary gland of the cat to study the influence of calcium on acetylcholine-evoked secretion. They found that the output of water and protein varied with the extracellular calcium concentration over the range of 0 to 8 mM. The stimulant effect of norepinephrine also required calcium. The fact that the fall in sodium and potassium output that occurred during calcium deprivation paralleled the fall in volume, indicated little change in electrolyte concentration. When excess calcium was added during constant exposure to acetylcholine, protein and water output increased. Although Douglas and Poisner (81) saw some similarities in the effects of various alterations in the ionic milieu of the salivary gland with those of the adrenal medulla, they also realized that other observed

phenomena did not make a strong case for the simple conclusion that calcium acts as a link in "stimulus-secretion coupling" in the salivary gland in a manner parallel to its action at the adrenal medulla. For example, the reintroduction of calcium after a period of calcium-free perfusion produced only an irregular and weak stimulation of salivary secretion when compared to the huge outpouring of catecholamine during the reintroduction of calcium to the perfused adrenal gland (85). In fact, even the small effects of calcium reintroduction on salivary secretion were apparently indirect through the action of calcium on the secretomotor nerve fibers within the salivary gland, since this phenomenon did not occur in the presence of hexamethonium (230); by contrast, this ganglion-blocking agent does not inhibit calcium-evoked adrenomedullary secretion (85). In addition, the depression of salivary secretion produced by magnesium (10 mM) (81) was very small when compared to its blockade of medullary secretion (86).

The importance of calcium has been corroborated for protein secretion on whole parotid glands of the mouse *in vitro* (136), and for salivary secretion on perfused submandibular glands of the cat (259), although in slices of rat parotid glands calcium was not required for protein secretion in response to epinephrine, this secretion being strongly dependent on potassium (11). Both potassium deprivation and excess potassium markedly enhance protein output from parotid slices (11). On the other hand, in the perfused salivary gland an increase in the potassium concentration depresses secretion of saliva (257). When chloride ions are replaced by nitrate (218) or sulfate (258) the ability of the salivary gland to secrete saliva in response to chemical stimulation is depressed by 75 to 100%. It has been postulated that an active electrogenic transport of chloride ions into the cell is the primary step in the secretion of saliva (219). By contrast, chloride ions are not required in other calcium-dependent secretory systems (15, 86, 137, 244). Up to now, studies with calcium^{45} have not been successful in helping to elucidate the role of calcium in salivary secretion (90–92). The transfer of calcium^{45} into or out of rat salivary and lacrimal glands is increased by parasympathetic and sympathetic agents, both *in vitro* (91) and *in vivo* (90), and is inhibited by metabolic inhibitors, sodium lack, and low temperature; these considerations suggest that calcium flux in salivary glands is an active process (91). However, the role of this calcium transport in the secretory process remains obscure, for whereas acetylcholine markedly increases residual calcium^{45} in parotid glands, no significant change in residual calcium^{45} could be demonstrated with whole submaxillary glands or pieces of submaxillary glands (91).

The problem of evaluating the role of calcium in salivary secretion is made more difficult by the fact that there is release of two basically different components, water (and electrolyte) and protein, and the effects of calcium on protein secretion do not always parallel the effects on secretion of saliva (81); thus, calcium may produce its effects in salivary glands by different mechanisms. If, indeed, calcium has more than one action on salivary secretion, it does not appear to be on the electrical properties of the acinar cells, since, in the absence of calcium, a secretory potential can still be obtained at a time when there is no response to acetylcholine (259). Calcium may not be important in the energy

metabolism of the secretory system of the salivary gland since calcium deprivation does not block the pilocarpine-induced enhancement of oxygen consumption of intact glands *in vitro*, although secretion is depressed (136). Also the action of calcium is not directly mediated through the cyclic AMP system, since, as Rasmussen and Tenenhouse (269) found, in the absence of calcium, epinephrine and high potassium can still increase cyclic AMP in slices of rat parotid glands. The importance of calcium in membrane permeability may account for the effects observed in secretion of water. It is well known that membranes require calcium for regulating ion and water movement (for reviews, see 32, 217, 223, 294), so that calcium may affect salivary secretion by an action on permeability to sodium and water. By contrast, calcium may affect protein secretion through a mechanism that involves the egress of the contents of the zymogen granules from the cell, *viz.*, exocytosis.

2. Hydrochloric acid from the gastric mucosa. During the secretion of gastric juice, hydrogen and chloride ions account for about 95 % of the ions transported, and sodium plus potassium ions account for most of the remaining 5 % (270). The calcium requirement for HCl secretion has been established from studies on the frog gastric mucosa *in vitro* (111, 127, 175, 292); and HCl, pepsin, and gastrin secretion are directly related to the plasma calcium concentration of man (10, 197, 318). The importance of calcium in the mechanism of HCl secretion is not only of theoretical interest, but of clinical interest as well, in view of the high incidence of peptic ulcers in the syndrome of hyperparathyroidism, which is associated with hypercalcemia. The removal of calcium from solutions bathing isolated frog gastric mucosa results in an initial increase in electrical resistance (first phase), accompanied by a 50 % fall in H^+ secretion after transmural stimulation. These effects are followed by a pronounced decrease in resistance and a fall in H^+ secretion to zero (second phase) (175). A complete reversal of the second phase is obtained by the restoration of calcium to the incubation medium. When the experiments are repeated in a chloride-free medium, the removal of calcium again gives rise to two phases; this demonstrates that the inhibition of H^+ secretion can still occur in the absence of chloride transport (175). Thus, although H^+ secretion can be suppressed to varying degrees by substituting large anions for chloride (94, 110, 146, 271), the effect of calcium deprivation does not appear to be due to an indirect action on chloride transport (175). A labile pool of calcium has been quantified and localized histochemically within the gastric mucosa. A marked fall in the tissue calcium and a decrease in the number of red-staining intracellular calcium-containing granules accompanies the removal of calcium, and a restoration of calcium results in a redistribution of the red granules corresponding to the control (292).

Just as with salivary secretion, it is difficult to pinpoint the site or mechanism of action of calcium in HCl secretion. A major question is whether calcium does exert a direct effect on the gastric secretory mechanism. *In vivo*, gastric secretion is blocked by surgical or pharmacologic denervation and by high magnesium content (10, 300), which may indicate that the calcium effects are mediated through the vagus nerve. In the frog gastric mucosa the use of calcium-chelating agents causes

a movement of normally impermeant substances (*e.g.*, NaCl and sucrose) across the mucosa into the cell, which suggests that the effects of calcium deprivation are on cell permeability and that the process of H^+ secretion is not directly inhibited (111). Indeed, electronmicroscopic studies show a widening of intercellular spaces when calcium is removed from solutions bathing the gastric mucosa, and magnesium, strontium, or barium cannot restore the calcium-depleted mucosa to normal (293). However, during the first phase of calcium deprivation there is an increase in resistance, with only a small decrease in the potential difference of the mucosal cells (175). This finding indicates that permeability is not radically altered during the initial stage of calcium deprivation, and supports the hypothesis that the removal of calcium from the bathing solution during the first phase reduces the H^+ secretory rate, at least in part, by a direct effect on the secretory mechanism (175). The decrease in resistance during the second phase, on the other hand, may be the result of an increase in the permeability of the intercellular space (292).

3. Granular proteins from polymorphonuclear leucocytes and exocrine pancreas. Excellent reviews on varying aspects of protein secretion have appeared recently (155, 290, 330); so, for the most part, only specific aspects of protein secretion as they relate to cations will be discussed here. The protein contained within granules is apparently synthesized by the ribosomes of the cell and is then transported to the Golgi region where it is incorporated into the zymogen granule. Once the mature granule is formed it remains in the apical region of the cell until secretion is initiated by the appropriate secretogogue. Calcium deprivation abolishes or markedly inhibits acetylcholine-stimulated amylase secretion from pigeon pancreas slices (154), and protein secretion (β-glucosidase, ribonuclease, and peroxidase) from rabbit polymorphonuclear leucocyte preparations in response to staphylococcal leucocidin, vitamin A, and steptrolysin 0 (333, 334). The finding of a requirement for calcium in these protein-secreting preparations is in general agreement with the finding of a calcium requirement for salivary amylase secretion, which was alluded to above. The role of calcium in protein secretion from leucocytes, which has been comprehensively studied by Woodin and Wieneke, has been reviewed in detail (330), so a discussion of these important findings will be limited. Protein secretion (β-glucosidase) in leucocidin-treated leucocytes varies with the calcium concentration; the optimal concentration of 0.1 mM is lower than that observed in most other secretory systems (333). By contrast, the small release of aldolase, a soluble cytoplasmic enzyme, is not enhanced by increasing the calcium concentration, and this suggests that the increased permeability is restricted to granular protein (333). Protein extrusion is sustained in media containing either high NaCl, KCl or even sucrose, provided that calcium is also present, but magnesium inhibits secretion by antagonizing the actions of calcium (334). Associated with leucocidin treatment, there is an increase in cell calcium (333) and a loss of potassium (330, 335). Potassium efflux may be an important step in the secretory process, since tetraethylammonium, which blocks potassium efflux, inhibits the action of leucocidin as a secretogogue (335); however, it is somewhat surprising that the local anesthetic, cocaine, had no such

inhibitory activity (335). In summary, leucocidin appears to cause an increase in the permeability of the leucocyte to cations, and the entry of calcium into the cell appears critical for the secretion of protein from the zymogen granules. It is very significant that although the efficiency of calcium as an inducer of secretion decreased with prolongation of the incubation in a calcium-free medium, efficiency was restored by adding tri- or diphosphonucleotides to the incubation medium (333). This important finding suggests that an interaction between calcium and high-energy phosphates may be critical in initiating the secretory process (330), and this point will be discussed more fully in the concluding sections. Electronmicrographs of leucocytes treated with leucocidin show no gross morphological changes in the absence of calcium, while in the presence of calcium leucocytes appear degranulated, a change that indicates the loss of secretory product (331). The authors offer a few electronmicrographs which they feel illustrate examples of granules fused with the cell membrane (334); but the morphological evidence for exocytosis in leucocidin-treated leucocytes is not convincing. The detailed investigations of Woodin and Wieneke on protein secretion from leucocidin-treated leucocytes show striking similarities to results obtained on other secretory organs which store a variety of secretory products in granules or vesicles.

4. Histamine and 5-hydroxytryptamine. In addition to catecholamines, two other biogenic amines, histamine and 5-hydroxytryptamine (serotonin), require calcium for their release from a variety of tissues. The physiological stimulus for the release of histamine is the antigen-antibody reaction, and omission of calcium or the addition of chelating agents markedly depresses or abolishes the antigen-induced histamine release from preparations of guinea-pig and rat lung (39, 240–243), mesentery mast cells *in situ* (153), human and rabbit leucocytes (128, 208), and isolated platelets (168, 313, 314). Since the inhibitory effect of calcium deprivation can be overcome by an increase in pH, Mongar and Schild (240, 241) have suggested that the anaphylactic reaction requires bound calcium and that the binding decreases with a fall in pH. The effect of calcium deprivation on histamine release is not related to any action of calcium on energy metabolism, since Mongar and Schild (240) found that the inhibition of histamine release produced by calcium lack was not accompanied by any change in oxygen consumption. The optimal calcium concentration for histamine release varies, depending upon the preparation employed, ranging from 0.1 to 5 mM (128, 153, 208, 240). High calcium concentrations may be inhibitory (208). Strontium can substitute for calcium in histamine release from human leucocytes (208), but barium (208) and magnesium (39, 128, 208) are ineffective substitutes. The addition of high magnesium concentrations, in the presence of calcium, depresses antigen-induced histamine release (153) or may potentiate the effect of calcium (208). When potassium is omitted or sucrose substituted for sodium chloride, the anaphylactic release of histamine is maintained (240).

Organic bases such as 48/80 also stimulate histamine release, and omission of calcium blocks their releasing activity in the rat (39, 153, 243, 284); but calcium deprivation does not affect the histamine-releasing activity of 48/80 from guinea-

pig lung (240) or from isolated mast cell preparations of the rat (284, 321). By contrast, histamine release induced by antiserum from isolated mast cell preparations is clearly inhibited in the absence of calcium (284). Such findings suggest that 48/80 might release histamine by a different mechanism from antigen (240, 284). Other investigators feel that their mechanisms are similar (177, 243), and that both antigen and 48/80 initiate a sequence of events that produces a rather selective increase in the permeability of the mast cell, which leads to the release of histamine (177, 178). Although the work of Mongar and Schild (240) on histamine release from preparations *in situ* indicates that calcium, but not sodium or potassium, is critical for histamine release, Uvnäs and his associates (2, 310, 320), on the basis of studies *in vitro*, have proposed that histamine release induced by 48/80 also involves an extracellular degranulating event, which is a cation exchange between histamine and sodium. This idea is based upon their findings that exposure of peritoneal mast cells to 48/80 in either isotonic salt solution or isotonic sucrose causes a degranulation and release of histamine from the mast cell; a subsequent exposure to a salt solution, but not to the ion-free solution, causes a release of histamine from the discharged isolated granules. In addition, although 48/80 causes an increase in sodium^{22} uptake by mast cells, it was concluded from the time course of sodium uptake and the amount of sodium entering the mast cells that histamine release and the intracellular accumulation of sodium are two unrelated processes (299). Since histamine release from isolated granules was stimulated by 48/80 at 0°C and there was no qualitative difference between the ability of sodium, potassium, and calcium to sustain release, further evidence, under more physiological conditions, is needed to substantiate the idea of a specific extracellular cation exchange between histamine and sodium. It should be stated, however, that an active transport of ions is probably somehow involved in the mechanism of histamine release since ouabain inhibits histamine release evoked by anaphylaxis (336).

ATP can induce histamine release from isolated mast cells of the rat; the action of ATP as a secretogogue depends upon the presence of calcium and is inhibited by magnesium (65, 304). Unlike other known histamine-releasing agents, the histamine release induced by ATP occurs without the visible expulsion of granules (66, 304). ATP-induced release appears to be associated with a splitting of ATP since the rat mast cell contains an ecto-ATPase, and ADP, which inhibits hydrolysis of ATP by mast cells, inhibits histamine release induced by ATP (64). The ability of ATP to stimulate histamine release is in harmony with the finding that inhibition of metabolism depresses the anaphylactic release of histamine (40, 153, 241, 243, 284). These findings bear a striking resemblance to those of the adrenal medulla where ATP releases the contents of isolated chromaffin granules (249, 265), and the simultaneous block of glycolysis and oxidative phosphorylation depresses calcium-evoked catecholamine release (275, 276), and they emphasize once more the important role of calcium and high-energy phosphate in the intimate mechanism of the secretory process (see section III).

5-Hydroxytryptamine (5-HT) is localized in blood platelets within specific membrane-bound intracellular organelles (251, 315), which also contain large

amounts of ATP (131, 158). Calcium appears to be necessary for the release of 5-HT induced by antigen (168) or by thrombin (130, 131, 224). The mere omission of calcium from the incubation medium is not always sufficient to depress significantly 5-HT release from isolated platelets (51, 224, 262); however, with the use of chelating agents the secretory response to thrombin is blocked and the blockade is readily reversed by calcium (224, 225). During thrombin-induced release the platelets remain intact in a calcium-free medium but there is no aggregation of platelets, which is usually observed in the presence of calcium (51, 131, 251). The need for calcium-chelating agents to manifest the effects of calcium deprivation on intact platelets suggests either that only minute amounts of calcium are necessary to sustain release or that an intracellular or firmly bound pool of calcium is important to sustain the release process. 5-HT release from rat brain slices induced by electrical stimulation is not modified by exposure to a calcium-free medium (41); however, calcium-chelating agents were not used in this study.

In isolated granule preparations, calcium enhances spontaneous 5-HT release in the absence of thrombin or any other stimulating agent (206, 262). In these isolated granule preparations, thrombin and ATP have no effect on release and excess magnesium inhibits spontaneous release. The 5-HT released both from isolated organelles and from intact platelets is accompanied by an enhanced loss of ATP, but only a small amount of protein (131, 156, 262). The parallel release of 5-HT and nucleotide in the same proportion (circa 6:1) as they exist in subcellular particles suggests that 5-HT release from platelets occurs by extrusion of material located in granules directly to the external medium (156, 158).

Electronmicrographs of isolated granules which have been exposed to calcium show ghost-like structures, with irregular, ill-defined membranes, which contain only very meager amounts of osmiophilic material (262). These findings are consistent with the idea that thrombin causes a specific increase in the permeability of the platelet cell membrane which permits an interaction of calcium with the intracellular granules containing 5-HT and leads to the release of 5-HT (131, 262). The site of calcium action on blood platelets is, of course, a matter of speculation. However, Grette (131) reported the isolation of a "contractile" protein from human blood platelets, whose physical properties are altered by low concentrations of ATP. Platelets do have ATPase activity (131), and ATP content of platelets decreases after stimulation with thrombin (157, 246). As a result of these findings, Grette (131) drew a parallel between 5-HT secretion and muscle contraction and suggested that an interaction between calcium, ATP, and a contractile protein may be the critical step in secretion just as this same interaction may be critical for muscle contraction and for many other specialized cellular activities (see also Stormorken, 303).

III. THE NATURE OF CALCIUM'S ROLE IN THE SECRETORY PROCESS

Although certain investigators have been able to show that secretion can continue, to some degree at least, under conditions of calcium deprivation, it still appears from the evidence already cited that calcium is a general requirement for

the secretory process. In order to attain some insight into the intimate mechanism by which calcium regulates secretion, one should first establish the means whereby the secretory product finds its way out of its respective organ. In most secretory systems electronmicroscopic and chemical studies have provided strong evidence that a large proportion of a tissue's secretory product is sequestered within membrane-bound organelles. Although this granular bound material might be just a stored form in equilibrium with readily releasable hormone in the cytoplasm, experiments carried out mainly on the adrenal medulla and neurohypophysis show that other components of the secretory granules such as protein and nucleotide also appear in the effluent (73, 105, 301). Such chemical findings support the idea originally put forward by the electronmicroscopists that secretion occurs by reverse pinocytosis (or exocytosis), which involves fusion of the granule membrane with the plasma membrane and the extrusion of the granule contents to the cell exterior (61, 252).

The apparent importance of the secretory granule in the secretory mechanism might suggest that calcium has some direct action on the granule. A number of studies on more primitive biological systems have shown that the addition of calcium to tissue suspensions causes lysis of granular components (133, 144, 167). In addition, in the amphibian, pituitary melanocyte-stimulating hormone causes darkening of the skin by a disruptive action on melanin-bearing chromatophores (melanophores)—a process which in some test systems requires calcium (203). However, despite such evidence from invertebrate and primitive vertebrate systems that calcium has a lytic action on certain granule systems, it has no marked or consistent stimulant effects on the release of secretory product from the following isolated granule preparations: catecholamine from chromaffin granules (6, 129, 148, 265); acetylcholine from cortical synaptic vesicles (328); vasopressin from pituitary granules (49, 264); and protein from zymogen granules (338). A stimulant action of calcium on release of catecholamines from chromaffin granules has been reported (248, 291), but the rather modest increase in release could not account for the tremendous outputs obtained with calcium in the intact adrenal gland (85). ATP, on the other hand, produces a more consistent and sometimes dose-dependent release of secretory product from isolated granule preparations *in vitro* (249, 264, 265, 338), and from intact mast cells (65). These findings suggest that the intracellular action of calcium requires some intermediate step that involves the presence of intracellular energy stores. The critical role of ATP or some other high-energy intermediate may explain why calcium has such a meager effect on isolated secretory granules. In all the calcium-dependent secretory systems in which an energy requirement for secretion has been studied, it appears as if energy is indeed required for maintaining secretion (121, 193, 275, 276, 290), and the activity of calcium as a secretogogue has been closely correlated with the ability of the tissue to produce metabolic energy (75, 275, 276, 325, 333). ATP splitting may be a necessary step in the secretory mechanism. ATPase is found in the granular fraction of many calcium-dependent systems (5, 120, 149, 204, 264, 334), and ATPase inhibitors prevent the ATP-induced release of product from a variety of preparations *in vitro* (64, 131, 264, 265, 330).

It is of interest that Puszkin *et al.* (266) have isolated an ATPase from mammalian brain which has properties similar to those of muscle actomyosin, and they suggest that the contractile protein may serve a universal function to control secretion. Grette (131) also has claimed to have isolated a contractile protein from human platelets. Thus, calcium may activate a "contractile" protein and the resulting contraction of membranes might be responsible for secretion. Such a theory, although somewhat speculative, fits the experimental evidence better than the hypothesis that calcium acts to neutralize opposing electrostatic forces between the granule (or vesicle) membrane and the cell membrane (6, 23, 298). The latter theory is made less tenable by the fact that it tends to minimize the importance of the direct participation of metabolic energy in the secretory process which has been clearly demonstrated in a number of secretory systems.

There is the possibility that calcium acts as an enzyme activator of some other macromolecular components of the secretory system. For example, the medullary catecholamine-containing granules contain a high content of lysolecithin (22, 301), and it has been suggested that phospholipase A activation by calcium releases catecholamines either by somehow facilitating the fusion of the granule membrane with lipids of the cell membrane (22), or by detaching prostaglandins from the cell membrane (267). Although Ramwell *et al.* (267) have demonstrated the release of prostaglandins from perfused adrenals of cats in response to acetylcholine, it is not likely that these unsaturated fatty acids have any direct role in the calcium-dependent secretory process, since catecholamine release induced by nicotine or calcium can be dissociated from prostaglandin release (295), and exogenously administered prostaglandin does not modify the secretory response to acetylcholine or calcium (231). Hokin and Hokin (see 155) have shown that stimulation of secretion in many secretory tissues leads to enhanced incorporation of precursors into phospholipids. However, it appears as if this phospholipid effect does not represent a causal mechanism for secretion, since calcium deprivation, which markedly depresses protein secretion from the pancreas and leucocytes, and catecholamine secretion from adrenal medullary slices, does not markedly affect the incorporation of phosphorus into phospholipids in these tissues (154, 316, 332).

On the other hand, perhaps the role of calcium must be sought in some action that does not involve the process of exocytosis. It has been found that lipids from isolated chromaffin granules (301) and cholinergic synaptic vesicles (329) differ in their composition from those of other cell constituents, including the plasma membrane. This finding might argue against the theory that secretion occurs by fusion of the cell membrane with the membrane of the secretory organelle, although it does not militate against the possibility that the empty granules remain in the cell after the secretory products are extruded. In addition, the theory of exocytosis as a mechanism of secretion does not seem applicable in the fetal rabbit pancreas, the adrenal cortex, or the H^+-secreting cells of the gastric mucosa, in which the secretory product may not be stored in membrane-bound organelles; yet calcium is also required for secretion in these systems. Thus, to overcome the difficulty of the ubiquity of the calcium requirement despite differ-

ences in the mode of secretion, a suggestion put forward by Whittaker (329) may merit serious consideration. He proposed the existence of intracellular tubular connections from synaptic vesicles to the external membrane, and that calcium in some way enlarges the lumen of these tubules to facilitate the egress of the secretory product. Alternatively, calcium may produce its effect by altering the physicochemical properties of the cytoplasm; this action would be analogous to its dispersive actions on squid axoplasm (151) or its coagulative effects on the cytoplasm of primitive organisms (144).

Finally, although it would be more satisfactory to the scientific purists to establish a unitary hypothesis for the action of calcium on secretion, the reader must be aware that future research may show that the role of calcium cannot be explained in terms of a single action, but will vary depending upon the mode of secretion. Any further conjecture will be withheld in lieu of direct experimental evidence. However, the rapidity and explosiveness with which calcium itself elicits secretion in certain tissues (85, 182, 183) suggest that the effect of calcium will, in the main, not be explained through an indirect action on some system such as cyclic AMP, as Geschwind (121) has proposed, but will be related to some more direct action on the secretory process.

IV. PARALLELISM BETWEEN STIMULUS-SECRETION COUPLING AND EXCITATION-CONTRACTION COUPLING IN MUSCLE

One of the most striking features of the plethora of data presented in this review is the parallelism between the processes regulating secretion and those regulating contraction. The most obvious similarity, of course, is the ability of calcium to activate both the secretory and the contractile mechanisms. The involvement of calcium in muscle contraction was clearly indicated by the experiments of Heilbrunn and Wiercinski (145), in which they recorded a shortening of myoplasm by an intracellular injection of calcium ions. To characterize the process in which calcium plays such a vital role, Sandow (286) coined the term "excitation-contraction coupling." Studies on the adrenal medulla (71, 85–87) initially revealed the many parallels between responses of secretory cells and those of muscle, which prompted the introduction of the term "stimulus-secretion coupling" to describe the sequence of events leading to the extrusion of secretory product.

The common denominator in secretion and contraction appears to be a transient intracellular accumulation or translocation of calcium ions, which is effected by a depolarization of the cell membrane. Cell depolarization leads to an enhanced calcium uptake and exchangeability in both types of tissue (14, 80, 247, 278). The properties of both calcium-dependent activation mechanisms are quite similar in that of all other cations tested only strontium and barium are able to stimulate directly both processes (50, 87, 145). Magnesium (27, 50, 86) and sodium (86, 221) inhibit the action of calcium in both systems, presumably by competing with calcium for sites involved with ion transport into the cell. Local anesthetics also depress both contraction (106, 107) and secretion (278, 282) by blocking calcium uptake and exchangeability.

Further analogies can be made with regard to the metabolic events involved in these two processes. Contraction (287), like secretion (275, 276), requires both ATP and calcium ions. ATP splitting is the immediate energy source for contraction, since contraction is inhibited when ATP splitting is depressed by inhibitors of ATPase (327). Likewise, ATPase inhibitors prevent ATP-induced secretion (64, 265); hence it appears that splitting of ATP may be required for secretion. Although it remains to be determined how much further one will be able to extend the parallels between "stimulus-secretion coupling" and "excitation-contraction coupling," it is tempting to speculate that a common mechanism may exist for the initiation of these two seemingly disparate processes. Thus, for example, studies have been cited in this review which at least hint at the possibility that a contractile protein with properties similar to muscle actomyosin exists in secretory cells. Douglas (73) has speculated on an interaction among calcium, ATP, and ATPase in the secretory process, and he drew analogies with events that may be involved in muscle contraction, where, according to Davies (52), the effect of calcium is to facilitate somehow the interaction of ATPase and ATP. It has been suggested from studies with certain tropomyosin preparations that calcium controls actomyosin contractility by reducing the affinity of ATP for some inhibitory site, which leads to ATP hydrolysis at the active site and contraction (207, 337). Thus, the hypothesis that calcium initiates contraction by removing an existing repression rather than by a direct activation (95) might also explain the effects of this cation on secretion. But whatever evidence future investigations uncover as to the intimate mechanism of calcium action in secretion and contraction, it appears reasonable at present to contemplate that nature in its usually efficient, organized, and uncomplicated way has devised a similar series of molecular events to control these two processes, which are so ubiquitous in biological systems.

REFERENCES

1. Abood, L. G.: Interrelationships between phosphates and calcium in bioelectric phenomena. Int. Rev. Neurobiol. **9**: 223-261, 1960.
2. Aborg, C. H., Novotny, J. and Uvnäs, B.: Ionic binding of histamine in mast cell granules. Acta Physiol. Scand. **69**: 276-283, 1967.
3. Baker, P. F., Blaustein, M. P., Hodgkin, A. L. and Steinhardt, R. A.: Influence of calcium on sodium efflux in squid axons. J. Physiol. (London) **200**: 431-458, 1969.
4. Baldessarini, R. J. and Kopin, I. J.: The effect of drugs on the release of norepinephrine-H^3 from central nervous system tissues by electrical stimulation *in vitro*. J. Pharmacol. Exp. Ther. **156**: 31-38, 1967.
5. Banks, P.: The adenosine-triphosphatase activity of adrenal chromaffin granules. Biochem. J. **95**: 490-496, 1965.
6. Banks, P.: An interaction between chromaffin granules and calcium ions. Biochem. J. **101**: 18c-20c, 1966.
7. Banks, P.: The effect of ouabain on the secretion of catecholamines and on the intracellular concentration of potassium. J. Physiol. (London) **193**: 631-637, 1967.
8. Banks, P., Biggins, R., Bishop, R., Christian, B. and Currie, N.: Sodium ions and the secretion of catecholamines. J. Physiol. (London) **200**: 797-805, 1969.
9. Bar, H. P. and Hechter, O.: Adenyl cyclase and hormone action. III. Calcium requirement for ACTH stimulation of adenyl cyclase. Biochem. Biophys. Res. Commun. **35**: 681-686, 1969.
10. Barreras, R. F. and Donaldson, R. M.: Effects of induced hypercalcemia on human gastric secretion. Gastroenterology **52**: 670-675, 1967.
11. Bdolah, A., Ben Zvi, R. and Schramm, M.: The mechanism of enzyme secretion by the cell. II. Secretion of amylase and other proteins by slices of rat parotid gland. Arch. Biochem. Biophys. **104**: 58-66, 1964.
12. Bennett, L. L., Curry, D. L. and Grodsky, G. M.: Calcium magnesium antagonism in insulin secretion by the perfused rat pancreas. Endocrinology **85**: 594-596, 1969.
13. Berlind, A. and Cooke, I. M.: Effect of calcium omission on neurosecretion and electrical activity of crab pericardial organs. Gen. Comp. Endocrinol. **11**: 458-463, 1968.
14. Bianchi, C. P.: Cell Calcium, London, Butterworths, 1968.

15. BIRKS, R. I.: The role of sodium ions in the metabolism of acetylcholine. Can. J. Biochem. Physiol. 41: 2573–2597, 1963.
16. BIRKS, R. I.: The role of sodium ions in the release of acetylcholine. *In* Pharmacology of Cholinergic and Adrenergic Transmission., ed. by G. B. Koelle, W. W. Douglas and A. Carlsson, pp. 3–19, Pergamon Press New York, 1965.
17. BIRKS, R. I., BURSTYN, P. G. R. AND FIRTH, D. R.: The form of sodium-calcium competition of the frog myoneural junction. J. Gen. Physiol. 52: 887–907, 1968.
18. BIRKS, R. I. AND COHEN, M. W.: The action of sodium pump inhibitors on neuromuscular transmission. Proc. Roy. Soc. Ser. B Biol. Sci. 170: 381–399, 1968.
19. BIRKS, R. I. AND COHEN, M. W.: The influence of internal sodium on the behavior of motor nerve endings. Proc. Roy. Soc. Ser. B Biol. Sci. 170: 401–421, 1968.
20. BIRKS, R. AND MACINTOSH, F. C.: Acetylcholine metabolism of a sympathetic ganglion. Can. J. Biochem. Physiol. 39: 787–827, 1961.
21. BIRMINGHAM, M. K., KURLENTS, E., LANE, R., MUHLSTOCK, B. AND TRAIKOV, H.: Effects of calcium on the potassium and sodium content of rat adrenal glands, on the stimulation of steroid production by adenosine 3′,5′-monophosphate, and on the response of the adrenal to short contact with ACTH. Can. J. Biochem. Physiol. 38: 1077–1085, 1960.
22. BLASCHKO, H.: Biochemical studies on chromaffin cells. Naunyn-Schmiedebergs Arch. Pharmakol. Exp. Pathol. 257: 143–147, 1967.
23. BLIOCH, Z. L., GLAGOLEVA, I. M., LIBERMAN, E. A. AND NENASHEV, V. A.: A study of the mechanism of quantal transmitter release at a chemical synapse. J. Physiol. (London) 199: 11–35, 1968.
24. BLOODWORTH, J. M. B. AND POWERS, K. L.: The ultrastructure of the normal dog adrenal. J. Anat. 102: 457–476, 1968.
25. BOELES, J. T., IRAUSQUIN-CATH, H. AND VELDSEMA, A.: Effects of various cations and drugs on the isolated hypogastric nerve-vas deferens preparation of the rat. Acta Physiol. Pharmacol. Néer. 12: 117–130, 1963.
26. BOGDANSKI, D. F. AND BRODIE, B. B.: The effects of inorganic ions on the storage and uptake of H^3-norepinephrine by rat heart slices. J. Pharmacol. Exp. Ther. 165: 181–189, 1969.
27. BOHR, D. F.: Electrolytes and smooth muscle contraction. Pharmacol. Rev. 16: 85–111, 1964.
28. BOROWITZ, J. L.: Effects of acetylcholine on the subcellular distribution of ^{45}Ca in the bovine adrenal medulla. Biochem. Pharmacol. 18: 715–723, 1969.
29. BOULLIN, D. J.: The action of extracellular cations on the release of the sympathetic transmitter from peripheral nerves. J. Physiol. (London) 189: 85–99, 1967.
30. BOYD, I. A. AND MARTIN, A. R.: Spontaneous subthreshold activity at mammalian neuromuscular junctions. J. Physiol. (London) 132: 61–73, 1956.
31. BOYD, I. A. AND MARTIN, A. R.: The end-plate potential in mammalian muscle. J. Physiol. (London) 132: 74–91, 1956.
32. BRINK, F.: The role of calcium ions in neural processes. Pharmacol. Rev. 6: 243–295, 1954.
33. BRYANT, S. H.: Transmission in squid giant synapses. The importance of oxygen supply and the effects of drugs. J. Gen. Physiol. 41: 473–484, 1958.
34. BURN, J. H.: Introductory Remarks. Second International Symposium on Catecholamines, Milano, Italy, July, 1965. Pharmacol. Rev. 18: 459–470, 1966.
35. BURN, J. H. AND GIBBONS, W. R.: The part played by calcium in determining the response to stimulation of sympathetic postganglionic fibres. Brit. J. Pharmacol. 22: 540–548, 1964.
36. BURN, J. H. AND GIBBONS, W. R.: The release of noradrenaline from sympathetic fibres in relation to calcium concentration. J. Physiol. (London) 181: 214–223, 1965.
37. CARE, A. D., COOPER, C. W., DUNCAN, T. AND ORIMO, H.: A study of thyrocalcitonin secretion by direct measurement of *in vivo* secretion rates in pigs. Endocrinology 83: 161–169, 1968.
38. CERF, J. A.: L'action physiologique et pharmacologique des ions alcalino-terreux sur les fibres nerveuses. *In* Ions Alcalino-Terreux, ed. by Z. M. Bacq, Handb. Exp. Pharmakol. 17 (1): 164–285, 1963.
39. CHAKRAVARTY, N.: The mechanism of histamine release in anaphylactic reaction in guinea pig and rat. Acta Physiol. Scand. 48: 146–166, 1960.
40. CHAKRAVARTY, N.: Further observations on the inhibition of histamine release by 2-deoxyglucose. Acta Physiol. Scand. 72: 425–432, 1968.
41. CHASE, T. N., KATZ, R. I. AND KOPIN, I. J.: Release of [^3H] serotonin from brain slices. J. Neurochem. 16: 607–615, 1969.
42. COLOMO, F. AND RAHAMIMOFF, R.: Interaction between sodium and calcium ions in the process of transmitter release at the neuromuscular junction. J. Physiol. (London) 198: 203–218, 1968.
43. COPP, D. H.: Calcitonin and parathyroid hormone. Annu. Rev. Pharmacol. 9: 327–344, 1969.
44. COPP, D. H. AND DAVIDSON, A. G. F.: Direct humoral control of parathyroid function in the dog. Proc. Soc. Exp. Biol. Med. 107: 342–344, 1961.
45. COWAN, S. L.: The actions of eserine-like compounds upon frog's nerve-muscle preparations, and conditions in which a single shock can evoke an augmented muscular response. Proc. Roy. Soc. Ser. B Biol. Sci. 129: 356–391, 1940.
46. CURRY, D. L., BENNETT, L. L. AND GRODSKY, G. M.: Requirement for calcium ion in insulin secretion by the perfused rat pancreas. Amer. J. Physiol. 214: 174–178, 1968.
47. CURRY, D. L., BENNETT, L. L. AND GRODSKY, G. M.: Dynamics of insulin secretion by the perfused rat pancreas. Endocrinology 83: 572–584, 1968.

48. CUTHBERT, A. W.: Membrane lipids and drug action. Pharmacol. Rev. 19: 59–106, 1967.
49. DANIEL, A. R. AND LEDERIS, K.: Hormone release from the neurohypophysis *in vitro*. Gen. Comp. Endocrinol. 3: 693–694, 1963.
50. DANIEL, E. E.: Attempted synthesis of data regarding divalent ions in muscle function. *In* Muscle, ed. by W. M. Paul, E. E. Daniel, C. M. Kay and G. Monckton, pp. 295–313, Pergamon Press, New York, 1965.
51. DAPRADA, M., BARTHOLINI, G. AND PLETSCHER, A.: Formation of 5-hydroxytryptophol by blood platelets after thrombin and reserpine. Experientia (Basel) 21: 135–136, 1964.
52. DAVIES, R. E.: The role of ATP in muscle contraction. *In* Muscle, ed. by W. M. Paul, E. E. Daniel, C. M. Kay and G. Monckton, pp. 49–69, Pergamon Press, New York, 1965.
53. DEAN, C. R. AND HOPE, D. B.: The isolation of neurophysin I and II from bovine pituitary neurosecretory granules separated on a large scale from other subcellular organelles. Biochem. J. 106: 565–573, 1968.
54. DEAN, P. M. AND MATTHEWS, E. K.: Electrical activity in pancreatic islet cells. Nature (London) 219: 389–390, 1968.
55. DEL CASTILLO, J. AND ENGBAEK, L.: The nature of the neuromuscular block produced by magnesium. J. Physiol. (London) 124: 370–384, 1954.
56. DEL CASTILLO, J. AND KATZ, B.: The effect of magnesium on the activity of motor nerve endings. J. Physiol. (London) 124: 553–559, 1954.
57. DEL CASTILLO, J. AND KATZ, B.: Quantal components of the endplate potential. J. Physiol. (London) 124: 560–573, 1954.
58. DEL CASTILLO, J. AND KATZ, B.: Biophysical aspects of neuromuscular transmission. Progr. Biophys. Biophys. Chem. 6: 122–170, 1956.
59. DEL CASTILLO, J. AND STARK, L.: The effect of calcium ions on the motor end-plate potentials. J. Physiol. (London) 116: 507–515, 1952.
60. DEROBERTIS, E. D. P. AND BENNETT, H. S.: Some features of the submicroscopic morphology of synapses in frog and earthworm. J. Biophys. Biochem. Cytol. 1: 47–58, 1955.
61. DEROBERTIS, E. D. P. AND SABATINI, D. D.: Submicroscopic analysis of the secretory process in the adrenal medulla. Fed. Proc. 19: (part II, suppl. 5) 70–73, 1960.
62. DESMEDT, J. E.: L'action des cations alcalino-terreux sur les transmissions neuromusculaire et synaptiques. *In* Ions Alcalino-Terreux, ed. by Z. M. Bacq, Handb. Exp. Pharmakol. 17 (1): 295–336, 1963.
63. DETTBARN, W. D. AND ROSENBERG, P.: Effect of ions on the efflux of acetylcholine from peripheral nerve. J. Gen. Physiol. 50: 447–460, 1966.
64. DIAMANT, B.: The mechanism of histamine release from rat mast cells induced by adenosine triphosphate. Acta Pharmacol. Toxicol. 25: suppl. 4, pp. 33, 1967.
65. DIAMANT, B. AND KRÜGER, P. G.: Histamine release from isolated rat peritoneal mast cells induced by adenosine-5'-triphosphate. Acta Physiol. Scand. 71: 291–302, 1967.
66. DIAMANT, B. AND KRÜGER, P. G.: Structural changes of isolated rat peritoneal mast cells induced by adenosine triphosphate and adenosine diphosphate. J. Histochem. Cytochem. 16: 707–716, 1968.
67. DICKER, S. E.: Release of vasopressin and oxytocin from isolated pituitary glands of adult and new-born rats. J. Physiol. (London) 185: 429–444, 1966.
68. DODGE, F. A., MILEDI, R. AND RAHAMIMOFF, R.: Strontium and quantal release of transmitter at the neuromuscular junction. J. Physiol. (London) 200: 267–283, 1969.
69. DODGE, F. A., JR. AND RAHAMIMOFF, R.: Co-operative action of calcium ions in transmitter release at the neuromuscular junction. J. Physiol. (London) 193: 419–432, 1967.
70. DOUGLAS, W. W.: A possible mechanism of neurosecretion: Release of vasopressin by depolarization and its dependence on calcium. Nature (London) 197: 81–82, 1963.
71. DOUGLAS, W. W.: Calcium dependent links in stimulus-secretion in the adrenal medulla and neurohypophysis. *In* Mechanisms of Release of Biogenic Amines, Proc. Int. Wenner-Gren Symposium, Stockholm, pp. 267–290, Pergamon Press, London, Feb., 1965.
72. DOUGLAS, W. W.: The mechanism of release of catecholamines from the adrenal medulla. Second International Symposium on Catecholamines, Milano, Italy, July, 1965, Pharmacol. Rev. 18: 471–480, 1966.
73. DOUGLAS, W. W.: Stimulus-secretion coupling: The concept and clues from chromaffin and other cells. First Gaddum Memorial Lecture, Cambridge, England. Brit. J. Pharmacol. 34: 451–474, 1968.
74. DOUGLAS, W. W. AND ISHIDA, A.: The stimulant effect of cold on vasopressin release from the neurophyophysis *in vitro*. J. Physiol. (London) 179: 185–191, 1965.
75. DOUGLAS, W. W., ISHIDA, A. AND POISNER, A. M.: The effects of metabolic inhibitors on the release of vasopressin from the isolated neurohypophysis. J. Physiol. (London) 181: 753–759, 1965.
76. DOUGLAS, W. W. AND KANNO, T.: The effect of amethocaine on acetylcholine-induced depolarization and catecholamine secretion in the adrenal chromaffin cell. Brit. J. Pharmacol. Chemother. 30: 612–619, 1967.
77. DOUGLAS, W. W., KANNO, T. AND SAMPSON, S. R.: Effects of acetylcholine and other medullary secretogogues and antagonists on the membrane potential of adrenal chromaffin cells: an analysis employing techniques of tissue culture. J. Physiol. (London) 188: 107–120, 1967.
78. DOUGLAS, W. W., KANNO, T. AND SAMPSON, S. R.: Influence of the ionic environment on the membrane potential of the adrenal chromaffin cells and on the depolarizing effect of acetylcholine. J. Physiol. (London) 191: 107–121, 1967.
79. DOUGLAS, W. W., LYWOOD, D. W. AND STRAUB, R. W.: The stimulant effect of barium on the release of acetylcholine from the superior cervical ganglion. J. Physiol. (London) 156: 515–522, 1961.
80. DOUGLAS, W. W. AND POISNER, A. M.: On the mode of acetylcholine in evoking adrenal medullary secretion: increased uptake of calcium during the secretory response. J. Physiol. (London) 162: 385–392, 1962.

81. 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.
82. DOUGLAS, W. W. AND POISNER, A. M.: Stimulus-secretion coupling in a neurosecretory organ: The role of calcium in the release of vasopressin from the neurohypophysis. J. Physiol. (London) 172: 1-18, 1964.
83. DOUGLAS, W. W. AND POISNER, A. M.: Calcium movement in the neurohypophysis of the rat and its relation to the release of vasopressin. J. Physiol. (London) 172: 19-30, 1964.
84. DOUGLAS, W. W. AND RUBIN, R. P.: Mechanism of nicotinic action at the adrenal medulla: Calcium as a link in stimulus-secretion coupling. Nature (London) 192: 1087-1089, 1961.
85. DOUGLAS, W. W. AND RUBIN, R. P.: The role of calcium in the secretory response of the adrenal medulla to acetylcholine. J. Physiol. (London) 159: 40-57, 1961.
86. DOUGLAS, W. W. AND RUBIN, R. P.: The mechanism of catecholamine release from the adrenal medulla and the role of calcium stimulus-secretion coupling. J. Physiol. (London) 167: 288-310, 1963.
87. DOUGLAS, W. W. AND RUBIN, R. P.: The effects of alkaline earths and other divalent cations on adrenal medullary secretion. J. Physiol. (London) 175: 231-241, 1964.
88. DOUGLAS, W. W. AND RUBIN, R. P.: Stimulant action of barium on the adrenal medulla. Nature (London) 203: 305-307, 1964.
89. DOUGLAS, W. W. AND RUBIN, R. P.: Unpublished observations.
90. DREISBACH, R. H.: Effect of pilocarpine on transfer of Ca^{45} and K^{42} to rat submaxillary gland and kidney. J. Pharmacol. Exp. Ther. 131: 257-276, 1961.
91. DREISBACH, R. H.: Regulation of Ca^{45} transfer in rat salivary and lacrimal glands *in vitro*. Amer. J. Physiol. 207: 1015-1020, 1964.
92. DREISBACH, R. H.: Calcium transfer in rat salivary and lacrimal glands. *In* Salivary Glands and Their Secretions, ed. by L. M. Sreebny and J. Meyer, vol. 3, pp. 237-251, Macmillan, New York, 1964.
93. DREISBACH, R. H.: Effect of isoproterenol on calcium, protein, and electrolytes of rat submaxillary gland. Proc. Soc. Exp. Biol. Med. 126: 279-281. 1967.
94. DURBIN, R. P.: Anion requirements for gastric acid secretion. J. Gen. Physiol. 47: 735-748, 1964.
95. EBASHI, S. AND ENDO, M.: Calcium ion and muscle contraction. Progr. Biophys. Mol. Biol. 18: 123-183, 1968.
96. ELMQVIST, D. AND FELDMAN, D. S.: Calcium dependence of spontaneous acetylcholine release at mammalian motor nerve terminals. J. Physiol. (London) 181: 487-497, 1965.
97. ELMQVIST, D. AND FELDMAN, D. S.: Effects of sodium pump inhibitors on spontaneous acetylcholine release at the neuromuscular junction. J. Physiol. (London) 181: 498-505, 1965.
98. ELMQVIST, D. AND FELDMAN, D. S.: Influence of ionic environment on acetylcholine release from the motor nerve terminals. Acta Physiol. Scand. 67: 34-42, 1966.
99. ELMQVIST, D. AND QUASTEL, D. M. J.: A quantitative study of end-plate potentials in isolated human muscle. J. Physiol. (London) 178: 505-529, 1965.
100. EMMELIN, N.: Secretory nerves of the salivary glands. *In* Salivary Glands and Their Secretions, ed. by L. M. Sreebny and J. Meyer, vol. 3, pp. 161-173, Macmillan, New York, 1964.
101. ENGBAEK, L.: The pharmacological actions of magnesium ions with particular reference to the neuromuscular and cardiovascular system. Pharmacol. Rev. 4: 396-414, 1952.
102. FARMER, J. B. AND CAMPBELL, I. K.: Calcium and magnesium ions: influence on the response of an isolated artery to sympathetic nerve stimulation, noradrenaline and tyramine. Brit. J. Pharmacol. Chemother. 29: 319-328, 1967.
103. FATT, P. AND KATZ, B.: Spontaneous subthreshold activity at motor nerve endings. J. Physiol. (London) 117: 109-128, 1952.
104. FATT, P. AND KATZ, B.: The effect of sodium ions on neuromuscular transmission. J. Physiol. (London) 118: 73-87, 1952.
105. FAWCETT, C. P., POWELL, A. E. AND SACHS, H.: Biosynthesis and release of neurophysin. Endocrinology, 83: 1299-1310, 1968.
106. FEINSTEIN, M. B. AND PAIMRE, M.: Pharmacological action of local anesthetics on excitation-contraction coupling in striated and smooth muscle. Fed. Proc. 28: 1643-1648, 1969.
107. FEINSTEIN, M. B., PAIMRE, M. AND LEE, M.: Effect of local anesthetics on excitation-coupling mechanisms. Trans. N. Y. Acad. Sci. 30: 1073-1081, 1968.
108. FENG, T. P.: Studies on the neuromuscular junction. II. The universal antagonism between calcium and curarizing agencies. Chin. J. Physiol. 10: 513-528, 1936.
109. FEX, J.: Calcium action at an inhibitory synapse. Nature (London) 213: 1233-1234, 1967.
110. FORTE, J. G., ADAMS, P. H. AND DAVIES, R. E.: Source of the gastric mucosal potential difference. Nature (London) 197: 874-876, 1963.
111. FORTE, J. G. AND NAUSS, A. H.: Effects of calcium removal on bullfrog gastric mucosa. Amer. J. Physiol. 205: 631-637, 1963.
112. FRANKENHAEUSER, B. AND HODGKIN, A. L.: The action of calcium on the electrical properties of squid axons. J. Physiol. (London) 137: 218-244, 1957.
113. FREDERICQ, E.: Physiochimie des ions alcalino-terreux. *In* Ions Alcalino-Terreux, ed. by Z. M. Bacq, Handb. Exp. Pharmakol. 17 (1): 20-73, 1963.
114. GAGE, P. W.: Effect of cardiac glycosides on neuromuscular transmission. Nature (London) 205: 84-85, 1965.
115. GAGE, P. W.: Depolarization and excitation-secretion coupling in presynaptic terminals. Fed. Proc. 26: 1627-1632, 1967.
116. GAGE, P. W. AND QUASTEL, D. M. J.: Dual effect of potassium on transmitter release. Nature (London) 206: 625-626, 1965.

117. GAGE, P. W. AND QUASTEL, D. M. J.: Competition between sodium and calcium ions in transmitter release at mammalian neuromuscular junctions. J. Physiol. (London) 185: 95–123, 1966.
118. GERHARDS, K. P., RÖTTCHER, M. AND STRAUB, R. W.: Wirkungen von Ca und Mg auf Freisetzung und Synthese von Acetylcholin am spontan aktiven Darm. Pflügers Arch. Gesamte Physiol. Menschen Tiere 279: 239–250, 1964.
119. GERHARDS, K. P., RÖTTCHER, M. AND STRAUB, R. W.: Wirkung von Ca und Mg auf Freisetzung und Synthese von Acetylcholin am ruhiggestellten Darm. Pflügers Arch. Gesamte Physiol. Menschen Tiere 279: 251–264, 1964.
120. GERMAIN, M. AND PROULX, P.: Adenosine triphosphatase activity in synaptic vesicles of rat brain. Biochem. Pharmacol. 14: 1815–1819, 1965.
121. GESCHWIND, I.: Mechanism of action of releasing factors. *In* Frontiers in Neuroendocrinology, ed. by W. F. Ganong and L. Martini, Oxford, New York, pp. 389–431, 1969.
122. GILLIS, C. N. AND PATON, D. M.: Cation dependence of sympathetic transmitter retention by slices of rat ventricle. Brit. J. Pharmacol. 29: 309–318, 1967.
123. GINSBURG, M.: Production, release, transportation and elimination of the neurohypophysial hormones. Handb. Exp. Pharmakol. XXIII: 286–371, 1968.
124. GINSBURG, M.: Molecular aspects of neurohypophysial hormone release. Proc. Roy. Soc. Ser. B Biol. Sci. 170: 27–36, 1968.
125. GINSBURG, M. AND IRELAND, M.: The role of neurophysin in the transport and release of neurohypophysial hormones. J. Endocrinol. 35: 289–298, 1966.
126. GINSBURG, M., JAYASENA, K. AND THOMAS, P. J.: The preparation and properties of porcine neurophysin and the influence of calcium on the hormone-neurophysin complex. J. Physiol. (London) 184: 387–401, 1966.
127. GRAY, J. S. AND ADKINSON, J. L.: The effect of inorganic ions on gastric secretion *in vitro*. Amer. J. Physiol. 134: 27–31, 1941.
128. GREAVES, M. W. AND MONGAR, J. L.: The mechanism of anaphylactic histamine release from rabbit leucocytes. Immunology 15: 743–749, 1968.
129. GREENBERG, R. AND KOLEN, C. A.: Effects of acetylcholine and calcium ions on the spontaneous release of epinephrine from catecholamine granules. Proc. Soc. Exp. Biol. Med. 121: 1179–1184, 1966.
130. GRETTE, K.: The release of 5-hydroxytryptamine (serotonin) from blood platelets during coagulation. Scand. J. Clin. Lab. Invest. 11: 50–56, 1959.
131. GRETTE, K.: Studies on the mechanism of thrombin-catalyzed hemostatic reactions in blood platelets. Acta Physiol. Scand. 56: (suppl. 195) 1–87, 1962.
132. GRODSKY, G. M. AND BENNETT, L. L.: Cation requirements for insulin secretion in the isolated perfused pancreas. Diabetes 15: 910–913, 1966.
133. GROSS, P. R.: On the mechanism of the yolk-lysis reaction. Protoplasma 43: 416–428, 1954.
134. HAEUSLER, G., THOENEN, H., HAEFELY, W. AND HUERLIMANN, A.: Electrical events in cardiac adrenergic nerves and noradrenaline release from the heart induced by acetylcholine and KCl. Naunyn-Schmiedebergs Arch. Pharmakol. Exp. Pathol. 261: 389–411, 1968.
135. HAGAN, N. H. AND ORMOND, J. K.: Relation of calcium to the cardioinhibitory function of the vagus. Amer. J. Physiol. 30: 105–113, 1912.
136. HAGEN, J. M.: Stimulation of secretion and metabolism in mouse parotid glands *in vitro*. Biochem. Pharmacol. 2: 206–214, 1959.
137. HALES, C. N. AND MILNER, R. D. G.: The role of sodium and potassium in insulin secretion from rabbit pancreas. J. Physiol. (London) 194: 725–743, 1968.
138. HALES, C. N. AND MILNER, R. D. G.: Cations and the secretion of insulin from rabbit pancreas *in vitro*. J. Physiol. (London) 199: 177–187, 1968.
139. HALLER, E. W., SACHS, H., SPERELAKIS, N. AND SHARE, L.: Release of vasopressin from isolated guinea pig posterior pituitaries. Amer. J. Physiol. 209: 79–83, 1965.
140. HAMILTON, J. W. AND COHN, D. V.: Studies on the biosynthesis *in vitro* of parathyroid hormone. I. Synthesis of parathyroid hormone by bovine parathyroid gland slices and its control by calcium. J. Biol. Chem. 244: 5421–5429, 1969.
141. HARVEY, A. M. AND MACINTOSH, F. C.: Calcium and synaptic transmission in a sympathetic ganglion. J. Physiol. (London) 97: 408–416, 1940.
142. HAZARD, R. AND WURMSER, L.: Magnesium et acetylcholine. Differenciation par le magnesium des effects de l'ion potassium de ceux de l'acetylcholine. C. R. Séances Soc. Biol. 130: 1424–1426, 1939.
143. HECHTER, O., ZAFFARONI, A., JACOBSEN, R. P., LEVY, H., JEANLOZ, R. W., SCHENKER, V. AND PINCUS, G.: The nature of the biogenesis of the adrenal secretory product. Recent Progr. Hormone Res. 6: 215–246, 1951.
144. HEILBRUNN, L. V.: The Dynamics of Living Protoplasm, Academic Press, New York, 1956.
145. HEILBRUNN, L. V. AND WIERCINSKI, F. J.: The action of various cations on muscle protoplasm. J. Cell Comp. Physiol. 29: 15–32, 1947.
146. HEINZ, E. AND DURBIN, R.: Evidence for an independent hydrogen-ion pump in the stomach. Biochim. Biophys. Acta 31: 246–247, 1959.
147. HEMSWORTH, B. A. AND MITCHELL, J. F.: The characteristics of acetylcholine release mechanisms in the auditory cortex. Brit. J. Pharmacol. 36: 161–170, 1969.
148. HILLARP, N. A.: The release of catecholamine from the amine containing granules of the adrenal medulla. Acta Physiol. Scand. 43: 292–302, 1958.
149. HILLARP, N. A.: Enzymic systems involving adenosine-phosphates in the adrenaline and noradrenaline containing granules of the adrenal medulla. Acta Physiol. Scand. 42: 144–165, 1958.

150. HIRSCH, P. F. AND MUNSON, P. L.: Thyrocalcitonin. Physiol. Rev. 49: 548–622, 1969.
151. HODGKIN, A. L. AND KATZ, B.: The effect of calcium on the axoplasm of giant nerve fibers. J. Exp. Biol. 26: 292–294, 1949.
152. HODGKIN, A. L. AND KEYNES, R. D.: Movements of labelled calcium in squid giant axons. J. Physiol. (London) 138: 253–281, 1957.
153. HOGBERG, B. AND UVNÄS, B.: Further observations on the disruption of rat mesentery mast cells caused by compound 48/80, antigen-antibody reaction, lecithinase A, and decylamine. Acta Physiol. Scand. 48: 133–145, 1960.
154. 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.
155. HOKIN, L. E.: Dynamic aspects of phospholipids during protein secretion. Int. Rev. Cytol. 23: 187–208, 1968.
156. HOLMSEN, H. AND DAY, H. J.: Thrombin-induced platelet release reaction and platelet lysosomes. Nature (London) 219: 760–761, 1968.
157. HOLMSEN, H., DAY, H. J. AND STORM, E.: Adenine nucleotide metabolism of blood platelets. VI. Subcellular localization of nucleotide pools with different functions in the platelet release reaction. Biochim. Biophys. Acta 186: 254–266, 1969.
158. HOLMSEN, H., DAY, H. J. AND STORMORKEN, H.: The blood platelet release reaction. Scand. J. Haematol. (suppl.) 8: 1–26, 1969.
159. HOLZBAUER, M.: The corticosterone content of rat adrenals under different experimental conditions. J. Physiol. (London) 139: 294–305, 1957.
160. HOUSSAY, B. A. AND MOLINELLI, E. A.: Excitabilité des fibres adrenaline-secretoires du nerf grand splanchnique. Fréquences, seuil et optimum des stimulus. Role de l'ion calcium. C. R. Séances Soc. Biol. 99: 172–174, 1928.
161. HOYLE, G.: The effects of some common cations on neuromuscular transmission in insects. J. Physiol. (London) 127: 90–103, 1955.
162. HUBBARD, J. I.: The effect of calcium and magnesium on the spontaneous release of transmitter from mammalian motor nerve endings. J. Physiol. (London) 159: 507–517, 1961.
163. HUBBARD, J. I., JONES, S. F. AND LANDAU, E. M.: On the mechanism by which calcium and magnesium affect the spontaneous release of transmitter from mammalian motor nerve terminals. J. Physiol. (London) 194: 355–380, 1968.
164. HUBBARD, J. I., JONES, S. F. AND LANDAU, E. M.: On the mechanism by which calcium and magnesium affect the release of transmitter by nerve impulses. J. Physiol. (London) 196: 75–86, 1968.
165. HUBBARD, J. I. AND KWANBUNBUMPEN, S.: Evidence for the vesicle hypothesis. J. Physiol. (London) 194: 407–420, 1968.
166. HUKOVIC, S. AND MUSCHOLL, E.: Die Noradrenalin-Abgabe aus dem isolierten Kaninchenherzen bei sympathischen Nervenreizung und ihre pharmakologische Beeinflussung. Naunyn-Schmiedebergs Arch. Pharmakol. Exp. Pathol. 244: 81–96, 1962.
167. HULTIN, T.: On the acid formation, breakdown of cytoplasmic inclusions, and increased viscosity of *Paracentrotus* egg homogenates after the addition of calcium. Exp. Cell Res. 1: 272–283, 1950.
168. HUMPHREY, J. H. AND JAQUES, R.: The release of histamine and 5-hydroxytryptamine (serotonin) from platelets by antigen-antibody reactions (*in vitro*). J. Physiol. (London) 128: 9–27, 1955.
169. HUTTER, O. F. AND KOSTIAL, K.: Effect of magnesium and calcium ions on the release of acetylcholine. J. Physiol. (London) 124: 234–241, 1954.
170. HUTTER, O. F. AND KOSTIAL, K.: The relationship of sodium ions to the release of acetylcholine. J. Physiol. (London) 129: 159–166, 1955.
171. ISHIDA, A.: The effect of tetrodotoxin on calcium-dependent link in stimulus-secretion coupling in neurohypophysis. Jap. J. Physiol. 17: 308–320, 1967.
172. ISHIDA, A.: Stimulus-secretion coupling on the oxytocin release from the isolated posterior pituitary lobe. Jap. J. Physiol. 18: 471–480, 1968.
173. IVERSEN, L. L. AND KRAVITZ, E. A.: Sodium dependence of transmitter uptake at adrenergic nerve terminals. Mol. Pharmacol. 2: 360–362, 1966.
174. JAANUS, S. D., ROSENSTEIN, M. J. AND RUBIN, R. P.: On the mode of action of ACTH on the isolated perfused adrenal gland. J. Physiol. (London) 209, in press.
175. JACOBSON, A., SCHWARTZ, M. AND REHM, W. S.: Effects of removal of calcium from bathing media on frog stomach Amer. J. Physiol. 209: 134–140, 1965.
176. JENKINSON, D. H.: The nature of the antagonism between calcium and magnesium ions at the neuromuscular junction. J. Physiol. (London) 138: 434–444, 1957.
177. JOHNSON, A. R. AND MORAN, N. C.: Release of histamine from rat mast cells: A comparison of the effects of 48/80 and two antigen-antibody systems. Fed. Proc. 28: 1716–1720, 1969.
178. JOHNSON, A. R. AND MORAN, N. C.: Selective release of histamine from rat mast cells by compound 48/80 and antigen. Amer. J. Physiol. 216: 453–459, 1969.
179. KAO, C. Y.: Tetrodotoxin, saxitoxin and their significance in the study of excitation phenomena. Pharmacol. Rev. 18: 997–1049, 1966.
180. KATZ, B.: Microphysiology of the neuromuscular junction. Bull. Johns Hopkins Hosp. 102: 275–312, 1958.
181. KATZ, B.: The transmission of impulses from nerve to muscle and the subcellular unit of synaptic action. Proc. Roy. Soc. Ser. B Biol. Sci. 155: 455–477, 1962.
182. KATZ, B. AND MILEDI, R.: The effect of calcium on acetylcholine release from motor nerve terminals. Proc. Roy. Soc. Ser. B Biol. Sci. 161: 496–503, 1965.

183. KATZ, B. AND MILEDI, R.: Modification of transmitter release by electrical interference with motor nerve endings. Proc. Roy. Soc. Ser. B Biol. Sci. **167:** 1–7, 1967.
184. KATZ, B. AND MILEDI, R.: The timing of calcium action during neuromuscular transmission. J. Physiol. (London) **189:** 535–544, 1967.
185. KATZ, B. AND MILEDI, R.: Tetrodotoxin and neuromuscular transmission. Proc. Roy. Soc. Ser. B Biol. Sci. **167:** 8–22, 1967.
186. KATZ, B. AND MILEDI, R.: A study of synaptic transmission in the absence of nerve impulses. J. Physiol. (London) **192:** 407–436, 1967.
187. KATZ, B. AND MILEDI, R.: Spontaneous and evoked activity of motor nerve endings in calcium Ringer. J. Physiol. (London) **203:** 689–706, 1969.
188. KATZ, R. I. AND KOPIN, I. J.: Electrical field-stimulated release of norepinephrine-H^3 from rat atrium: Effects of ions and drugs. J. Pharmacol. Exp. Ther. **169:** 229–236, 1969.
189. KELLY, J. S.: Antagonism between Na^+ and Ca^{+2} at the neuromuscular junction. Nature (London) **205:** 296–297, 1965.
190. KELLY, J. S.: The antagonism of Ca^{+2} by Na^+ and other monovalent ions at the frog neuromuscular junction. Quart. J. Exp. Physiol. Cog. Med. Sci. **53:** 239–249, 1968.
191. KEYNES, R. D. AND LEWIS, P. R.: The intracellular calcium contents of some invertebrate nerve fibers. J. Physiol. (London) **134:** 399–407, 1956.
192. KIRPEKAR, S. M. AND MISU, Y.: Release of noradrenaline by splenic nerve stimulation and its dependence on calcium. J. Physiol. (London) **188:** 219–234, 1967.
193. KIRPEKAR, S. M., PRAT, J. C. AND YAMAMOTO, H.: Effects of metabolic inhibitors on norepinephrine release from the perfused spleen of the cat. J. Pharmacol. Exp. Ther. **172:** 342–350, 1970.
194. KIRPEKAR, S. M. AND WAKADE, A. R.: Release of noradrenaline from the cat spleen by potassium. J. Physiol. (London) **194:** 595–608, 1968.
195. KIRPEKAR, S. M. AND WAKADE, A. R.: Factors influencing noradrenaline uptake by the perfused spleen of the cat. J. Physiol. (London) **194:** 609–626, 1968.
196. KIRSHNER, N. AND SMITH, W. J.: Requirements for secretion from the adrenal medulla. Life Sci. **8:** 799–803, 1969.
197. KOCIAN, J. AND HLAVACKOVA, D.: Calcium and gastric secretion. Rev. Czech. Med. **15:** 55–61, 1969.
198. KRAICER, J., MILLIGAN, J. V., GOSBEE, J L., CONRAD, R. G. AND BRANSON, C. M.: *In vitro* release of ACTH: Effects of potassium, calcium and corticosterone. Endocrinology **85:** 1144–1153, 1969.
199. KUFFLER, S. W.: The effect of calcium on the neuromuscular junction. J. Neurophysiol. **7:** 17–26, 1944.
200. KURIYAMA, H.: Effect of calcium and magnesium on neuromuscular transmission in the hypogastric nerve-vas deferens preparation of the guinea pig. J. Physiol. (London) **175:** 211–230, 1964.
201. LAMBERT, A. E., JEANRENAUD, B., JUNOD, A. AND RENOLD, A E.: Organ culture of fetal rat pancreas. II. Insulin release induced by amino and organic acids, by hormonal peptides, by cationic alterations of the medium and by other agents. Biochim. Biophys. Acta **174:** 540–553, 1969.
202. LANDAU, E. M.: The interaction of presynaptic polarization with calcium and magnesium in modifying spontaneous transmitter release from mammalian motor nerve terminals. J. Physiol. (London) **203:** 281–299, 1968.
203. LANDE, S. AND LERNER, A. B.: The biochemistry of melanotropic agents. Pharmacol. Rev. **19:** 1–20, 1967.
204. LAZARUS, S., BARDEN, H. AND BRADSHAW, M. S.: Pancreatic beta cells and alloxan toxicity. Enzymatic histochemistry and toxic mechanisms. Arch. Pathol. **73:** 210–222, 1962.
205. LEHNINGER, A. L., CARAFOLI, E. AND ROSSI, C. S.: Energy-linked ion movements in mitochondrial systems. Advan. Enzymol. **29:** 259–320, 1967.
206. LEMBECK, F. AND HELD, H.: Serotoninfreisetzung aus isolierten Granula enterochromaffiner Zellen. Naunyn-Schmiedebergs Arch. Pharmakol. Exp. Pathol. **253:** 409–420, 1966.
207. LEVY, H. M. AND RYAN, E. M.: Evidence that calcium activates the contraction of actomyosin by overcoming substrate inhibition. Nature (London) **205:** 703–705, 1965.
208. LICHTENSTEIN, L. M. AND OSLER, A. G.: Studies on the mechanisms of hypersensitivity phenomena. IX. Histamine release from human leucocytes by ragweed pollen antigen. J. Exp. Med. **120:** 507–530, 1964.
209. LILEY, A. W.: The quantal components of the mammalian end-plate potential. J. Physiol. (London) **133:** 571–587, 1956.
210. LILEY, A. W.: The effects of presynaptic polarization on the spontaneous activity at the mammalian neuromuscular junction. J. Physiol. (London) **134:** 427–443, 1956.
211. LINDMAR, R., LÖFFELHOLZ, K. AND MUSCHOLL, E.: Unterschiede zwischen Tyramin und Dimethylphenylpiperzin in der Ca^{++}-Abhängigkeit und im zeitlichen Verlauf der Noradrenalin-Freisetzung am isolierten Kaninchenherzen. Experientia (Basel) **23:** 933–934, 1967.
212. LIPICKY, R. J., HERTZ, L. AND SHANES, A. M.: Ca^{45} transfer and acetylcholine release in the rabbit superior cervical ganglion. J. Cell. Comp. Physiol. **62:** 233–242, 1963.
213. LITTLEDIKE, E. T., WITZEL, D. A. AND WHIPP, S. C.: Insulin: Evidence for inhibition of release in spontaneous hypocalcemia. Proc. Soc. Exp. Biol. Med. **129:** 135–139, 1968.
214. LOCKE, F. S.: Notiz uber den Einfluss physiologischer Kochsalzlösung auf die elektrische Erregbarkeit von Muskel und Nerv. Zentralbl. Physiol. **8:** 166–167, 1894.
215. LÖFFELHOLZ, K.: Unterschungen über die Noradrenalin Freisetzung durch Acetylcholin am perfundierten Kaninchenherzen. Naunyn-Schmiedebergs Arch. Pharmakol. Exp. Pathol. **258:** 108–122, 1967.
216. LONG, J. A. AND JONES, A. L.: The fine structure of the zona glomerulosa and the zona fasciculata of the adrenal cortex of the opposum. Amer. J. Anat. **120:** 463–471, 1967.
217. LUCKE, B. AND MCCUTCHEON, M.: The living cell as an osmotic system and its permeability to water. Physiol. Rev. **12:** 68–139, 1932.

218. LUNDBERG, A.: Anionic dependence of secretion and secretory potentials in the perfused sublingual gland. Acta Physiol. Scand. **40**: 101–112, 1957.
219. LUNDBERG, A.: Electrophysiology of salivary glands. Physiol. Rev. **38**: 21–40, 1958.
220. LUNDBERG, A. AND QUILISCH, H.: On the effect of calcium on presynaptic potentiation and depression at the neuromuscular junction. Acta Physiol. Scand. **30**: (suppl. 111) 121–129, 1953.
221. LUTTGAU, H. C.: The role of calcium ions in excitation-contraction coupling. *In* Electrophysiology of the Heart, ed. by B. Taccardi and G. Marchetti, pp. 87–95, Pergamon Press, New York, 1965.
222. MAMBRINI, J. AND BENOIT, P. R.: Action du calcium sur la jonction neuromusculaire chez la Grenouille. C. R. Séances Soc. Biol. **158**: 1454–1458, 1964.
223. MANERY, J. F.: Effects of Ca ions on membranes. Fed. Proc. **25**: 1804–1810, 1966.
224. MARKWARDT, F. AND BARTHEL, W.: Unterschungen über die Freisetzung von Serotonin aus Blutplattchen durch Thrombin. Naunyn-Schmiedebergs Arch. Pharmakol. Exp. Pathol. **249**: 176–186, 1964.
225. MARKWARDT, F., BARTHEL, W., HOFFMANN, A. AND WITTIER, E.: Unterschungen über den Mechanismus der aminfreisetzenden Wirkung von proteolytischen Fermenten an Blutplättchen. Naunyn-Schmiedeberg Arch. Pharmacol. Exp. Pathol. **251**: 255–264, 1965.
226. MARTIN, A. R.: Quantal nature of synaptic transmission. Physiol. Rev. **46**: 51–66, 1965.
227. MARTIN, A. R. AND PILAR, G.: Quantal components of the synaptic potential in the ciliary ganglion of the chick. J. Physiol. (London) **175**: 1–16, 1964.
228. MATTHEWS, E. K. AND SAFFRAN, M.: Steroid production and membrane potential measurement in cells of the adrenal cortex. J. Physiol. (London) **189**: 149–161, 1967.
229. MATTHEWS, E. K. AND SAFFRAN, M.: Effect of ACTH on the electrical properties of adrenocortical cells. Nature (London) **219**: 1369–1370, 1968.
230. MCCARTHY, D. M. AND SHEEHAN, J. D.: The role of calcium ions in salivary secretion. J. Physiol. (London) **184**: 81–82P, 1968.
231. MIELE, E.: Lack of effect of prostaglandin E_1 and $F_{1\alpha}$ on adrenomedullary catecholamine secretion evoked by various agents. *In* Prostaglandins, Peptides and Amines, ed. by P. Mantegazza and E. W. Horton, pp. 85–93, Academic Press, London, 1969.
232. MIKITEN, T. M. AND DOUGLAS, W. W.: Effect of calcium and other ions on vasopressin release from rat neurohypophyses stimulated electrically *in vitro*. Nature (London) **207**: 302–303, 1965.
233. MILEDI, R.: Strontium as a substitute for calcium in the process of transmitter release at the neuromuscular junction. Nature (London) **212**: 1233–1234, 1966.
234. MILEDI, R. AND SLATER, C. R.: The action of calcium on neuronal synapses in the squid. J. Physiol. (London) **184**: 473–498, 1966.
235. MILNER, R. D. G. AND HALES, C. N.: The role of calcium and magnesium in insulin secretion from rabbit pancreas studied *in vitro*. Diabetologia **3**: 47–49, 1967.
236. MILNER, R. D. G. AND HALES, C. N.: Cations and the secretion of insulin. Biochim. Biophys. Acta **156**: 165–167, 1968.
237. MILNER, R. D. G. AND HALES, C. N.: The interaction of various inhibitors and stimuli of insulin release studied with rabbit pancreas *in vitro*. Biochem. J. **113**: 473–479, 1969.
238. MINES, G. R.: On the replacement of calcium in certain neuromuscular mechanisms by allied substances. J. Physiol. (London) **42**: 251–266, 1911.
239. MITCHELL, J. F., NEAL, M. J. AND SRINIVASAN, V.: The release of ^{3}H-gamma-aminobutyric acid (GABA) from rat cerebral cortex. Brit. J. Pharmacol. Chemother. **34**: 661P–662P, 1968.
240. MONGAR, J. L. AND SCHILD, H. O.: The effect of calcium and pH on the anaphylactic reaction. J. Physiol. (London) **140**: 272–284, 1958.
241. MONGAR, J. L. AND SCHILD, H. O.: Cellular mechanisms in anaphylaxis. Physiol. Rev. **42**: 226–270, 1962.
242. MOTA, I.: Effect of antigen and octylamine on mast cells and histamine content of sensitized guinea pig tissues. J. Physiol. (London) **147**: 425–436, 1959.
243. MOTA, I. AND ISHII, T.: Inhibition of mast cell disruption and histamine release in rat anaphylaxis *in vitro*. Comparison with compound 48/80. Brit. J. Pharmacol. Chemother. **15**: 82–87, 1960.
244. MUCHNIK, S. AND GAGE, P. W.: Effect of bromide ions on junctional transmission. Nature (London) **217**: 373–374, 1968.
245. MUCHNIK, S. AND VENOSA, R. A.: Role of sodium ions in the response of the frequency of miniature end-plate potentials to osmotic changes in the neuromuscular junction. Nature (London) **222**: 169–171, 1969.
246. MURER, E. H.: A comparative study of the action of release inducers upon platelet release and phosphorus metabolism. Biochim. Biophys. Acta **192**: 138–140, 1969.
247. NAYLER, W. G.: Influx and efflux of calcium in the physiology of muscle contraction. Clin. Orthop. Related Res. **46**: 157–182, 1966.
248. OKA, M., OHUCHI, T., YOSHIDA, H. AND IMAIZUMI, R.: The importance of calcium in the release of catecholamine from the adrenal medulla. Jap. J. Pharmacol. **15**: 348–356, 1965.
249. OKA, M., OHUCHI, T., YOSHIDA, H. AND IMAIZUMI, R.: Stimulatory effect of adenosine triphosphate and magnesium on the release of catecholamines from adrenal medullary granules. Jap. J. Pharmacol. **17**: 199–207, 1967.
250. OKADA, K.: Effects of calcium and magnesium ions on the frequency of miniature end-plate potential discharges in amphibian muscle in the presence of ethyl alcohol. Experientia (Basel) **23**: 363–364, 1967.
251. PAASONEN, M. K.: Release of 5-hydroxytryptamine from blood platelets. J. Pharm. Pharmacol. **17**: 681–697, 1965.
252. PALADE, G. E.: Functional changes in the structure of cell components. *In* Subcellular Particles, Society of General Physiologists Symposium, ed. by T. Hayashi, pp. 64–80, Ronald Press, New York, 1959.
253. PARSONS, J. A.: Calcium ion requirement for prolactin secretion by rat adenohypophyses *in vitro*. Amer. J. Physiol. **217**: 1599–1603, 1969.

254. PARSONS, R. L. HOFMANN, W. W. AND FEIGEN, G. A.: Presynaptic effects of potassium ion on the mammalian neuromuscular junction. Nature (London) **208**: 590–591, 1965.
255. PATT, H. M. AND LUCKHARDT, A. B.: Relationship of a low blood calcium to parathyroid secretion. Endocrinology **31**: 384–392, 1942.
256. PERON, F. G. AND MCCARTHY, J. L.: Corticosteriodogenesis in the rat adrenal gland. *In* Functions of the Adrenal Cortex, vol. I, ed. by K. W. McKerns, pp. 261–337, Appleton-Century-Croft, New York, 1968.
257. PETERSEN, O. H. AND POULSEN, J. H.: The effects of varying the extracellular potassium concentration on the secretory rate and on the resting and secretory potentials in the perfused cat submandibular gland. Acta Physiol. Scand. **70**: 293–298, 1967.
258. PETERSEN, O. H. AND POULSEN, J. H.: Secretory potentials, potassium transport and secretion in the cat submandibular gland during perfusion with sulphate Locke's solution. Experientia (Basel) **24**: 919–920, 1968.
259. PETERSEN, O. H., POULSEN, J. H. AND THORN, N. A.: Secretory potentials, secretory rate, and water permeability of the duct system in the cat submandibular gland during perfusion with calcium-free Locke's solution. Acta Physiol. Scand. **71**: 203–210, 1967.
260. PHILIPPU, A. AND SCHÜMANN, H. J.: Der Einfluss von Calcium auf die Brenzcatechinamin freisetzung. Experientia (Basel) **18**: 138–140, 1962.
261. PHILIPPU, A. AND SCHÜMANN, H. J.: Über die Bedeutung der Calcium und Magnesiumionen für die Speicherung der Nebennierenmark-Hormone. Naunyn-Schmiedebergs Arch. Pharmakol. Exp. Pathol. **252**: 339–358, 1966.
262. PLETSCHER, A., DA PRADA, M. AND TRANZER, J. P.: The action of calcium and thrombin on isolated 5-hydroxytryptamine organelles of blood platelets. Experientia (Basel) **24**: 1202–1203, 1968.
263. POISNER, A. M. AND DOUGLAS, W. W.: The need for calcium in adrenomedullary secretion evoked by biogenic amines, polypeptides, and muscarinic agents. Proc. Soc. Exp. Biol. Med. **123**: 62–64, 1966.
264. POISNER, A. M. AND DOUGLAS, W. W.: A possible mechanism of release of posterior pituitary hormones involving adenosine triphosphate and an adenosine triphosphatase in the neurosecretory granules. Mol. Pharmacol. **4**: 531–540, 1968.
265. POISNER, A. M. AND TRIFARÓ, J. M.: The role of ATP and ATPase in the release of catecholamines from the adrenal medulla. I. ATP-evoked release of catecholamines, ATP and protein from isolated chromaffin granules. Mol. Pharmacol. **3**: 561–571, 1967.
266. PUSZKIN, S., BERL, S., PUSZKIN, E. AND CLARK, D. D.: Actomyosin-like protein isolated from mammalian brain. Science **161**: 170–171, 1968.
267. RAMWELL, P. W., SHAW, J. E., DOUGLAS, W. W. AND POISNER, A. M.: Efflux of prostaglandin from adrenal glands stimulated with acetylcholine. Nature (London) **210**: 273–274, 1966.
268. RANDIC, M. AND PADJEN, A.: Effect of calcium ions on the release of acetylcholine from the cerebral cortex. Nature (London) **215**: 990, 1967.
269. RASMUSSEN, H. AND TENENHOUSE, A.: Cyclic adenosine monophosphate, Ca^{++} and membranes. Proc. Nat. Acad. Sci. U.S.A. **59**: 1364–1370, 1968.
270. REHM, W. S.: Hydrochloric acid secretion, ion gradients, and the gastric potential. *In* The Cellular Functions of Membrane Transport, ed. by J. F. Hoffman, pp. 231–250, Prentice-Hall, Englewood Cliffs, N.J., 1963.
271. REHM, W. S., DAVIS, T. L., CHANDLER, C., GOHMANN, E., JR. AND BASHIRELAHI, A.: Frog gastric mucosae bathed in chloride-free solutions. Amer. J. Physiol. **204**: 233–242, 1963.
272. ROBINSON, R. L.: Stimulation of the catecholamine output of the isolated perfused adrenal gland of the dog by angiotensin and bradykinin. J. Pharmacol. Exp. Ther. **156**: 252–257, 1967.
273. ROTHSTEIN, A. P.: Membrane phenomena. Annu. Rev. Physiol. **30**: 15–72, 1968.
274. RUBIN, R. P.: On the Mode of Action of Acetylcholine on the Adrenal Medulla. Ph.D. Thesis, Albert Einstein College of Medicine, 1963.
275. RUBIN, R. P.: The metabolic requirements for catecholamine release from the adrenal medulla. J. Physiol. (London) **202**: 197–209, 1969.
276. RUBIN, R. P.: The role of energy metabolism in calcium-evoked secretion from the adrenal medulla. J. Physiol. (London) **206**: 181–192, 1970.
277. RUBIN, R. P.: Unpublished observations.
278. RUBIN, R. P., FEINSTEIN, M. B., JAANUS, S. D. AND PAIMRE, M.: Inhibition of catecholamine secretion and calcium exchange in perfused cat adrenal glands by tetracaine and magnesium. J. Pharmacol. Exp. Ther. **155**: 463–471, 1967.
279. RUBIN, R. P. AND JAANUS, S. D.: A study of the release of catecholamines from the adrenal medulla by indirectly acting sympathomimetic amines. Naunyn-Schmiedebergs Arch. Pharmakol. Exp. Pathol. **254**: 125–137, 1966.
280. RUBIN, R. P., JAANUS, S. D. AND MIELE, E.: Calcium-dependent corticosteroid release from the perfused cat adrenal gland. Experientia (Basel) **25**: 1327–1328, 1969.
281. RUBIN, R. P. AND MIELE, E.: A study of the differential secretion of epinephrine and norepinephrine from the perfused cat adrenal gland. J. Pharmacol. Exp. Ther. **164**: 115–121, 1968.
282. RUBIN, R. P. AND MIELE, E.: The relation between the chemical structure of local anesthetics and inhibition of calcium-evoked secretion from the adrenal medulla. Naunyn-Schmiedebergs Arch. Pharmakol. Exp. Pathol. **260**: 298–308, 1968.
283. SABINE, J. R. AND BENTLEY, G. A.: Effects of ionic alterations on various sympathetic nerve-smooth muscle preparations. Aust. J. Exp. Biol. Med. Sci. **44**: 485–502, 1966.
284. SAEKI, K.: Effects of compound 48/80, chymotrypsin, and antiserum on isolated mast cells under aerobic and anaerobic conditions. Jap. J. Pharmacol. **14**: 375–390, 1964.
285. SAMLI, M. H. AND GESCHWIND, I. I.: Some effects of energy-transfer inhibitors and of Ca^{++}-free or K^{+}-enhanced

media on the release of luteinizing hormone (LH) from the rat pituitary gland *in vitro*. Endocrinology **82**: 225–231, 1968.

286. SANDOW, A.: Excitation-contraction coupling in muscular response. Yale J. Biol. Med. **25**: 176–201, 1952.
287. SANDOW, A.: Excitation-contraction coupling in skeletal muscle. Pharmacol. Rev. **17**: 265–320, 1965.
288. SCHNEIDER, F. H.: Drug-induced release of catecholamines, soluble protein and chromogranin A from the isolated bovine adrenal gland. Biochem. Pharmacol. **18**: 101–107, 1969.
289. SCHNEYER, L. H. AND SCHNEYER, C. A.: Electrolyte secretion by rat salivary glands *in vivo* and *in vitro*. *In* Salivary Glands and Their Secretions, ed. by L. M. Sreebny and J. Meyer, vol. 3, pp. 217–235, Macmillan, New York, 1964.
290. SCHRAMM, M.: Secretion of enzymes and other macromolecules. Annu. Rev. Biochem. **36**: 307–320, 1967.
291. SCHÜMANN, H. J. AND PHILIPPU, A.: Zum Mechanismus der durch Calcium und Magnesium verursachten Freisetzung der Nebennierenmarkhormone. Naunyn-Schmiedebergs Arch. Pharmakol. Exp. Pathol. **244**: 466–476, 1963.
292. SCHWARTZ, M., KASHIWA, H. K., JACOBSON, A. AND REHM, W.: Concentration and localization of calcium in frog gastric mucosa. Amer. J. Physiol. **212**: 241–246, 1967.
293. SEDAR, A. W. AND FORTE, J. G.: Effects of calcium depletion on the junctional complex between oxyntic cells of gastric glands. J. Cell. Biol. **22**: 173–188, 1964.
294. SHANES, A. M.: Electrochemical aspects in excitable cells. Pharmacol. Rev. **10**: 59–273, 1958.
295. SHAW, J. E. AND RAMWELL, P. W.: Prostaglandin release from the adrenal gland. *In* Prostaglandins, Proceedings of the Second Nobel Symposium, Stockholm, June, 1966, ed. by S. Bergstrom and B. Samuelson, pp. 291–299, Almquist and Wiksell, Stockholm and Interscience, New York, 1967.
296. SHERWOOD, L. M.: Relative importance of parathyroid hormone and thyrocalcitonin in calcium homeostasis. N. Engl. J. Med. **278**: 663–670, 1968.
297. SHERWOOD, L. M., MAYER, G. P., RAMBERG, C. F., KRONFELD, D. S., AURBACH, G. D. AND POTTS, J. T.: Regulation of parathyroid hormone secretion: Proportional control by calcium, lack of effect of phosphate. Endocrinology **83**: 1043–1051, 1968.
298. SIMPSON, L. L.: The role of calcium in neurohumoral and neurohormonal extrusion processes. J. Pharm. Pharmacol. **20**: 889–910, 1968.
299. SLORACH, S. A. AND UVNÄS, B.: Dissociation of histamine release and $^{22}Na^{+}$ uptake in rat mast cells exposed to compound 48/80 *in vitro*. Acta Physiol. Scand. **76**: 201–212, 1969.
300. SMALLWOOD, R. A.: Effects of intravenous calcium administration on gastric secretion of acid and pepsin in man. Gut **8**: 592–598, 1967.
301. SMITH, A. D.: Biochemistry of adrenal chromaffin granules. *In* The Interaction of Drugs and Subcellular Components in Animal Cells, ed. by P. N. Campbell, pp. 239–292, Churchill, London, 1967.
302. SMITH, M. W. AND THORN, N. A.: The effects of calcium on protein-binding and metabolism of arginine vasopressin in rats. J. Endocrinol. **32**: 141–151, 1965.
303. STORMORKEN, H.: The release reaction of secretion. Scand. J. Haematol. (suppl.) **9**: 1–24, 1969.
304. SUGIYAMA, K. AND YAMASAKI, H.: Calcium-dependent histamine release by ATP from isolated rat mast cells. Jap. J. Pharmacol. **19**: 175–176, 1969.
305. TAKEUCHI, A. AND TAKEUCHI, N.: On the permeability of end-plate membrane during the action of transmitter. J. Physiol. (London) **154**: 52–67, 1960.
306. TAKEUCHI, A. AND TAKEUCHI, N.: Electrical changes in pre- and postsynaptic axons of the giant synapse of Loligo. J. Gen. Physiol. **45**: 1181–1193, 1962.
307. TAKEUCHI, N.: Effects of calcium on the conductance change of the end-plate membrane during the action of transmitter. J. Physiol. (London) **167**: 141–155, 1963.
308. TAUGNER, G. AND HASSELBACH, W.: Über den Mechanismus der Catecholamin-Speicherung in den "chromaffinen Granula" des Nebennierenmarks. Naunyn-Schmiedeberg Arch. Pharmakol. Exp. Pathol. **255**: 266–286, 1966.
309. THOENEN, H., HUERLIMANN, A. AND HAEFELY, W.: Cation dependence of the noradrenaline-releasing action of tyramine. European J. Pharmacol. **6**: 29–37, 1969.
310. THON, I. L. AND UVNAS, B.: Degranulation and histamine release, two consecutive steps in the response of rat mast cells to compound 48/80. Acta Physiol. Scand. **71**: 303–315, 1967.
311. THORN, N. A.: Role of calcium in the release of vasopressin and oxytocin from posterior pituitary protein. Acta Endocrinol. **50**: 357–364, 1965.
312. THORN, N. A., SMITH, M. W. AND SKADHAUGE, E.: The antidiuretic effect of intravenous and intracarotid infusion of calcium chloride in hydrated rats. J. Endocrinol. **32**: 161–165, 1965.
313. TIDBALL, M. E.: Heparin inhibition of histamine release and its relation to calcium. Fed. Proc. **26**: 231–232, 1967.
314. TIDBALL, M. E. AND SHORE, P. A.: Release of rabbit platelet histamine by serum *in vitro*. Amer. J. Physiol. **202**: 265–267, 1962.
315. TRANZER, J. P., DAPRADA, M. AND PLETSCHER, A.: Electron microscopic study of the storage site of 5-hydroxytryptamine in blood platelets. Advan. Pharmacol. **6A**: 125–128, 1968.
316. TRIFARO, J. M.: The effect of Ca^{++} omission on the secretion of catecholamines and the incorporation of orthophosphate-^{32}P into nucleotides and phospholipids of bovine adrenal medulla during acetylcholine stimulation. Mol. Pharmacol. **5**: 420–431, 1969.
317. TRILLER, H. AND BIRMINGHAM, M. K.: Steroid production by incubated mouse adrenals. II. Control of steroid output. Gen. Comp. Endocrinol. **5**: 624–630, 1965.
318. TRUDEAU, W. L. AND MCGUIGAN, J. E.: Effects of calcium on serum gastrin levels in the Zollinger-Ellison syndrome. N. Engl. J. Med. **281**: 862–866, 1969.

319. USHERWOOD, P. N. R., MACHILI, P. AND LEAF, G.: L-Glutamate at insect excitatory nerve-muscle synapses. Nature (London) 219: 1169–1172, 1968.
320. UVNÄS, B.: Release processes in mast cells and their activation by injury. Ann. N.Y. Acad. Sci. 116: 880–890, 1964.
321. UVNÄS, B. AND THON, I. L.: Evidence for enzymatic histamine release from isolated rat mast cells. Exp. Cell. Res. 23: 45–57, 1961.
322. VALE, W., BURGUS, R. AND GUILLEMIN, R.: Presence of calcium ions as a requisite for the *in vitro* stimulation of TSH-release by hypothalamic TRF. Experientia (Basel) 23: 853–855, 1967.
323. VALE, W. AND GUILLEMIN, R.: Potassium-induced stimulation of thyrotropin release *in vitro*. Requirement for presence of calcium and inhibition by thyroxine. Experientia (Basel) 23: 855–857, 1967.
324. VINCENZI, F. F. AND WEST, T. C.: Modification by calcium of the release of autonomic mediators on the isolated sino-atrial node. J. Pharmacol. Exp. Ther. 150: 349–360, 1965.
325. WARBERG, J. AND THORN, N. A.: *In vitro* studies of the release mechanism for vasopressin in rats. III. Effect of metabolic inhibitors on the release. Acta Endocrinol. 61: 415–424, 1969.
326. WATANABE, A., TASAKI, I., SINGER, I. AND LERMAN, L.: Effects of tetrodotoxin on excitability of squid giant axons in sodium-free media. Science 155: 95–97, 1967.
327. WEBER, H. H.: The Motility of Muscle and Cells, pp. 1–69, Harvard University Press, Cambridge, Mass., 1958.
328. WHITTAKER, V. P.: The isolation and characterization of acetylcholine containing particles from brain. Biochem. J. 72: 694–706, 1959.
329. WHITTAKER, V. P.: Structure and function of animal-cell membranes. Brit. Med. Bull. 24: 101–106, 1968.
330. WOODIN, A. M.: The basis of leucocidin action. *In* The Biological Basis of Medicine, ed. by E. E. Bittar, vol. 2, pp. 373–396, Academic Press, New York, 1968.
331. WOODIN, A. M., FRENCH, J. E. AND MARCHESI, V. T.: Morphological changes associated with the extrusion of protein induced in the polymorphonuclear leucocyte by staphylococcal leucocidin. Biochem. J. 87: 567–571, 1963.
332. WOODIN, A. M. AND WIENEKE, A. A.: The incorporation of radioactive phosphorus in the leucocyte during the extrusion of protein by staphylococcal leucocidin. Biochem. J. 87: 480–487, 1963.
333. WOODIN, A. M. AND WIENEKE, A. A.: The accumulation of calcium by the polymorphonuclear leucocyte treated with staphylococcal leucocidin and its significance in the extrusion of protein. Biochem. J. 87: 487–495, 1963.
334. WOODIN, A. M. AND WIENEKE, A. A.: The participation of calcium, adenosine triphosphate, and adenosine triphosphatase in the extrusion of the granular proteins from the polymorphonuclear leucocyte. Biochem. J. 90: 498–509, 1964.
335. WOODIN, A. M. AND WIENEKE, A. A.: Role of leucocidin and triphosphoinositide in the control of potassium permeability. Nature (London) 220: 283–286, 1968.
336. YAMASAKI, H. AND ENDO, K.: Effects of 2-deoxyglucose, phlorizin, insulin, and ouabain on glucose-dependent anaphylactic histamine release from guinea pig lung. Jap. J. Pharmacol. 15: 48–56, 1965.
337. YASUI, B., FUCHS, F. AND BRIGGS, F. N.: The role of the sulfhydryl groups of tropomyosin and troponin in the calcium control of actomyosin contractility. J. Biol. Chem. 243: 735–742, 1968.
338. YOSHIDA, H., MIKI, N., ISHIDA, H. AND YAMAMOTO, I.: Release of amylase from zymogen granules by ATP and a low concentration of Ca^{+2}. Biochim. Biophys. Acta 158: 489–490, 1968.
339. YOUNG, J. A. AND SCHÖGEL, E.: Micropuncture investigation of sodium and potassium excretion in rat submaxillary saliva. Pflügers Arch. Gesamte Physiol. Menschen Tiere 291: 85–98, 1966.