ELECTROLYTES AND SMOOTH MUSCLE CONTRACTION[1]

DAVID F. BOHR

Department of Physiology, University of Michigan, Ann Arbor, Michigan

Rather than just one subject, a variety of topics is concealed in the deceptive simplicity of this title. Each of at least six physiologically important ions may have a specific action on each of several steps in the overall contractile process of smooth muscle. The relative importance of each action differs greatly among the various types of smooth muscle, and even in a given smooth muscle under different conditions. However, among the many specific observations recently reported there appear threads of recurring relationships from which at least a weak fabric of generalizations may now be woven.

Although Bozler (17) has cautioned that the extreme variability of smooth muscle phenomena "has at least the advantage that it protects us from premature generalization," some generalizations now seem justified because of an overall similarity that exists in the basic components of the contractile process, among the several smooth muscles and even between smooth muscle and striated muscle. Qualitative differences in electrolyte effects on contraction often represent merely quantitative differences in the action of the electrolyte shift on individual steps of the overall process which leads to contraction. For example, doubling the potassium concentration in the environment of smooth muscle from the aorta potentiates its response to epinephrine; the same procedure depresses the response of smooth muscle from the mesenteric arteriole (11). The potentiating effect of an increase in extracellular potassium on membrane excitability appears to be dominant in the aorta, whereas the plasticizing effect of an elevated intracellular K concentration on the contractile protein seems to limit the response of the arertiole. The net effects of this electrolyte shift on these two tissues are opposite, but the actions of this shift on the individual components of the overall contractile response are probably similar.

This review is concerned mainly with uterine, gastrointestinal, and vascular smooth muscle of vertebrates. Extra consideration will be given to the last because of the reviewer's particular interest in it, and because this one of the three major smooth muscles has been relatively neglected since the excellent review by Furchgott in 1955 (60).

At the outset, it should be noted that we do not recognize that adequate evidence exists for a difference in the basic contractile processes involved in normal phasic and tonic shortening. We assume that the failure to demonstrate such a difference is based on the fact that there is none. Further, we assume that there is no such thing as active dilatation or relaxation at the level of smooth muscle mechanics. Relaxation of this muscle is effected by a lessening of its

[1] Personal studies described in this review, and neglected during its preparation, have been supported by the following grants: Life Insurance Medical Research Fund, American Heart Association, and National Institutes of Health, HE-03756.

contractile activity. Definition of the roles of H, HCO_3 and PO_4 ions in the contractile process of smooth muscle is left as a challenge to a future reviewer.

DISTRIBUTION AND MOBILITY OF IONS

The distribution and mobility of a specific ion in smooth muscle have direct bearing on the interpretation that may be placed on the role of this ion in the contractile process. Four factors, critical in the resolution of the problems relating electrolyte composition to function of smooth muscle, remain poorly defined. These are 1) the volume of the extracellular space, 2) the binding or sequestration of ions, 3) the change in ion content that occurs when smooth muscle is transferred from an *in vivo* to an *in vitro* environment, and 4) transient states that exist immediately after an electrolyte shift. Since much of our insight into the mechanism of action of ions on the contractile process rests on these uncertainties it is appropriate to come to grips with them at the outset. Ionic content of smooth muscle and related problems have been more extensively reviewed by Burnstock *et al.* (31).

Extracellular space

Tools are not yet available for direct measurement of the intracellular concentration of physiologically active ions and their transmembrane gradients. Currently this information is sought from measurement of the total amount of the electrolyte and of water in the tissue, and estimation of the portion of this water that is outside the cell. On the assumption that the remaining volume of water is intracellular, and that the extracellular concentration of the electrolyte is the same as that known to be present in the environment of the tissue, it is simple arithmetic to calculate extracellular and intracellular amounts of the ion. If, for convenience, we assume that all of the intracellular ion is in simple solution, the intracellular concentration of the ion can then be calculated.

Difficulties in the measurement of the amount of water outside the cell account for some of the differences among estimates of the concentration of an electrolyte inside the cell. The most satisfying handling of the problems of measuring the elusive extracellular space in smooth muscle is that presented by Goodford and Hermansen in their study of the guinea pig taenia coli (61). They selected inulin, which occupies 320 ml/kg of this tissue, as the most representative measure of extracellular fluid volume. In making this selection they studied four other methods that gave equally reproducible results but ones which for one reason or another they felt were not as representative of the true extracellular fluid volume. Laborious measurements from electron micrographs of this tissue indicate that the extracellular space occupies approximately 200 ml/kg, but concern was expressed about error that might have been introduced because of tissue shrinkage. This value is not far from that estimated by use of a large polyglucose molecule; after 4 hours this had entered 220 ml/kg of the tissue. In other approaches, using the assumption that a given ion enters the extracellular space more rapidly than it does the intracellular space, they were able with the aid of isotopically labeled ions to calculate the volume of the

space entered at the more rapid rate. They found that Li entered 400 ml/kg of the tissue at a rapid rate; Na, on the other hand, entered 700 ml/kg of the tissue at a very fast rate.

Most authors have selected inulin space as the most reliable measure of extracellular fluid volume (2, 42, 46, 57, 77, 95). Values so obtained range from 110 ml/kg for the circular muscle of cat intestine (2) to 440 ml/kg for dog aorta (95). Sources of error are present, *e.g.*, 1) failure of this large molecule to be distributed uniformly in narrow chinks and crevices of the extracellular space, 2) intracellular penetration of inulin, and 3) binding of inulin. Nevertheless, the variability reported probably represents real differences between extracellular space volume in different tissues. This is corroborated by electron microscope studies (99) in which the relative amounts of extracellular space in various tissues are proportional to the values for inulin space found elsewhere in the literature. The main problem with electron microscope space measurements is the indeterminable amount of shrinkage that occurs. However, when comparative studies are made using identical fixation and slicing techniques, the values obtained for relative magnitudes of this space in the several tissues are probably reliable; they are inversely related to tissue conductivity (29).

Sucrose space is frequently used as a measure of extracellular space, but sucrose occupies a larger space than does inulin. This has been considered to represent merely access to smaller crevices between cells than could be reached by inulin. However, Bozler (15, 18) has presented evidence indicating that sucrose, fructose, and other sugars rapidly penetrate the smooth muscle cell, where they occupy an intracellular space smaller than that occupied by water. He has demonstrated also that the intracellular sugars have osmotic activity capable of causing cell swelling. He visualizes the intracellular sugar molecules as having access to only that amount of water which exists in spaces between the solids, spaces large enough to accept these specific sugar molecules.

Daniel and Robinson (44) have preferred to use the measurement of tissue chloride for an estimate of extracellular space in the uterus. The calculation requires estimates of both the amount of bound Cl and the concentration of Cl within the cell. This approach depends on an estimate of intracellular Cl based on the assumption that the distribution of this ion is determined by Donnan potential of 50 mV (negative inside). Estimates of extracellular volume by this method give results considerably larger than those obtained with inulin.

Although it is evident that the relative intracellular and extracellular fluid volume in smooth muscle cannot be defined precisely, the estimates establish the fact that there is great variability among smooth muscle from different sources and that, in general, there is a proportionally greater extracellular space in smooth muscle than in skeletal muscle regardless of whether measurements are made *in vitro* or *in vivo* (*e.g.*, 57).

Ion binding

There is convincing evidence that some electrolytes in some smooth muscle are bound in an osmotically and electrochemically inactive state (31). The

subject of ion binding is introduced in this section of the review because of the problem it presents in the estimation of concentrations and gradients of freely diffusible ions. This opportunity will also be taken to review the findings which indicate that the bound ion itself plays an important role in various parts of the contractile process of smooth muscle.

The amount of bound electrolyte is highly variable from tissue to tissue, and in any one tissue it may vary considerably with changes in physiological conditions. In the uterus, for example, progesterone decreases the amount of bound monovalent cation (77), whereas it increases the binding of the divalent ion, Ca (6, 62). Before reviewing some of the estimates that have been made of the amount of bound electrolyte in smooth muscle it is relevant to consider the nature of the sites to which the electrolytes may be bound. Nanninga (91) considers that adenosine triphosphate (ATP), creatine phosphate (CP), and myosin are quantitatively the most important intracellular binding sites for K, Ca, and Mg in skeletal muscle. By using measured individual affinities of these cations for each of these binding sites, together with estimates of the intracellular content of all of the reactants, this investigator was able to calculate the amount of each cation bound to each of the sites. These calculations indicate that most of the K is in a free ionized form, and that the bound portion is in association with myosin. In contrast, the divalent cations are for the most part bound to ATP and to CP. Recognizing the hazards of extrapolating information from skeletal muscle to smooth muscle, and from studies *in vitro* to the situation *in vivo*, this basic principle of a complex interaction of various affinities still must be accepted as operative.

The following are representative estimates of the amount of bound electrolyte in the three major types of smooth muscle. Kao (77) found that in the estrogen-dominated uterus soaked for 6 to 8 days in neutral isotonic sucrose there remained K in an amount of 14 mmol/l water and Na, 29 mmol/l water. Comparable unextractable amounts of K and Na in the progesterone-dominated myometrium were 4 and 9 mmol/l water, respectively. Daniel and Robinson (44), also using uterus, obtained evidence that the amounts of electrolytes held in a bound state (expressed in mmol/kg wet weight) are as follows: Na, 15; K, 22; and Cl, 10 to 19. Goodford and Hermansen (61), in studies of the taenia coli, interpreted their findings as indicating that 15 mmol/kg wet weight of Na was bound. Headings, Rondell, and Bohr (68) found that after the medial layer of the dog carotid artery was damaged so that the cell membrane no longer formed a barrier to ion distribution, the amount of K in the tissue was such that if distributed uniformly in the tissue water it would establish a concentration similar to that in the surrounding physiological salt solution. However, this tissue contained much more Na than could be accounted for as being in equilibrium with its environment; this excess was 125 mmol of Na/kg of tissue solid. They give evidence that this Na is bound to the mucopolysaccharide which is present in large amounts in the extracellular space of the media of the dog carotid. In normal extracellular concentration K is not bound, but if the K concentration is elevated, K displaces Na from its binding sites. H-ion may also replace Na, and does so at pH 3.

Recent studies (67) suggest that there is a parallel increase in mucopolysaccharides and in bound Na in the vessel wall, in both old age and hypertension. Peterson (98) has reviewed numerous studies of the electrolyte composition of blood vessels of normal and hypertensive animals. The latter consistently have more Na. The possibility that this elevation reflects an increase in bound Na lessens its value as evidence of the importance of altered transmembrane gradients of sodium in the etiology of hypertension (55, 100, 115). Schatzmann (104) has found that nearly half of the Ca in intestinal smooth muscle is present in an electrochemically inactive form. The amount of this cation in a bound form, and its changes from a bound to a free form, constitute a most important aspect of contraction and relaxation of smooth muscle.

The importance of the bound cations in the contraction of smooth muscle will be dealt with in more detail in connection with the functions of the individual electrolytes. The roles played by binding of electrolytes can be itemized as: 1) Binding of Na, K, and Ca removes these ions from an electrochemically active state, hence they do not enter into the forces involved in electrochemical gradients. 2) Bound Mg may play an important part in the link between ATP and myosin (*e.g.*, 73). 3) The release of bound Ca is an important link between membrane excitation and tension development by the contractile protein (51), possibly through its action on the relaxing factor (49). 4) There is much evidence that bound Ca in the smooth muscle cell membrane acts to alter cell membrane permeability to Na and hence to influence cellular excitability (26, 31). Goto and Csapo (62) suggest that progesterone stabilizes uterine muscle cell membrane by increasing its bound Ca. They postulate that the release from the progesterone dominance at term removes this "block" and facilitates the onset of labor.

Smooth muscle in vitro and in vivo

A study of the effect of electrolytes on smooth muscle can be carried out in most controlled fashion *in vitro*, where this sensitive tissue is not subjected to the chemical and physical variables occurring in its setting *in vivo*. Unfortunately, smooth muscle in the standard muscle bath differs appreciably from the same tissue in its environment *in vivo*. In all three of the major smooth muscles comparable changes occur in electrolyte composition and in performance when they are shifted from the *in vivo* to an *in vitro* environment. When the environmental transfer is made there is first an extreme change in chemical composition; subsequently there is a gradual recovery toward the chemical composition *in vivo*, but this is never complete, and the electrolyte composition at steady state differs measurably from that *in vivo*. However, Goodford has found that when sufficiently small pieces of tissue are used, isolated taenia coli can recover its *in vivo* ionic composition (see 31).

When rat aorta was transferred from an *in vivo* to an *in vitro* situation there were rapid changes in Na and K content (47). After 15 seconds in a physiological salt solution (PSS)[2] at 37°C the K content had fallen from 33 to 13 mEq/kg wet

[2] In the hall of fame for investigators who have contributed recipes for solutions containing the electrolyte requirements of specific tissues for specific functions, Drs. Ringer,

weight. In this same period the Na content rose from 92 to 113 mEq/kg wet weight. Since about half of the water in this tissue is intracellular (68), and since nearly three-fourths of the K in the tissue was lost, much of the K which moved out into the PSS bath during this 15-second period must have traveled from an intracellular position through the cell membrane and extracellular space. Apparently during the period immediately after the removal of this smooth muscle from its environment *in vivo*, the membrane failed to function as it normally does as a barrier to movement of this cation. A factor which is undoubtedly contributory to the speed of this movement is the very small size of the smooth muscle cell and the consequently large surface-to-volume ratio.

In order to determine whether the dramatic initial change in electrolyte composition was due to changes in chemical environment of the aortic tissue or to the mechanical handling involved in the removal of the tissue, two procedures were carried out (47): In one it was found that incubation in rat plasma rather than in PSS did not prevent the initial rapid change in electrolyte composition. On the other hand, when the whole rat was perfused with the PSS, these rapid changes in electrolyte composition did not occur. It appears that the rapid movement of cations is due to the mechanical handling of the tissue during its removal rather than to a change in the chemical environment. Qualitatively similar changes have been observed (61) in K and Na content of the taenia coli when it was transferred to PSS. Neither change of temperature nor degree of oxygenation influenced these cation shifts. The speed of Na movement into the taenia coli was particularly striking; when it was dipped momentarily in a PSS the tissue acquired 13.6 mmol/kg of Na.

These initial rapid changes in electrolyte composition suggest that the cations were running downhill along their concentration gradients across the cell membrane. Thereafter, a reversal in the direction of electrolyte shifts occurred (44, 47, 61). These observations indicate that after the initial period the membrane regained some of its characteristics as a barrier, and maintained Na and K gradients by an active transport system. Recovery was not complete, however, and this fact suggests that an unknown factor *in vivo* is necessary to maintain the high transmembrane cation gradients of the intact animal. This is illustrated by the observation that whereas the intracellular K concentration in the dog carotid was 125 mEq/l intracellular water *in vivo* (68), it reached only about one-half this value after 2 hours incubation in PSS of normal K concentration. Furthermore, if the K concentration was increased to 4 times normal (24 mmol/l), intracellular K still failed to reach its concentration *in vivo* (5). It has been shown that incubation in plasma fails to maintain the normal ionic equilibrium in uterus (44) or in rat aorta (47). Further suggestion that there is a persistent inadequacy of the

---

Tyrode, Henseleit, Krebs, and Modified have places. The last named has been the most prolific. It is impossible for the ordinary reader to remember the concentrations or even the ingredients in each recipe. This reviewer enters a plea to each investigator to define the composition of the physiological solution he uses in mmole/liter; any such solution might be referred to as a physiological salt solution (PSS).

barrier properties of the membrane *in vitro* is found in the observation that the extracellular fluid volume increases as the smooth muscle is incubated in a PSS (44, 47). Mechanical handling does not seem to be the only factor responsible, since after 90 minutes of perfusion of an intact rat with PSS the K content of the aorta had fallen and the Na content had increased to values similar to those of a rat aorta removed and incubated in an isolated bath for this same period of time.

In addition to these transient and sustained changes in electrolyte composition, functional differences are seen when smooth muscle is transferred from its *in vivo* to an *in vitro* environment. When smooth muscle is first immersed in an isolated bath, of whatever composition, it has little or no mechanical response. Depending on the tissue, 15 minutes to 3 hours of incubation in PSS is required before the muscle responds optimally and reproducibly. Details of the recovery of the mechanical responsiveness have been described (5) and the time course of recovery has been shown to be dependent on the K concentration of the bath. The observation that the time course of recovery of the mechanical response can be greatly accelerated by stretching (125) is surprising indeed. Similar periods of incubation in PSS are required for the development of steady state electrical potentials and activity (14).

Since these differences in electrolyte composition and in functional activity exist, a degree of caution must be exercised in extrapolating from findings *in vitro* to conditions *in vivo*. This seems to be particularly true in the case of vascular smooth muscle, which in the usual PSS remains completely relaxed (12, 60). In the normal basal state *in vivo* vascular smooth muscle is partially contracted; in some "resting" vascular beds resistance can be reduced to a small fraction of its rest value by maximum dilatation (35). While it may be tempting to associate the functional differences between smooth muscle *in vivo* and *in vitro* with known electrolyte differences, one must also consider such factors as the active humoral agents (hormones, local metabolites, enzymes), neurogenic stimulation, and mechanical influences (*e.g.*, pressure pulse, peristalsis) that occur *in vivo*.

Understanding of the complex and sensitive contractile machinery of smooth muscle calls for evaluation of the effect of a single rigidly controlled variable rather than an analysis of the multiple unknowns that influence the performance of the muscle *in vivo*. Furthermore, when clear differences are evident between the performance *in vivo* and *in vitro*, they provide clues to guide the search for control systems *in vivo*. Although the performance of smooth muscle *in vivo* differs from its performance in the isolated bath, the environment *in vitro* does not reduce all smooth muscle to an identical performance. Individual characteristics of the muscle are retained when it is transferred to the isolated bath. This generalization is convincingly demonstrated by the differences in electrolyte composition (43) and both mechanical (6) and electrical (87) performance of uterine smooth muscle obtained from animals under different endocrine influence. The retention of some *in vivo* characteristics argues that the isolated system retains some of the properties it had in the intact animal.

Transient effects of electrolyte shifts

Mechanisms responsible for some of the transient responses to environmental changes can be reasonably explained in terms of our current understanding of the basic processes; others, however, can not. Recently Kuriyama (80) has described a series of such transients produced by shifts in electrolyte composition of the environment of the guinea pig taenia coli. Increasing K concentration in the bath from 5.9 to 59 mM caused an initial extreme depolarization lasting 15 seconds, followed by a gradual repolarization to an intermediate potential. The initial acute change probably can be explained by a decrease in K potential "which is then, by diffusion of K into the cell, gradually raised to a new equilibrium potential." An example of an unexplained transient is the depolarization that he observed when the taenia coli was initially exposed to a K-free environment. This depolarization, which was accompanied by increased spike frequency, gave way after several minutes to the expected hyperpolarization, with decrease in spike activity. Obviously an adequate description of the effect of an electrolyte shift can be made only if the effects are followed in time until a new steady state develops.

COMPONENTS OF THE OVERALL CONTRACTILE PROCESS

When the mechanical response occurs *in vivo* it is the end product of a fixed series of events. Attachment of the terms, "*membrane phenomena*," "*coupling process*," "*protein contraction*," and "*energy metabolism*," to four of these events should not imply that these are separate and unrelated processes or that they necessarily embrace all the happenings. The effect of an electrolyte shift on the end product, muscle contraction, can best be analyzed by considering it in relation to each of these events.

Membrane phenomena

The largest number of recent contributions to the understanding of the workings of the smooth muscle cell have dealt with affairs of its membrane. The challenge of invading the privacy of these tiny cells with glass pipettes has been an important stimulus which has led to detailed information about the characteristics of the cell's resting and action potentials, the dependence of these potentials on specific electrolytes, and the effect of stimulating and inhibiting agents on its membrane.

The role of electrolytes in the electrophysiology of smooth muscle has just been presented in excellent summations arising from the two ends of the earth (26, 27, 31, 80). Additional coverage of this subject in breadth and depth is also noteworthy (25, 40, 45, 70, 87). The current review will merely paraphrase this material in a form intended to satisfy only the most casual reader and to serve as a background for the more detailed consideration of the role of electrolytes in the remainder of the overall contractile process of smooth muscle.

Reassurance is developing that the intracellular concentrations of Na and Cl are higher in smooth muscle than in other muscle cells. The permeability of smooth muscle cell membranes to these ions is also relatively high. These properties

explain the basic individualities of the resting and action potentials of smooth muscle; the resting potential is low and unstable, and in the action potential the rate of depolarization is slow. When the smooth muscle cell is in a PSS the primary determinant of the *resting potential* is the K diffusion potential. To this, however, is added an appreciable Cl diffusion potential, and from the sum of these is subtracted an important, inwardly directed, Na diffusion potential. Substitution of a nondiffusible anion (SO_4) for Cl, therefore, results in some depolarization, whereas a decrease in Na outside the cell causes hyperpolarization. Removal of Ca from the environment also results in depolarization of the cell. This depolarization may be secondary to a resultant increased membrane permeability to Na, and hence to an augmented Na diffusion potential. The existence of an electrogenic Na pump must be seriously considered as contributory to the instability of the resting membrane potential of smooth muscle (25).

The *action potential* of smooth muscle in a PSS probably reflects an increase in membrane conductance for Na. This situation is peculiar, however, in that the action potential remains unchanged until extracellular Na concentration is reduced to a small fraction of that normally present in extracellular fluid. This seems best explained by the possibility that the smooth muscle cell membrane has only a limited number of sites that can be made available for the permeation of sodium during the action potential. In this situation the Na permeation sites or carriers, or both, become saturated at a lower extracellular concentration; available sites, rather than Na gradient, are then the bottleneck for the rate of depolarization. Here again, the role of the Ca ion seems to be all-important in the control of the transmembrane diffusion of Na. Rates of depolarization are accelerated by increased Ca concentration and slowed by low-Ca environment. A valuable working hypothesis is that poor fixation of Ca in the smooth muscle membrane is responsible, on the one hand, for the high permeability of this membrane to Na, and, on the other hand, for the limited "number of sites at which Na permeability, which is already high in the resting state, can be further increased during the spike" potential (26). It seems that Ca "unfixation" during excitation makes available additional channels for Na permeation. An increase in Ca fixed to the membrane would, therefore, augment the possibility of an increased influx of Na during depolarization, and would accelerate the rate of rise of the action potential. Important evidence can be mustered to support the hypothesis that the fixation of Ca to the membrane, and therefore the stability of the membrane, is dependent on metabolic processes (26). The action of epinephrine in increasing metabolic rate stabilizes the membrane only in the presence of Ca. Factors which decrease metabolic activity, such as cooling or treatment with dinitrophenol (DNP), decrease membrane stability.

Burnstock *et al.* (31) have presented a schematic model which is helpful in understanding how the smooth muscle membrane may be carried into and out of a zone of excitability by shifts in the electrolyte environment or by the action of specific physiological and pharmacological agents.

In general the contractile response of smooth muscle seems to be initiated by the action potential. Although many examples can be cited in which 1) action

potentials occur without a mechanical response (uncoupling), or 2) tension is developed or maintained in the absence of action potentials, the latter seem to be the usual triggers for either tonic or phasic contractions.

The application of this generalization to vascular smooth muscle has not yet been established. Although reported membrane and action potentials of vascular smooth muscle (58, 102, 116) are similar to those of other smooth muscle, we are faced by the unexplained observations that the contractile response to norepinephrine occurs in the absence of action potentials or change in resting potential (3, 113), and without loss of K from the cells (66). Waugh (120) has indirect evidence that contraction occurs in vascular smooth muscle in response to epinephrine in the absence of changes in membrane potential. The validity of this possible peculiarity of vascular smooth muscle is not established, since other investigators (59, 102, 116) have observed action potentials in vascular smooth muscle and have noted an increase in K efflux accompanying contraction (3, 21).

Coupling process

In addition to the satisfying role that smooth muscle has played as a pin cushion for the investigator with a microelectrode, this tissue is important because of its contractile response. The coupling process links the excitatory event of the membrane with the chemo-mechanical transducing effected by the contractile protein. From extensive studies with striated muscle it is postulated that the structural site of coupling resides in the endoplasmic reticulum and that the functional components of the system include Ca and a relaxing factor. There is little direct evidence that the endoplasmic reticulum and a relaxing factor are involved in coupling in smooth muscle. Electron microscope studies (97) indicate that the endoplasmic reticulum is poorly developed in smooth muscle, and efforts to demonstrate the presence of relaxing factor in uterus (65) and vascular smooth muscle (85) have failed. However, it has been inferred from two recent studies (20, 53) that the relaxing factor of smooth muscle from these two sources may be peculiar in that it may have a tendency to be bound to the contractile protein and be extracted with it. The functional role of Ca in excitation-contraction coupling in smooth muscle appears to correspond very closely to its role in this process in striated muscle. Evidence supporting this role of Ca will be described under the section devoted to this ion.

Protein contraction

Contractile protein from uterine (20, 38, 65, 93, 94), gastrointestinal (96, 117), and vascular (10, 53, 81, 85, 127) smooth muscle has been studied. When these smooth muscle tissues are extracted in high concentrations of KCl (0.5 M) a soluble protein is obtained which has the following properties, very similar to those of actomyosin obtained from skeletal muscle under similar conditions (10, 93): 1) It forms a very viscous solution which undergoes a reversible fall in viscosity in response to the addition of ATP. This fall in viscosity is caused by a dissociation of actin from myosin, and is a highly specific characteristic of actomyosin. 2) Its ATPase activity differs from that of actomyosin of skeletal

muscle in potency and in dependence on divalent cations and pH, but these dissimilarities cannot be interpreted as indicating that the two are basically different. Furthermore, Needham and Williams (94) have observed that when uterine actomyosin is treated with trypsin to split light-meromyosin from acto-heavy-meromyosin, the ATPase activity is enormously increased. The acto-heavy-meromyosin, which is the active ATPase component of actomyosin, then has activity equivalent to a similar preparation of acto-heavy-meromyosin from skeletal muscle. 3) The actomyosins from both smooth and striated muscle show superprecipitation in response to ATP (53, 85). 4) Threads can be made from the actomyosin-like protein of smooth muscle and they develop tension in response to ATP (53).

It is probable that the unusual protein solubility properties found in some smooth muscle (81) are caused by the presence of a solubilizing factor rather than by a basic difference in the contractile protein (53). Evidence of important differences in the solubility spectrum of protein from blood vessels of normal and of hypertensive subjects (127) requires confirmation.

In the more intact form in glycerol-extracted muscle the contractile protein of smooth muscle still behaves very much like that of striated muscle (10, 20, 65, 117). In each of these models ATP causes tension development which is dependent on the same divalent cation (Mg), and has the same pH optimum (around 6.5). Ulbrecht and Ulbrecht (117) were able to obtain glycerol-extracted models from smooth and skeletal muscle which were capable of developing tension equivalent to that which the muscle developed *in vivo*. These models do not respond to electrical (110) or humoral (20, 21) stimuli.

Energy metabolism

The metabolic processes in smooth muscle have an important and direct influence on its contraction. Inhibition of metabolism with DNP causes a rapid cessation of contraction in uterine (51), gastrointestinal (110), and vascular (83) smooth muscle. DNP prevents contraction when the muscle is depolarized as well as when it is in its normal, polarized state. In vascular smooth muscle under anaerobic conditions, stimulating agents of wide variety cause an increase in lactic acid production which shows a quantitative and temporal correlation with contraction (83). Singh and Raju (108) have recently reported that DNP caused an initial relaxation of frog stomach muscle which was followed by a contraction. During the secondary period of contraction the muscle was insensitive to either stimulating or relaxing agents. In this time ATP content had fallen from a control level of 1.5 to 0.11 mmol/kg wet weight. The authors interpret their findings as indicating that ATP in this smooth muscle performs the two functions that it is known to fulfill in striated muscle: 1) to provide energy for phasic and tonic contraction which is susceptible to metabolic inhibitors, and 2) to keep the muscle relaxed by plasticizing its contractile protein.

Metabolic processes not only have a direct influence on muscle contraction, they also influence excitation-contraction coupling of the cell membrane. Bülbring and Lüllmann (28) have reported that after DNP treatment of the guinea pig

taenia coli, there is a period when acetylcholine continues to cause its typical increase in spike frequency but fails to increase tension. Bülbring (25) has argued that factors which decrease metabolic rate, such as a decrease in temperature, removal of substrate, and DNP, tend to cause depolarization and an increase in membrane activity. Conversely, factors that increase metabolic rate, including epinephrine (109), lead to hyperpolarization and inactivity of the membrane. Epinephrine causes an increase in production of ATP and CP which make more energy available for stabilization of the membrane (Na pump) (23, 24). Although this increase in energy-rich phosphate does not appear to be due to an increased phosphorylase activity, it has been noted that epinephrine causes an increase in phosphorylase activity in tracheal muscle, which is relaxed by epinephrine, while it fails to increase phosphorylase activity in mesenteric artery, in which tone is increased (89).

Multiple actions of electrolyte shifts

Shifts in the electrolyte environment can influence each of the intracellular phenomena as well as the membrane phenomenon of excitation. In this complex interrelationship of unknowns it is realistic to recognize that a given electrolyte shift may change the processes in more than one of these categories. The direction of the change produced by a given electrolyte shift may add to the mechanical response through its influence on one component while it subtracts from the response by its influence elsewhere. The resultant effect, then, will be the algebraic sum of its effects on the several components. But even such algebraic addition will not adequately describe the magnitude of the change in contraction. This may also be dependent on one of the steps in the contractile process being a bottleneck; changes could then occur in other components without altering the contractile end-product.

The unrealistic objective of this review is to identify the specific effects of shifts in electrolyte composition on each of the components of the contractile process. The best that can be expected in most instances is a descriptive account of the net results of a given change in ionic composition on the mechanical output of the whole contractile process. The actual site at which, and mechanism through which, the altered response is effected are subjects for free conjecture. There is an obvious need for a more intimate understanding and quantification of the effects of various electrolytes on the physico-chemical events of muscle contraction.

CALCIUM

Ca is essential for the contraction of smooth muscle. It participates in at least two links of the chain of events leading to a normal contraction: it modulates membrane excitability, and it triggers the release of mechanical energy. Because of the direct dependence of the contractile process of smooth muscle on the Ca ion it has proven fruitful to attribute the action of other electrolytes, and of physiological and pharmacological agents, to their effect on the Ca ion (27, 105, 120). A summation of the evidence bearing on the role played by Ca is probably the most important contribution that can be made in a review of the

role of electrolytes in smooth muscle contraction. The overall contractile response is lost completely in the absence of Ca and, within limits, the magnitude of the response is a function of the Ca concentration (4, 6, 8, 16, 33, 34, 50, 51, 63, 76, 105, 110, 120).

It should be profitable first to look at the intimate bases of this Ca dependence. Ca is a potent activator of the ATPase activity of the actomyosin-like protein obtained from smooth muscle (10, 53, 93); however, its physiological importance as a direct activator of the chemo-mechanical transducing effected by the interaction of ATP and actomyosin is not clear. Added Ca does little to support tension development by glycerol-extracted smooth muscle in the presence of ATP (10, 20); Mg here seems to be the essential cofactor. The possibility remains, however, that the minute amount of bound Ca which remains with the contractile protein throughout glycerol extraction is essential for tension development (65) as it seems to be for superprecipitation (122).

Two lines of evidence are in accord with the possibility that Ca may inactivate a relaxing factor bound to the extracted contractile protein of smooth muscle. Since relaxing factor prevents the chemo-mechanical transduction by the ATP-actomyosin system, this inhibitory action of Ca would cause the initiation of contraction. Briggs (20) has noted that the addition of Ca causes an augmentation of tension development by freshly prepared, glycerol-extracted uterine fibers, but has no effect on these fibers if they have undergone prolonged storage. He suggests that attached relaxing factor is responsible for the effects of Ca on the freshly extracted strips, and that the relaxing factor becomes inactive with the passage of time. Filo, Ruegg, and Bohr (53) have observed that an actomyosin-like protein may be extracted from vascular smooth muscle at low ionic strengths (0.05 M). They confirm the observation of Laszt and Hamoir (81) that the protein extracted in this manner does not show superprecipitation. This protein precipitated immediately upon the addition of $CaCl_2$, and following this treatment it did show the characteristic property of superprecipitation. They suggest that both the solubility at low ionic strengths and the failure to superprecipitate were caused by an attached relaxing factor which was inactivated by Ca. The possibility that relaxing factor may be bound closely to the contractile protein in smooth muscle may account for the failure to isolate this factor in particles extracted from smooth muscle (65, 85). It has been observed, however, that relaxing factor (Ca-sensitive) from skeletal muscle can prevent superprecipitation of actomyosin-like protein from vascular smooth muscle (85). Thus a case can be made for the possibility that Ca may trigger the mechanical response by inhibiting a relaxing factor, as may also be the case in striated muscle (49).

The ability of other divalent cations to substitute for Ca may reveal the individual effects of Ca in the overall contraction process if it is found that a given substitute can functionally replace or compete with Ca in one but not all of its actions. It has been observed (13) that in vascular smooth muscle made nonresponsive by Ca depletion, Ba is more potent than Ca in re-establishing the response to epinephrine. Sr is much less effective than Ca, and Mg is ineffective. Daniel *et al.* (45) have noted that both Sr and Ba restore the response lost by

the Ca-depleted uterine muscle. In contrast to Ca, however, the effects of which persisted for a long period after the bath fluid was replaced by a Ca-free PSS, the corresponding effects of Sr and Ba lasted only as long as these cations were in the bath. The difference may be due to differences in binding to ATP: the binding constant for Ca-ATP approximates 8,500 while that for Sr-ATP is 3,900 and for Ba-ATP 2,350 (90).

Sperelakis (110) has made far more detailed replacement studies using the circular layer of the cat intestine. Equal maximal tensions were developed in response to electrical stimulation of polarized and depolarized (K_2SO_4) muscle; however, 0.5 to 2 V/cm were sufficient for the former, whereas 5 to 25 V/cm were required for the latter. Glycerol-extracted fibers failed to respond to strong electrical stimulations and the responses of the depolarized smooth muscle were abolished by the metabolic poison DNP. He considers that electrical stimulation of the depolarized smooth muscle caused a direct displacement of charged particles which led to the tension development; that the excitation-contraction coupling appeared to be by-passed. Nevertheless, the responses of both the normal and depolarized muscle were dependent on the presence of Ca. Using a log scale he obtained a straight line relationship between contractile response and Ca concentration, in the range from 0 to 7 mM. Ba, Sr and Mg failed to restore contraction in Ca-depleted intact muscle, but Sr was actually more effective than Ca in supporting a response of the depolarized muscle. Arguments are made that Sr is substituting in some direct role that Ca plays in the contractile process and that in the intact muscle in addition to this action, Ca influences membrane excitability and is responsible for excitation-contraction coupling. Sperelakis (112) has evidence that Mg shares the action of Ca on the membrane, while Ba acts like Ca on the excitation-contraction coupling process. In measurements of the influx rate of Ca^{45}, he has found support for his thesis that Ca plays separate roles in excitation-contraction coupling on the one hand, and in the contractile machinery on the other (111). Whereas an increase in Ca^{45} influx occurred with stimulation of both intact and depolarized muscle, no such increase was seen in the intact muscle when uncoupling (action potentials remained but contraction was lost) was produced by adding 20 mM Mg to the bath. This procedure did not alter the contraction or the increase in Ca influx resulting from stimulation of the depolarized muscle. Sperelakis has made the case for these three roles of Ca quite convincing. His speculation that Ca may initiate contraction by releasing some intracellular humoral agent is more tenuous (111).

Consistent results have emerged from many recent studies measuring the Ca ion fluxes that accompany smooth muscle contraction. With contraction there is a greater entry of Ca into the cell, while Ca bound intracellularly or in the membrane is released (*vide infra*). Such observations do not add much to our insight into the mechanism of action of Ca in the contractile machine, but they constitute valuable supporting evidence that this cation does have a role in the contractile process.

Several isotope studies have demonstrated that Ca influx increases during contraction of smooth muscle (19, 21, 33, 101, 111). Many more studies have

presumed that an increase in Ca influx occurred because a greater response was observed when the extracellular Ca concentration was increased over a given range (34, 48, 51, 76, 105, 110, 120, 123). The fact that this dependence on Ca exists irrespective of the type of stimulus used implies a universal need for Ca in the contractile process. Inferences have been made about the role played by specific excitatory agents in effecting this Ca-influx in smooth muscle contraction. Woolley (123), noting that high concentrations of Ca caused a contraction similar to that produced by 5-hydroxytryptamine (5-HT) and that this agent failed to produce a contraction in the absence of Ca, concluded that 5-HT produces stimulation by facilitating the entry of Ca into the cell. Subsequently, he and Campbell (124) isolated from hog duodenum a lipid-soluble substance to which Ca attachment was effected by the action of 5-HT. They proposed that this substance may be a receptor for 5-HT, and that the mechanism of action of 5-HT is to augment the binding of Ca to the receptor-5-HT complex. Since the substance is lipid-soluble, movement of Ca through the membrane is facilitated. Waugh (120) has noted that the presence of epinephrine greatly increases the constrictor effect of acute increases in Ca in isolated vascular smooth muscle. This he interpreted as indicating that epinephrine produces contraction by increasing membrane permeability to Ca.

The influx of Ca associated with activity does not seem to be dependent on membrane action potentials; it is as prominent in the depolarized muscle as it is in the intact muscle (111). Furthermore, the dependence of the magnitude of the response on the external Ca concentration is equivalent in the normal and depolarized muscle (48, 51, 76, 110, 120). Actually, the depolarized muscle is clearly more responsive to elevations in Ca concentration than is the intact muscle (51, 120); this suggests that the normal polarized membrane offers an added barrier to the inward movement of Ca ions. Further evidence that Ca influx is associated with internal affairs of the smooth muscle cell rather than with membrane excitation is seen in the observation that no increase in Ca influx occurs when membrane excitation is divorced from the contractile process (111).

The responsiveness of the uterus to acetylcholine during Ca depletion, while being alternately in Na PSS and K PSS (polarized and depolarized membrane, respectively), has been studied by Edman and Schild (51). Polarization of the membrane potentiated subsequent responses to acetylcholine when the membrane was depolarized. This suggests that the polarized membrane may attract additional Ca to critical sites intracellularly or in the membrane, and that this Ca is then available for activation by acetylcholine subsequently, when the membrane is depolarized by K_2SO_4. This interpretation utilizes the important concept that stimulating agents, in addition to increasing Ca influx, are also capable of activating or releasing bound Ca from critical sites, and that the free Ca ion thus released is capable of triggering the contractile process. The fact that contractions in response to stimulating agents persist for a period after the smooth muscle is transferred to a Ca-free environment supports this interpretation. Further evidence for this action of stimulating agents is present in that repeated stimulation in a Ca-free solution accelerates the decline of the response (4, 51); each stimulus

seems then to add to the exhaustion of the Ca complex. The fact that the response to acetylcholine cannot be faithfully reproduced by adding external Ca suggests that the freeing of Ca ions from the bound sites in the cell may be a more important aspect of the response to stimulation than is the increased entrance of Ca from outside the cell (51).

Two additional types of information that give superficial insight into the nature of the site that binds Ca and then, with activity, releases it, are documented in the current literature: 1) Contractility of smooth muscle, which is lost slowly in a Ca-free environment, is regained very rapidly when Ca is added back to the environment. Robertson (101) observed that 10 to 12 minutes were required for a major reduction of responsiveness of the rabbit ileum, whereas significant recovery occurred in 15 seconds after the return of Ca. This suggested that Ca ions are active at the membrane, and that acetylcholine, the stimulant used, increases the permeability of the membrane to Ca ions as well as to monovalent ions. In any case, the restoration of the Ca-dependent process in a Ca environment requires less than one-tenth the time required for the loss of this activity in a Ca-free environment.

2) The persistence of Ca-binding in a Ca-free medium depends on the composition of the medium. Responsiveness of smooth muscle may persist for an hour or more in an electrolyte-free sucrose solution (16, 79); this suggests that Ca ions remain bound at essential sites longer when other ions are not present to replace them. Much consideration has been given to the Lüttgau-Niedergerke (84) hypothesis that Na competes with Ca for essential binding sites in smooth muscle (11, 21, 51, 88, 120) and the possibility that the depressing effect of high Na may be caused by its replacement of Ca at these sites.

In 1911, Cow (37) described an inverse relationship between the magnitude of the response of isolated vascular smooth muscle to epinephrine and the concentration of Ca in the bath. It was not then appreciated that an increase in Ca has a facilitating action on excitation-contraction coupling, and that under proper conditions Ca itself will cause a contraction. This apparent paradox is now well documented. Current studies indicate that whereas small increases in Ca concentration may augment smooth muscle contraction, greater increases will depress it (51, 106). Conversely, decreases in Ca concentration are commonly accompanied by initial transient increases in tension development (1, 27). An explanation for this negative influence of Ca on tension development is probably to be found in the well-documented observation (31, 80) that Ca causes hyperpolarization and stabilization of the membrane, decreasing its permeability to Na. However, hyperpolarization is not required for the depressant effect of high Ca since this occurs in the depolarized as well as the polarized muscle (51).

Hurwitz (71) has reviewed the evidence for this dual effect of Ca on smooth muscle. Subsequently, in studies in that laboratory (72) it was observed that an elevation of Ca concentration consistently inhibits transmembrane efflux of K, but may either enhance or inhibit contraction. Thus, whereas an increase in Ca concentration facilitates one link in the chain of events leading to muscle contraction, excitation-contraction coupling, it depresses another, membrane permeabil-

ity or excitability. A situation has recently been described in which these two effects of Ca have been differentiated within a single response of vascular smooth muscle to epinephrine (8). The response of rabbit aorta in the isolated bath has been found to be composed of a fast and a slow component, each of which can be altered independently by shifts in electrolyte composition or temperature, or the use of various stimulating and blocking agents (22). Later (8) it was observed that elevation in concentration of Ca over a specific range caused a depression of the fast component, whereas it enhanced the slow component of the response to epinephrine. This observation was interpreted as indicating that the fast component had as a rate-limiting factor membrane excitation (depressed by increased Ca), and the slow component had as its rate-limiting factor excitation-contraction coupling (enhanced by increased Ca). This then constitutes a circuitous argument that the little-explored membrane of the vascular smooth muscle cell may conform in this respect to the properties of smooth muscle cell membranes that are more adequately known.

MAGNESIUM

Mg, like Ca, has both an enhancing and a depressing action on smooth muscle contraction. In contrast to Ca, however, its greater effect is in decreasing the magnitude of the smooth muscle contraction. Definition of the means by which Mg produces these effects is tempting but the resulting propositions are tenuous. Mg can activate the contractile response of a glycerol-extracted smooth muscle to ATP; Ca cannot (10, 20, 65). Conversely, Mg is much less effective than Ca in supporting the ATPase activity of actomyosin-like protein from smooth muscle (10, 53, 94). The possibility that the contrasting effectiveness of Mg in the activation of these two different systems might be related to its ambivalent effect on the intact system does not seem worth considering. Nanninga (90, 91) has made a series of estimations based on the affinities of various intracellular contents and has presented relationships that may bear on the possible intracellular mechanism of action of Mg: 1) 90% of the ATP in the cell is bound as Mg-ATP, 2) 7% of this Mg-ATP is bound to myosin, presumably at the enzymatic site, 3) cellular activities which decrease ATP and CP and increase intracellular H-ion concentration all tend to increase the amount of unbound intracellular Mg. This last observation raises the question of whether increased free Mg plays a role in the depressed response of the fatigued muscle.

It has been generally reported that an increase in Mg concentration causes a relaxation and a decrease in responsiveness of vascular smooth muscle (63, 64, 106). Bohr (13) has observed that the magnitude of depression produced by 6 mM Mg is similar to that produced by the same concentration of Ca. In both cases it is the fast component (for which membrane excitation seems to be the rate-limiting factor) that is primarily affected. Sperelakis (110) has observed that treatment with 20 mM Mg arrests the mechanical activity of intestinal smooth muscle but permits continuation of action potentials. Mg will not support a mechanical response in a Ca-depleted muscle (45, 110). Not only will an increase in Mg depress a response in these smooth muscle preparations, but reduction or

elimination of Mg in the muscle environment results in an enhanced response. Haddy and his co-workers (63) have wondered whether the hypomagnesemia observed in hypertension may play a role in the increase in total peripheral resistance.

Responses of uterine smooth muscle are not affected by Mg in such a unidirectional fashion. Its spontaneous activity (34) and its response to histamine (54) are depressed by Mg; its response to vasopressin is potentiated by Mg over a range of 0 to 10 mM. Because an increase in Mg content of the uterus has been observed with estrogen treatment (118), it has been suggested that the change in responsiveness brought about by this hormone may be in part the result of the increase in Mg (7).

POTASSIUM

The influence that a shift in external K has on the contractile process, mediated through its effect on the membrane potential, has been extensively studied (70, 75, 87, 113). Under normal conditions each action potential is associated with an increase in tension (27, 31). When external K concentration is increased the membrane potential falls and spike frequency increases. This increase in frequency of action potentials is accompanied by an increase in contraction. When K concentration is elevated to the point where action potentials stop, the tension usually declines. However, this fall in tension does not always occur (45). When only the mechanical response of the smooth muscle is considered, the effect of an increase in K concentration is usually an increase in tension development (9, 69, 75, 114). Conversely, in a K-free solution the contractility decreases (1, 9, 70). Possibly related to its effect on membrane excitability is the interesting observation that the tachyphylaxis that occurs following a large response of the guinea pig intestine to histamine, acetylcholine, or barium chloride may be prevented by increasing the K concentration in the bath (32). However, many reported effects of altered K concentration *in vivo* and in the isolated bath are not in accord with the simple idea that an increase in membrane excitability is produced by an increase in extracellular K (11, 78, 82, 106, 107).

Potassium plays an essential role in the intracellular workings of the contractile machine. Although smooth muscle can contract in a nearly normal fashion in the extremely high concentrations of intracellular K achieved by placing the tissue in isotonic KCl or K_2SO_4 (*vide infra*), in the absence or near absence of intracellular K the smooth muscle is incapable of developing a normal reversible contraction (5, 82). Refrigerated storage in PSS of vascular smooth muscle (5) results in a loss of intracellular K (replaced by Na). Ability to respond to electrical and humoral stimuli is also lost. Subsequently, when this smooth muscle is incubated at 38°C in PSS containing K, it regains its intracellular K and its responsiveness. If incubated in PSS without K it fails to regain its responsiveness but undergoes an irreversible contracture. Dependence of the contractile response on intracellular K has also been demonstrated by a less drastic procedure. When vascular smooth muscle has been equilibrated at 38°C with PSS containing 3 or 6 mM KCl, and is then stimulated by increasing external K to 24 mM an immediate

contraction results. On the other hand, if the muscle has been equilibrated in PSS containing 1.5 mM K or less, a latent period of 5 to 10 minutes follows such stimulation before contraction occurs. Responsiveness of the internal contractile machine appears to require some threshold concentration of intracellular K.

In this study (5) it was observed also that the rates of contraction and of relaxation were both direct functions of the K concentration. An interesting corollary of this finding, that gives a more intimate insight into the role played by K in the contractile machine, is found in observations of Briggs (20) on glycerol-extracted preparations of uterine smooth muscle. Although the magnitude of tension developed in response to the addition of ATP did not vary over a range of K concentrations from 50 to 200 mM, the rate of relaxation was a direct function of the K concentration. In a 10-minute period the tension fell only 2% in 50 mM KCl, whereas it fell 90% in 200 mM KCl. Such higher concentrations of KCl appear to have a dissociating or plasticizing effect on the actomyosin molecule. The lack of responsiveness and the contracture of smooth muscle in the absence of intracellular K (5) may well be caused by the inability of actomyosin to dissociate in a K-free environment. Rigidity then develops as in rigor mortis except that in rigor mortis it is caused by a lack of ATP.

The existence of a unique type of structural protein in vascular smooth muscle has been proposed by Laszt and Hamoir (81). This protein, called "tonoactomyosin," is said to undergo syneresis, resulting in the development of a tonic contraction, in the presence of high K concentrations. However, further studies on the protein have cast doubt on its individuality (53).

SODIUM

A satisfactory evaluation of the role of Na in the mechanical response of mammalian smooth muscle cannot now be made. Understanding of the relationship has been confused by a large number of descriptive studies, using various techniques and approaches, which have developed conflicting results and have been subjected to loose interpretations. The primary contributors to this situation have been those of us interested in the responses of vascular smooth muscle and the mechanism by which Na might influence this tissue in its effect on hypertension (9, 11, 69, 86, 92, 103, 126).

It should be instructive to identify some of the sources of the confusion: 1) The action of Na on the contraction of smooth muscle is usually studied by observing the effect of a change in the Na concentration of the environment. The effect of altered Na on the mechanical response of smooth muscle is greatly influenced by the length of time the muscle is exposed to the altered Na environment. For instance, a given reduction in Na concentration may initially enhance the mechanical performance and subsequently depress it (88). Furthermore, if the tissue is permitted to equilibrate in a low Na environment and is then returned to control Na concentration the mechanical response will be temporarily depressed below control levels (9). 2) The substance substituted for Na to maintain isotonicity in the environment, may itself have a specific effect on smooth muscle contractility. The most obvious of these is choline chloride; perhaps its

cholinergic effect is not suppressed by atropine (88). It is generally accepted that if a consistent effect is obtained with several Na substitutes, such as lithium, THAM, hydrazine, choline, and sucrose, the interpretation that the effect is due to the Na lack and not to a specific effect of Na substitute (1, 119) is justified. It is difficult, however, to rule out the possibility that the decrease in mechanical performance usually seen after long exposures to very low concentrations of Na is not due to the entrance of the Na substitute into the cell and its deleterious effect on the contractile system. Bozler (15, 18) has presented evidence that even sucrose enters the smooth muscle cell. The replacement of NaCl by K_2SO_4 has obvious effects on both polarization and permeability of the membrane, and on tension development by the contractile protein. 3) Finally, real differences exist in the effects of altered Na on different tissues (86) and on the responses to different agents (69), and it must be recognized that the Na ion may have multiple sites of action among the various events of the contractile process.

It has been demonstrated that smooth muscle is capable of a contractile response when NaCl is completely replaced either by KCl or by K_2SO_4 (45, 48, 51, 52, 119). After such replacement, responses may occur at lower threshold concentrations of the stimulating agent. Measurements of the efflux of Na (44, 46, 61) indicate that this cation moves so rapidly that it is unlikely that the observed response is due to residual Na. Although the tissues in the K environment undoubtedly retain a small amount of bound Na, this material is so firmly fixed that it seems unlikely that it could play an essential role in the contractile event. Major consideration has been given to the mechanism by which the normal membrane events accompanying excitation are by-passed in the depolarized muscles. A selection among the possibilities suggested by Evans *et al.* (52) is yet to be made. These possibilities include: 1) The stimulating agent may cause the release of a specific substance or ion which initiates contraction, 2) the stimulating agent may produce permeability changes in a depolarized membrane which permit diffusion of an activating substance, 3) permeability changes may produce membrane potential changes even in the depolarized membrane, 4) the stimulating agent may act directly on the contractile elements. The last named possibility seems unlikely since none of these physiological stimulating agents has been shown to have a contractile effect on glycerol-extracted contractile systems (20, 110), nor do any of these agents alter the response of these isolated systems to ATP. It is noteworthy that the cell with depolarized membrane does not respond to mechanical stimulation nor does it respond to an electrical stimulus which produces a maximal contraction in a PSS that contains normal Na. Csapo (39) and more recently Sperelakis (110) have observed, however, that if the strength of the electrical stimulus is increased by 5- or 10-fold, a mechanical response can be obtained. Both of these investigators concluded that the strong electrical currents "by-pass" the initial steps of excitation and activate some process further along in the normal excitation-contraction sequence of events, so that the final common endpoint, the mechanical change, is achieved. This can happen in the complete absence of Na.

There appear to be consistent differences between the responses occurring in a

Na environment and those occurring in a Na-free solution. When K is substituted for Na the response to stimulation is slower and the maximum response may not be as great (51). The photic response of frog iris occurs in a K-substituted PSS but it is smaller than when the membrane is polarized (4). These observations are interpreted as indicating that membrane potentials do play a part in this response but that they are not necessary to it. Furthermore, spontaneous activity of smooth muscle may disappear when Na is replaced by K (52). On the other hand, the contractile response to added Ca is less consistent and smaller in "normal" Na PSS than it is when the membrane is depolarized with KCl (51).

Two additional observations have emphasized the possibility that the Na ion has an important influence on the normal mechanical response. As has been noted earlier in this review, during Ca deprivation when the tissue was switched back and forth from a Na- to a K-containing salt solution the exposure to the Na solution potentiated subsequent responses in the K solution (51). It was proposed that the polarized membrane might attract additional Ca and by this mechanism enhance future responses. The other evidence supporting a specific "contractile effect of Na" has been proposed from the following observations (45). In an isotonic salt solution containing equal parts of Na_2SO_4 and K_2SO_4, replacement of Na by either choline or sucrose results in a decrease in smooth muscle responsiveness. Furthermore, when a partial contraction is established in a K_2SO_4 environment, substitution in the environment of a solution containing equal parts of K_2SO_4 and Na_2SO_4 results in a sustained maximal shortening. This represents an instance in which depolarization does not initiate the contraction. It is proposed that Na is a contractile agent by virtue of its ability to displace Ca from its binding sites and prevent relaxation by interfering with the normal mechanisms for Ca immobilization. In the normal Na environment the action potential may initiate the contraction by a similar process, in which entering Na displaces Ca to trigger the contractile mechanism.

These observations and others (36) indicating that Na may facilitate the contraction are at odds with the commonly expressed hypothesis (11, 21, 51, 88, 120) that Na ions may be inhibitory merely by competing with Ca for anionic sites which require the divalent cation to effect a mechanical response.

Another way in which varying the Na has been observed to alter the mechanical response of smooth muscle seems to be related more to the direction of shifts in Na concentration than to the absolute amount of Na present in the environment. The immediate effect of a decrease in Na concentration in the environment seems to be to increase contractility, and the immediate effect of an increase in Na concentration appears to be to diminish contractility (9, 55). These changes are compatible with the interpretation that a decrease in the $[Na]_o:[Na]_i$ gradient permits greater responsiveness of the smooth muscle (100). Subsequently this gradient rises to control levels as intracellular Na is pumped out and $[Na]_i$ falls. Once a new equilibrium is established in a low-Na solution, a return of Na to control concentration results in a transient abnormally high $[Na]_o:[Na]_i$ ratio, and this situation in some manner depresses the mechanical response. Although this effect of altered Na gradient does fit some of the experimental

observations it is difficult to imagine a mechanism by which a decrease in gradient may increase the mechanical response. Perhaps the decreased demand on the active transport system for Na leaves a greater portion of the cells' metabolic energy available for the contractions.

Friedman *et al.* (56) have noted that a reduction in Na content (replaced by sucrose) of the perfusing solution caused a rapid efflux of Na from the rat tail. They believe that some of the lost Na came from intracellular sites and suggest that the $[Na]_o$:$[Na]_i$ gradient may not be reduced by the decrease in extracellular Na concentration. Since the procedure caused a reduction in vascular resistance, they consider that the intracellular content of Na may be an important determinant of vascular resistance. However, they summarize by stating, "the transcellular distribution of Na . . . is causally involved in the maintenance of vascular tone." This is based on their hypothesis that "environmental Na^+ is relatively less affected than that of cells so that the gradient actually becomes steeper" in this low-Na environment. These interpretations permit them to use the same hypothesis to account for both their current observation that low Na causes a decrease in vascular tone and their earlier observation that low Na increases the response of intestinal smooth muscle (55).

The following generalizations regarding the role of Na in the overall contractile process seem justified: 1) Contraction can occur in the absence of the Na ion, 2) the presence of the Na ion in normal amounts plays a major role in the normal contractile response, 3) shifts in Na concentration in the environment are accompanied by transient changes in the contractile response, which suggests that the tissue gradually adapts to the altered Na environment.

ANIONS

The usual approximate anionic composition of the PSS employed in studies of the response of isolated smooth muscle is: Cl, 120 mEq; HCO_3, 30 mEq; and PO_4, 5 mEq. None of these ions appears to be essential for the contractile response of smooth muscle, but the characteristics of the response can be altered by the substitution of other, nonphysiological, anions. Substitution of SO_4 for Cl seems to have little effect on the contractile response, particularly if the muscle is depolarized by K (52, 119). However, in studies where the membrane is polarized by a normal K gradient the substitution of SO_4 for Cl causes an initial depolarization accompanied by an increase in action potentials (80). This is followed after a half hour by a decrease in action potentials while the membrane still remains depolarized. Tension development here seems to parallel the degree of spike activity (30, 41, 80). Replacement of the Cl in the PSS by NO_3, Br, or I causes an initial depolarization followed by a hyperpolarization (80). Substitution of any one of these ions for Cl causes an augmentation of the contractile response (41, 120, 121). Waugh considers that the role played by these anions in increasing the contractile response is related to their ability to form ion pairs with Ca and thus facilitate the passage of Ca through the cell membrane (120). Sperelakis (112) has observed that while I and NO_3 potentiate, and SO_4 depresses, the normal smooth muscle response to an electrical stimulus, these anions have little

effect on the response when the contractile mechanism is stimulated directly following cell membrane depolarization.

Holman (70) has made an interesting suggestion concerning the role that the Cl ion may play in determining the membrane potential of smooth muscle, and therefore the way in which the Cl ion may alter its ability to develop tension. After a tissue has been exposed to high KCl concentration and then returned to a solution containing a "normal" K concentration the resting potential transiently remains low (and the responsiveness remains elevated). This may be due to an accumulation of intracellular KCl during the previous exposure, so that now when the tissue is back in "normal" KCl concentration the Cl ion gradient, and hence the Cl potential, is low; the total membrane potential remains low until this Cl gradient returns to its control level. In such subtle ways the anions may alter smooth muscle responsiveness, but the generalization that their effects are mediated through affairs of the membrane rather than through the internal workings of the cell seems correct.

SUMMARY

Attention to details concerning the individual components of the contractile process of smooth muscle creates the impression that there is a striking difference not only between this muscle and striated muscle, but also among smooth muscles from various sources. Examples of its dissimilarity are found in the great and variable permeability of the membrane to Na; the individuality of endoplasmic reticulum, relaxing factor, and response to Ca; and its relatively weak but very extensible contractile protein. Although the individualities may be only quantitative, they are responsible for large differences in the mechanical event which is the end-product of the overall contractile process. Emphasis in the past has been placed on the uniqueness, variability, and lability of the smooth muscle contractile system. However, when one looks within the basic mechanisms of the smooth muscle contractile process, its excitation, coupling, chemo-mechanical transducing, and energy metabolism, one sees processes that are generally similar to those of striated muscle.

Ca modulates membrane excitability, is essential for excitation-contraction coupling, and may have another more direct function in the relationship between the chemical energy source (ATP) and the contractile machine (actomyosin). Mg depresses membrane excitability or excitation-contraction coupling but, in some cases, has a positive influence on contraction. It does not seem to be essential for contraction and its usual effect reflects depressed excitation or coupling. The action of K is strikingly ambivalent; an increase in concentration of K in the environment of the cell results in depolarization, increased excitability, and increased contraction; whereas intracellular K is essential for the normal reversible contractile process. In its absence a rigid contracture develops and in excess it plasticizes the contractile machine so that tension development is impaired. Na is not essential for the contraction-relaxation cycle, but in the intact system it has an important controlling influence on membrane excitability and on excitation-contraction coupling. The action of Na is largely dependent on interrela-

tionships between the concentrations of Na and Ca. The influence of anions on smooth muscle contraction is determined primarily by the ease with which they permeate the cell membrane. In accordance with this facility they influence membrane potential and may have a more direct effect on the rate of movement of Ca through the membrane.

The generalization most profitable to live with in dealing with the influence of electrolytes on the contractile process of smooth muscle is that each may affect several of the components of the overall contractile process. The net effect of a shift in electrolyte concentration on the size of the contraction will depend on the relative magnitude and direction of the influence of this change on each process. In a general way the basic components of the overall contractile process of smooth muscle resemble those of skeletal muscle.

ACKNOWLEDGMENT

The reviewer is indebted to Dr. Ruth McVaugh for her indispensable contributions to the accuracy and comprehensibility of this manuscript. Special thanks also go to Miss Paula Levy for her diligence and care in its clerical preparation.

REFERENCES

1. Axelsson, J.: Dissociation of electrical and mechanical activity in smooth muscle. J. Physiol. **158**: 381–398, 1961.
2. Barr, L.: Distribution of ions in intestinal smooth muscle. Proc. Soc. exp. Biol., N. Y. **101**: 283–285, 1959.
3. Barr, L.: The responsiveness of arterial smooth muscle. In: Biophysics of Physiological and Pharmacological Actions, ed. by A. Shanes, pp. 579–589. Publ. No. 69, Amer. Ass. Advanc. Sci., Washington, D. C., 1961.
4. Barr, L. and Alpern, M.: Photosensitivity of the frog iris. J. gen. Physiol. **46**: 1249–1265, 1963.
5. Barr, L., Headings, V. E. and Bohr, D. F.: Potassium and the recovery of arterial smooth muscle after cold storage. J. gen. Physiol. **46**: 19–33, 1962.
6. Berger, E. and Marshall, J. M.: Interactions of oxytocin, potassium, and calcium in the rat uterus. Amer. J. Physiol. **201**: 931–934, 1961.
7. Best, F. A. and Pickles, V. R.: A myometrial effect of oestradiol, imitated by high Mg^{2+} concentrations. J. Physiol. **166**: 12–13P, 1963.
8. Bohr, D. F.: Vascular smooth muscle: Dual effect of calcium. Science **139**: 597–599, 1963.
9. Bohr, D. F., Brodie, D. C. and Cheu, D. H.: Effect of electrolytes on arterial muscle contraction. Circulation **17**: 746–749, 1958.
10. Bohr, D. F., Filo, R. S. and Guthe, K. F.: Contractile protein in vascular smooth muscle. Physiol. Rev. **42**: suppl. 5, 98–107, 1962.
11. Bohr, D. F. and Goulet, P. L.: Role of electrolytes in the contractile machinery of vascular smooth muscle. Amer. J. Cardiol. **8**: 549–556, 1961.
12. Bohr, D. F., Goulet, P. L. and Taquini, A. C., Jr.: Direct tension recording from smooth muscle of resistance vessels from various organs. Angiology **12**: 478–485, 1961.
13. Bohr, D. F.: Contraction of vascular smooth muscle. Canad. med. Ass. J. **90**: 174–179, 1964.
14. Bortoff, A.: Slow potential variations of small intestine. Amer. J. Physiol. **201**: 203–208, 1961.
15. Bozler, E.: Osmotic effects and diffusion of nonelectrolytes in muscle. Amer. J. Physiol. **197**: 505–510, 1959.
16. Bozler, E.: Contractility of muscle in solutions of low electrolyte concentration. Amer. J. Physiol. **199**: 299–300, 1960.
17. Bozler, E.: Initiation of contraction in smooth muscle. Physiol. Rev. **42**: suppl. 5, 179–186, 1962.
18. Bozler, E. and Lavine, D.: Permeability of smooth muscle. Amer. J. Physiol. **195**: 45–49, 1958.
19. Briggs, A. H.: Calcium movements during potassium contracture in isolated rabbit aortic strips. Amer. J. Physiol. **203**: 849–852, 1962.
20. Briggs, A. H.: Characteristics of contraction in glycerinated uterine smooth muscle. Amer. J. Physiol. **204**: 739–742, 1963.
21. Briggs, A. H. and Melvin, S.: Ion movements in isolated rabbit aortic strips. Amer. J. Physiol. **201**: 365–368, 1961.
22. Brodie, D. C., Bohr, D. F. and Smit, J.: Dual contractile response of the aorta strip. Amer. J. Physiol. **197**: 241–246, 1959.
23. Bueding, E., Bülbring, E., Gercken, G. and Kuriyama, H.: The effect of adrenaline on the adenosine-triphosphate and creatine-phosphate of intestinal smooth muscle. J. Physiol. **166**: 8-9P, 1963.
24. Bueding, E., Bülbring, E., Kuriyama, H. and Gercken, G.: Lack of activation of phosphorylase by adrenaline during its physiologic effect on intestinal smooth muscle. Nature, Lond. **196**: 944–946, 1962.

25. BÜLBRING, E.: Electrical activity in intestinal smooth muscle. Physiol. Rev. 42: suppl. 5, 160–174, 1962.
26. BÜLBRING, E. AND KURIYAMA, H.: Effects of changes in the external sodium and calcium concentrations on spontaneous electrical activity in smooth muscle of guinea-pig taenia coli. J. Physiol. 166: 29–58, 1963.
27. BÜLBRING, E. AND KURIYAMA, H.: Effects of changes in ionic environment on the action of acetylcholine and adrenaline on the smooth muscle cells of guinea-pig taenia coli. J. Physiol. 166: 59–74, 1963.
28. BÜLBRING, E. AND LÜLLMANN, H.: The effect of metabolic inhibitors on the electrical and mechanical activity of the smooth muscle of the guinea-pig's taenia coli. J. Physiol. 136: 310–323, 1957.
29. BURNSTOCK, G. AND PROSSER, C. L.: Conduction in smooth muscles: Comparative electrical properties. Amer. J. Physiol. 199: 553–559, 1960.
30. BURNSTOCK, G. AND STRAUB, R. W.: A method for studying the effects of ions and drugs on the resting and action potentials in smooth muscle with external electrodes. J. Physiol. 140: 156–167, 1958.
31. BURNSTOCK, G., HOLMAN, M. E. AND PROSSER, C. L.: Electrophysiology of smooth muscle. Physiol. Rev. 43: 482–527, 1963.
32. CANTONI, G. L. AND EASTMAN, G.: On the response of the intestine to smooth muscle stimulants. J. Pharmacol. 87: 392–399, 1946.
33. CHUJYO, N. AND HOLLAND, W. C.: Na, K, Ca, and Cl exchange in guinea pig ileum. Amer. J. Physiol. 202: 909–912, 1962.
34. CLEGG, P. C., HOPKINSON, P. AND PICKLES, V. R.: Some effects of calcium and magnesium ions on guinea-pig uterine muscle. J. Physiol. 167: 1–17, 1963.
35. CONWAY, J.: The nature of the increased peripheral resistance in hypertension. Amer. J. Cardiol. 5: 649–651, 1960.
36. COREY, E. L., MCGAUGHEY, H. S., JR., YON, J. L. JR. AND THORNTON, W. N.: Effect, of environmental pH, sodium concentration, and temperature on the spontaneous motility of isolated human uterine muscle. Amer. J. Obstet. Gynec. 82: 1201–1206, 1961.
37. COW, D: Some reactions of surviving arteries. J. Physiol. 42: 125–143, 1911.
38. CSAPO, A.: Actomyosin formation by estrogen action. Amer. J. Physiol. 162: 406–410, 1950.
39. CSAPO, A.: A link between 'models' and living muscle. Nature, Lond. 173: 1019–1021, 1954.
40. CSAPO, A.: Smooth muscle as a contractile unit. Physiol. Rev. 42: suppl. 5, 7–33, 1962.
41. CSAPO, A. AND KURIYAMA, H. A.: Effect of ions and drugs on cell membrane activity and tension in the postpartum rat myometrium. J. Physiol. 165: 575–592, 1963.
42. DANIEL, E. E.: Smooth muscle electrolytes. Canad. J. Biochem. Physiol. 36: 805, 1958.
43. DANIEL, E. E. AND DANIEL, B. N.: Effects of ovarian hormones on the content and distribution of cation in intact and extracted rabbit and cat uterus. Canad. J. Biochem. Physiol. 35: 1205–1223, 1957.
44. DANIEL, E. E. AND ROBINSON, K.: The secretion of sodium and uptake of potassium by isolated uterine segments made sodium rich. J. Physiol. 154: 421–444, 1960.
45. DANIEL, E. E., SEHDEV, H. AND ROBINSON, K.: Mechanisms for activation of smooth muscle. Physiol. Rev. 42: suppl. 5, 228–260, 1962.
46. DAVENPORT, H. W.: Sodium and potassium in canine gastric mucosa and smooth muscle. Amer. J. Physiol. 205: 413–416, 1963.
47. DAWKINS, O. AND BOHR, D. F.: Sodium and potassium movement in the excised rat aorta. Amer. J. Physiol. 199: 28–30, 1960.
48. DURBIN, R. P. AND JENKINSON, D. H.: The calcium dependence of tension development in depolarized smooth muscle. J. Physiol. 157: 90–96, 1961.
49. EBASHI, S.: Calcium binding activity of vesicular relaxing factor. J. Biochem. 50: 236–244, 1961.
50. EDMAN, K. A. P. AND SCHILD, H. O.: Interaction of acetylcholine, calcium and depolarization in the contraction of smooth muscle. Nature, Lond. 190: 350–352, 1961.
51. EDMAN, K. A. P. AND SCHILD, H. O.: The need for calcium in the contractile responses induced by acetylcholine and potassium in the rat uterus. J. Physiol. 161: 424–441, 1962.
52. EVANS, D. H. L., SCHILD, H. O. AND THESLEFF, S.: Effects of drugs on depolarized plain muscle. J. Physiol. 143: 474–485, 1958.
53. FILO, R., RUEGG, J. C. AND BOHR, D. F.: Actomyosin-like protein of arterial wall. Amer. J. Physiol. 205: 1247–1252, 1963.
54. FRASER, A. M.: The effect of magnesium on the response of the uterus to posterior pituitary hormones. J. Pharmacol. 66: 85–94, 1939.
55. FRIEDMAN, S. M., JAMIESON, J. D. AND FRIEDMAN, C. L.: Sodium gradient, smooth muscle tone, and blood pressure regulation. Circulation Res. 7: 44–53, 1959.
56. FRIEDMAN, S. M., NAKASHIMA, M. AND FRIEDMAN, C. L.: Sodium, potassium, and peripheral resistance in the rat tail. Circulation Res. 13: 223–231, 1963.
57. FRIEDMAN, S. M., SRÉTER, F. A., NAKASHIMA, M. AND FRIEDMAN, C. L.: Adrenal cortex and neurohypophyseal deficiency in salt and water homeostasis of rats. Amer. J. Physiol. 203: 697–701, 1962.
58. FUNAKI, S.: Electrical activity of single vascular smooth muscle fibers. In: Electrical Activity of Single Cells, ed. by Y. Katsuki, pp. 233–241. Igaku Shoin, Ltd., Tokyo, Japan, 1960.
59. FUNAKI, S.: Spontaneous spike discharges of vascular smooth muscle. Nature, Lond. 191: 1102–1103, 1961.
60. FURCHGOTT, R. F.: The pharmacology of vascular smooth muscle. Pharmacol. Rev. 7: 183–265, 1955.
61. GOODFORD, P. J. AND HERMANSEN, K.: Sodium and potassium movements in the unstriated muscle of the guinea-pig taenia coli. J. Physiol. 158: 426–448, 1961.
62. GOTO, M. AND CSAPO, A.: The effect of the ovarian steroids on the membrane potential of uterine muscle. J. gen. Physiol. 43: 455–466, 1960.
63. HADDY, F. J., SCOTT, J. B., FLORIO, M. A., DOUGHERTY, R. M., JR. AND HUIZENGA, J. N.: Local vascular effects of hypokalemia, alkalosis, hypercalcemia, and hypomagnesemia. Amer. J. Physiol. 204: 202–212, 1963.

64. HANENSON, I. B.: Effect of electrolytes and hormones on contraction of arterial smooth muscle. Circulation **26:** 727, 1962.
65. HASSELBACH, W. AND LEDERMAIR, O.: Der Kontraktionscyclus der isolierten contractilen Strukturen der Uterusmuskulatur und seine Besonderheiten. Pflüg. Arch. ges. Physiol. **267:** 532–542, 1958.
66. HEADINGS, V. E. AND RONDELL, P. A.: Arterial muscle contraction and potassium movement in vitro. Amer. J. Physiol. **202:** 17–20, 1962.
67. HEADINGS, V. E. AND RONDELL, P. A.: Changes in arterial ground substance, electrolyte and water accompanying aging and hypertension. Univ. of Mich. med. Bull., in press, 1964.
68. HEADINGS, V. E., RONDELL, P. A. AND BOHR, D. F.: Bound sodium in artery wall. Amer. J. Physiol. **199:** 783–787, 1960.
69. HINKE, J. A. M. AND WILSON, M. L.: Effects of electrolytes on contractility of artery segments in vitro. Amer. J. Physiol. **203:** 1161–1166, 1962.
70. HOLMAN, M. E.: Membrane potentials recorded with high-resistance micro-electrodes; and the effects of changes in ionic environment on the electrical and mechanical activity of the smooth muscle of the taenia coli of the guinea-pig. J. Physiol. **141:** 464–488, 1958.
71. HURWITZ, L.: Electrochemistry of smooth muscle and its relationship to contraction. In: Biophysics of Physiological and Pharmacological Actions, ed. by A. Shanes, pp. 563–577. Publ. No. 69, Amer. Ass. Advanc. Sci., Washington, D. C., 1961.
72. HURWITZ, L., BATTLE, F. AND WEISS, G. B.: Action of the calcium antagonists cocaine and ethanol on contraction and potassium efflux of smooth muscle. J. gen. Physiol. **46:** 315–332, 1962.
73. INGRAHAM, L. L. AND GREEN, D. E.: Role of magnesium in enzyme-catalyzed synthesis involving adenosine triphosphate. Science **128:** 310–312, 1958.
74. JUNG, H.: Die Bedeutung der Natrium-Leitfähigkeit der Uterusmuskelzelle für den Geburtsbeginn. Arch. Gynäk. **191:** 224–234, 1958.
75. JUNG, H.: Der Einfluss veränderter extracellulärer K-Konzentration auf das Ruhepotential und die Motilität des Uterus. Arch. Gynäk. **192:** 96–108. 1959.
76. JUNG, H.: The effect of oxytocin on the mechanism of uterine excitation. In: Oxytocin, Proceedings of an International Symposium held in Montevideo, 1959, ed. by R. Caldeyro-Barcia and H. Heller. Pergamon Press, New York, 1961.
77. KAO, C. Y.: Contents and distributions of potassium, sodium, and chloride in uterine smooth muscle. Amer. J. Physiol. **201:** 717–722, 1961.
78. KHAIRALLAH, P. A. AND PAGE, I. H.: Effect of adrenergic agents on responses of smooth muscle to angiotensin. Amer. J. Physiol. **202:** 841–844, 1962.
79. KOLODNY, R. L. AND VAN DER KLOOT, W. G.: Contraction of smooth muscles in non-ionic solutions. Nature, Lond. **190:** 786–788, 1961.
80. KURIYAMA, H.: The influence of potassium, sodium and chloride on the membrane potential of the smooth muscle of taenia coli. J. Physiol. **166:** 15–28, 1963.
81. LASZT, L. AND HAMOIR, G.: Étude par electrophorèse et ultracentrifugation de la composition protéinique de la couche musculaire des carotides de bovidé. Biochim. biophys. Acta **50:** 430–449, 1961.
82. LEONARD, E.: Alteration of contractile response of artery strips by a potassium-free solution, cardiac glycosides and changes in stimulation frequency. Amer. J. Physiol. **189:** 185–190, 1957.
83. LUNDHOLM, L. AND MOHME-LUNDHOLM, E.: Studies on the effects of drugs upon the lactic acid metabolism and contraction of vascular smooth muscle. Acta physiol. scand. **55:** 45–63, 1962.
84. LÜTTGAU, H. C. AND NIEDERGERKE, R.: The antagonism between Ca and Na ions on the frog's heart. J. Physiol. **143:** 486–505, 1958.
85. MALLIN, M. L. AND HANENSON, I. B.: Personal communication.
86. MALLOV, S.: Effects of sodium ion and solution tonicity on reactivities of hypertensive rat aortic strips. Amer. J. Physiol. **198:** 1019–1022, 1960.
87. MARSHALL, J. M.: Regulation of activity in uterine smooth muscle. Physiol. Rev. **42:** suppl. 5, 213–227, 1962.
88. MARSHALL, J. M.: Behavior of uterine muscle in Na-deficient solutions; effects of oxytocin. Amer. J. Physiol. **204:** 732–738, 1963.
89. MOHME-LUNDHOMM, E.: Phosphorylase activity of smooth muscle. Acta physiol. scand. **54:** 200–208, 1962.
90. NANNINGA, L. B.: The association constant of the complexes of adenosine triphosphate with magnesium, calcium, strontium, and barium. Biochim. biophys. Acta **54:** 330–338, 1961.
91. NANNINGA, L. B.: Calculation of free magnesium, calcium, and potassium in muscle. Biochim. biophys. Acta **54:** 338–344, 1961.
92. NAPODANO, R. J., CALIVA, F. S., LYONS, C., DE SIMONE, J. AND LYONS, R. H.: The reactivity to angiotensin of rabbit aorta strips after either alterations of external sodium environment or addition of benzydroflumethiazide. Amer. Heart J. **64:** 498–502, 1962.
93. NEEDHAM, D. M. AND CAWKWELL, J. M.: Some properties of the actomyosin-like protein of the uterus. Biochem. J. **63:** 337–344, 1956.
94. NEEDHAM, D. M. AND WILLIAMS, J. M.: Some properties of uterus actomyosin and myofilaments. Biochem. J. **73:** 171–181, 1959.
95. NORMAN, A., RONDELL, P. A. AND BOHR, D. F.: Determinations of extracellular fluid space (ECF) from small samples of tissue. Amer. J. clin. Path. **32:** 465–467, 1959.
96. OPPEL, V. V. AND SEREBRENIKOVA, T. P.: Contribution to contractile proteins of smooth muscles. Biokhimiia **24:** 648–656, 1959.

97. PEACHEY, L. D. AND PORTER, K. R.: Intracellular impulse conduction in muscle cells. Science **129**: 721–722, 1959.
98. PETERSON, L. H.: Systems behavior, feed-back loops, and high blood pressure research. Circulation Res. **12**: 585–594, 1963.
99. PROSSER, C. L., BURNSTOCK, G. AND KAHN, J.: Conduction in smooth muscle: comparative structural properties. Amer. J. Physiol. **199**: 545–552, 1960.
100. RAAB, W.: The integrated role of catecholamines, mineralocorticoids and sodium in hyper- and hypotension (a working hypothesis). J. Mt. Sinai Hosp. **19**: 233–239, 1952.
101. ROBERTSON, P. A.: Calcium and contractility in depolarized smooth muscle. Nature, Lond. **186**: 316–317, 1960.
102. RODDIE, I. C.: The transmembrane potential changes associated with smooth muscle activity in turtle arteries and veins. J. Physiol. **163**: 138–150, 1962.
103. RONDELL, P. A. AND GROSS, F.: Method for isometric recording from isolated vessels under various conditions. Helv. physiol. acta **18**: 366–375, 1960.
104. SCHATZMANN, H. J.: Calciumaufnahme und abgabe am Darmmuskel des Meerschweinchens. Pflüg. Arch. ges. Physiol. **274**: 295–310, 1961.
105. SCHATZMANN, H. J. AND ACKERMANN, H.: Die Strophanthinwirkung am Darmmuskel und ihre Beziehung zum Kationengehalt des Mediums. Helv. physiol. acta **19**: 196–213, 1961.
106. SCOTT, J. B., FROLICH, E. D., HARDIN, R. A. AND HADDY, F. J.: Na^+, K^+, Ca^{++}, and Mg^{++} action on coronary vascular resistance in the dog heart. Amer. J. Physiol. **201**: 1095–1100, 1961.
107. SHARMA, A. P.: Relationship between histamine and dual action of local ions (sodium, potassium and calcium) on involuntary musculature. Ind. J. med. Res. **47**: 434–436, 1959.
108. SINGH, I. AND RAJU, N. V.: Role of adenosine-triphosphate in muscular contraction. Nature, Lond. **199**: 288, 1963.
109. SINGH, I., SINGH, S. I., AND DHALLA, N. S.: Active relaxation of unstriated muscle produced by epinephrine. Amer. J. Physiol. **200**: 955–958, 1961.
110. SPERELAKIS, N.: Contraction of depolarized smooth muscle by electric fields. Amer. J. Physiol. **202**: 731–742, 1962.
111. SPERELAKIS, N.: Ca^{45} and Sr^{89} movements with contraction of depolarized smooth muscle. Amer. J. Physiol. **203**: 860–866, 1962.
112. SPERELAKIS, N.: Personal communication.
113. SU, CHE, BEVAN, J. A. AND URSILLO, R. C.: Electrical quiescence of pulmonary artery smooth muscle during sympathomimetic stimulation. Circulation Res., in press, 1964.
114. THOMPSON, J. W.: Studies on the responses of the isolated nictitating membrane of the cat. J. Physiol. **141**: 46–72, 1958.
115. TOBIAN, L.: Interrelationship of electrolytes, juxtaglomerular cells and hypertension. Physiol. Rev. **40**: 280–312, 1960.
116. TRAIL, W. M.: Intracellular studies on vascular smooth muscle. J. Physiol. **167**: 17–18P, 1963.
117. ULBRECHT, M. AND ULBRECHT, G.: Der isolierte Arbeitscyclus glätter Muskulatur. Z. Naturf. **7b**: 434–443, 1952.
118. WALAAS, O.: The effect of estrogenic hormone on the content of calcium and magnesium in uterus. Acta physiol. scand. **21**: 27–33, 1950.
119. WAUGH, W. H.: Adrenergic stimulation of depolarized arterial muscle. Circulation Res. **11**: 264–276, 1962.
120. WAUGH, W. H.: Role of calcium in contractile excitation of vascular smooth muscle by epinephrine and potassium. Circulation Res. **11**: 927–940, 1962.
121. WAUGH, W. H.: Vascular smooth muscle contractions induced by epinephrine in chloride-deficient electrolyte media. Physiologist **5**: 228, 1962.
122. WEBER, A. AND HERZ, R.: The binding of calcium to actomyosin systems in relation to their biological activity. J. biol. Chem. **238**: 599–605, 1963.
123. WOOLLEY, D. W.: A probable mechanism of action of serotonin. Proc. nat. Acad. Sci., Wash. **44**: 197–201, 1958.
124. WOOLLEY, D. W. AND CAMPBELL, N. K.: Serotonin receptors. II. Calcium transport by crude and purified receptor. Biochim. biophys. Acta **40**: 543–544, 1960.
125. WURZEL, M., PRUSS, T., WEISS, W. AND MAENGWYN-DAVIES, G. D.: Modification of rabbit aortic strip technic for catecholamine (4-point) assay and pharmacological studies. Proc. Soc. exp. Biol., N. Y. **105**: 659–661, 1960.
126. YAMABAYASHI, H. AND HAMILTON, W. F.: Effect of sodium ion on contractility of the dog's aortic strip in response to catecholamines. Amer. J. Physiol. **197**: 993–996, 1959.
127. YUR'EV, V. A.: Muscle proteins of blood vessel walls, and changes in their composition during hypertensive disease. Biull. Exsp. Biol. Med. **51**: 569, 1961.