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VASCULAR SMOOTH MUSCLE

I. NORMAL STRUCTURE, PATHOLOGY, BIOCHEMISTRY, AND BIOPHYSICS[1]

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Numerous studies of vascular smooth muscle have appeared since the publication of Furchgott's excellent review (200). Also, during these years major advances have taken place in general membrane and muscle physiology. The latter include the development of a cohesive theory of excitation based on ion fluxes and of the sliding-filament theory of muscle contraction. It is also during these recent years that the role of Ca as the activator of muscle contraction has been accepted. Our aim has been to correlate the literature on vascular smooth muscle within the general context of excitable membranes and contractile tissues. We have stressed the comparative approach to general biological processes, also believing that this will simplify the task of understanding an otherwise bewildering array of specialized cases. Therefore, we will discuss several studies relevant to the general problems of excitation and contraction, but not dealing specifically with vascular smooth muscle.

We have enlarged on Bozler's (75) dual classification of single-unit and multiunit muscle and also distinguish between spike-generating (phasic) and gradedly responsive (tonic) multiunit (smooth and striated) muscles. This treatment then is based not solely on the presence or absence of conducted response in the whole muscle, but also on the electrophysiological properties and excitation-contraction coupling mechanisms of individual fibers (section VI F). The reader should be warned of the still incomplete experimental foundation of this approach, which is presented to stimulate further comparative studies.

It is customary to discuss the action of drugs on vascular smooth muscle in terms of the acute pharmacology of excitation and contraction. The morphogenetic function of smooth muscle (section I C) implies, however, that the long-term effects of chemical and physical agents upon vascular connective tissue may also be caused by alterations in smooth muscle function. A particularly significant development is the recognition of the participation of smooth muscle in the atherosclerotic process (section III B). We have, therefore, extended the scope of this review to introduce some aspects of the chronic pharmacology of vascular smooth muscle.

Part I of the review deals primarily with the general biophysical properties that determine the pharmacological responses of vascular smooth muscle and with chronic pharmacology and pathology. In the forthcoming Part II, the responses of normal and pathological vascular smooth muscle to specific physical and chemical agents will be discussed.

I. ULTRASTRUCTURE

A. The cell envelope: plasma membrane, pinocytic vesicles, and basement membrane

Electronmicroscopy established that smooth muscle cells do not constitute a true anatomic syncytium: a distinct plasma membrane approximately 80 Å thick surrounds each cell with no evidence of direct cytoplasmic continuity between adjacent cells (62, 170, 403, 461, 468, 478, 490, 493). The plasma membrane and the basement membrane, when the latter is present, constitute the sarcolemma. The presence of a distinct basement membrane, on the external surface of the plasma membrane, has been noted about the smooth muscle of a great many blood vessels (62, 403, 490, 493, 494, 511) and is considered to be one of the ultrastructurally diagnostic features of smooth muscle. The basement membrane may be less conspicuous about smooth muscle cells of the rodent aorta (241, 320, 468, 472). By definition, in the areas of close apposition or nexus (section I D) the fused plasma membranes of adjacent cells are devoid of basement membranes.

The connective tissue components separating smooth muscle cells of different vessels vary, and vascular collagen-content tends to increase with age (114, 320, 468). Aortic smooth muscle cells run obliquely between adjacent elastic lamellae into which they insert. In some instances, there is an area of increased cytoplasmic density adjacent to the elastic insertion (62, 114, 241, 329, 472). Fingerform processes of smooth muscle insert on the elastic lamellae of the rabbit aorta, and there is a tendency towards a more pointed, end-to-end, insertion with increasing size of animal species (536).

Micropinocytic vesicles are spherical invaginations of the plasma membrane, ranging between about 450 Å (403) and 800 Å (114) in diameter. Similar vesicles are also seen lying free in the cytoplasm. The vesicles are common, and perhaps universal, components of the vascular smooth muscle membrane (170, 241, 320, 329, 461, 471, 490, 493, 570). There has been some disagreement regarding their actual number and distribution. According to some authors (424, 637), vesicles are more common on the inner, endothelial surface of smooth muscle, while according to others (361, 426) there is a greater number on the outer, adventitial surface. Lever *et al.* (361) found a significantly greater number of vesicles on the outer, adventitial surface and the lateral interfaces, than on the endothelial face of the smooth muscle in the rabbit coronary arterioles. The vesicles are not fixed structures (493) and their distribution may reflect the functional state of vascular smooth muscle. There often is a concentration of pinocytic vesicles in the smooth muscle processes adjacent to nerve endings (81, 139a, 178, 403). The careful study of Lever *et al.* on the neuromuscular relationship in coronary arterioles (361) may have favored selection of smooth muscle in relatively close association with nerve endings (see section I E), and this may account for the high density of vesicles found on the outer face of smooth muscle cells. It should be pointed out, however, that pinocytic vesicles are not always demonstrable in smooth muscle cells that are in close (600 Å) association with nerve bundles (18).

The functional significance of vesicles is apparent when considering the 25% increase in cell surface produced by them (493). The Ca content of the surface

vesicles is at least as high as that of the extracellular fluid with which they communicate, and therefore several orders of magnitude higher than the myoplasmic free Ca. Furthermore, some of the particulate relaxing factor isolated from smooth muscle (section VI C) may be composed of closed pinocytic vesicles capable of concentrating Ca. The localization of ATPase activity to pinocytic vesicles will be discussed later (section II C).

The profusion of vesicles in smooth muscle raises the possibility that certain agonists may act as pinocytosis-inducing agents, with the resultant intracellular uptake of agonist and extracellular fluid. This is suggested by the frequent concentration of pinocytic vesicles in the vicinity of adrenergic nerve endings and the recently demonstrated intracellular uptake of norepinephrine by vascular smooth muscle (214). Electronmicroscopic studies of the effects of drugs on pinocytosis would clearly be of great interest.

B. The contractile elements: myofilaments and dense bodies

The light-microscopic appearance of striation in striated muscle is due to a regular lateral alignment of sarcomeres bound by Z lines, into which insert thin (50 Å) actin filaments in the I bands, overlapping with thick (species variable 100 to 1500 Å) filaments in the A band (134, 298–299, 469). Contractile force is thought to be generated between crossbridges containing the H-meromyosin heads of the myosin molecules, constituting the thick filaments (299, 473, 474), and the adjacent thin filament containing actin and native tropomyosin (251). The sliding-filament model of muscle contraction depends on the existence of two types of myofilament within a muscle fiber. The interaction between the filaments leads to the generation of force or to shortening, or both, through a sliding movement of the two sets of filaments relative to each other. The primary structure and therefore length of myofilaments remains constant during contraction.

The light-microscopic definition of smooth muscle merely implies the absence of a regular alignment of filaments into sarcomeres. Electronmicroscopic studies have distinguished two ultrastructural types of smooth muscle. The first type, generally seen in invertebrates, is characterized by the presence of two grossly different sets of filaments: one thin, containing actin, the other thick (up to 1000 Å), containing myosin or a related protein, tropomyosin A (for review see 250). Because the two sets of filaments are not arranged in sarcomeres, the light-microscopic appearance of the muscle is smooth, but the presence of crossbridges and the invariant filament length and substructure during contraction provide good evidence for the sliding mechanism of contraction (162, 252). The second ultrastructural type, represented by most forms of mammalian smooth muscle, is characterized by the absence of a well-defined (or easily demonstrable) set of thick filaments. Whether the chemically demonstrable (section IV A) myosin in mammalian smooth muscle is organized in a filamentous form, which is merely technically difficult to demonstrate, or whether it is present as dissolved monomers, are the critical questions relevant to the operation of a sliding-filament mechanism in smooth muscle. The material reviewed here includes studies on several types, not solely vascular, smooth muscle.

Myofilaments are characteristic cytoplasmic components of vascular smooth muscle, although there are quantitative differences between the filament content of different vascular areas (see section I F). The filaments are usually denser in the peripheral region of the cytoplasm, and they run somewhat irregularly, but with an average vector directed parallel to the long axis of the muscle fiber (403). The latter directionality is more apparent in the contracted, than in the relaxed smooth muscle (352). The filaments have been followed for up to 1 μ in longitudinal section, but because of their tortuous course out of the plane of sectioning, this is probably not the true maximal length. The diameter of myofilaments varies in different reports from about 30 Å (471) to about 100 Å (403). Rhodin (493) observed 50 Å and 80 Å filaments in his material, but did not feel that there was sufficient evidence of two separate sets of filaments. An exception to the general difficulty in differentiating filaments is the report (118) of two distinct sets of filaments, 90 Å and 125 Å respectively, in bovine carotid artery. It is probable that the wide range of myofilament diameters reported is largely due to differences in the techniques of fixation, embedding, and staining and the calibration of electron optics in different laboratories. It is also probable that some of the filaments represent noncontractile material. The substructure of elastin, which is produced by vascular smooth muscle (section I C), is a 25 Å thick filament (537).

A large proportion of vascular myofilaments are typical thin, actin filaments, which are probably identical in all types of muscle studied (251). Shoenberg *et al.* (542) isolated thin 60 to 80 Å myofilaments from uterine smooth muscle, which had the appearance of a two-stranded helix composed of globular (50 to 55 Å diameter) subunits typical of actin filaments (251). X-Ray diffraction of living and contracting taenia coli, the only mammalian smooth muscle so studied to date, also reveals the presence of actin reflections, but not the reflection characteristic of myosin organized into filaments (162). There are three possibilities to be considered regarding the state of myosin in mammalian smooth muscle: 1) thick filaments are present but are destroyed during preparatory and extraction procedures for electronmicroscopy; 2) myosin filaments are built of a lesser number of myosin molecules than in striated muscle, consequently are shorter and thinner, and therefore are poorly distinguishable from actin filaments; 3) myosin is not organized into filaments but exists in the colloid dispersed phase in relaxed muscle: this implies at least the theoretical possibility that it becomes so organized during contraction (162, 446, 542).

The myofilaments in smooth muscle are intimately related to dense bodies, and the interrelationships between the two may have some bearing on the question of myosin aggregation. Dense bodies are dark-staining, spindle-shaped areas, which vary in length from 4000 to 9000 Å and in width from 2000 to 5000 Å (403), frequently adhering to the cytoplasmic side of the plasma membrane or dispersed within the cytoplasm. Filaments are seen entering dense bodies and, during contraction, the plasma membrane in the area of a dense body appears retracted. This finding indicates that a considerable amount of tension is transmitted to and through the sarcolemma. Pease *et al.* (471–472) suggested that dense bodies represent attachment devices that anchor the myofilaments to the

plasma membrane. This view has been generally accepted (for reviews, see 490, 493), in particular since Prosser *et al.* (485) reported that dense bodies were not extracted by the procedures that remove contractile proteins from smooth muscle. Similar structures in invertebrate (insect) muscle are clearly analogous to the Z line of striated muscle, and may form a regular lateral array of dense bodies producing a typical striated pattern.

A second suggestion about the nature of dense bodies has been that they represent areas of "increased contractile material" or the regions of overlap between thin actin and somewhat thicker myosin filaments (352) in analogy with the A band of striated muscle. The number and length of the dense bodies in rodent intestinal smooth muscle (no similar studies of vascular smooth muscle having been reported at this writing) is increased in the contracted state (352, 493). This could mean that they are sites of interaction between actin and filamentous myosin that are absent from relaxed areas.

The considerable variation in the morphology of free, cytoplasmic dense bodies, and the occasional appearance of an area of "increased density" traversed by filaments, between two typical dense bodies (*e.g.*, fig. 7 in 471) suggests to us the possibility that the term "dense bodies," as currently used, may describe two functionally different units: one an analogue of the Z line, the other an area of increased electron density at the sites of actin-myosin interaction. The refined fluorescent-antibody techniques applied to striated muscle by Pepe (473–474), combined with electronmicroscopy, may provide a more clear-cut identification of the nature of dense bodies and the location of myosin in mammalian smooth muscle.

In a study of mouse jejunum fixed during different states of contraction, Lane (352) described two types of filament, of 20 to 30 Å and 50 to 60 Å, respectively (although his fig. 1c describes filaments 80 Å thick); these intermingled only in dense areas. There remains, however, the discrepancy between the 20 to 30 Å filament thickness reported and the generally accepted 50 Å thickness of actin filaments in sectioned material (251).

In contrast to Lane's observations in mouse jejunum, and the report of Choi (111), who also described two filament sizes in chicken gizzard (less than 30 Å and 50 to 80 Å thick), observers (459) recently claimed the existence of only one type of filament, with an average 40 by 60 Å dimension in the turkey gizzard. According to these authors, individual myofilaments (particularly in material embedded in methacrylate) exhibit areas of increased density, rather than two separate classes of myofilament. The authors' description and the illustration (see fig. 6 in 459), seem equally compatible with the presence of fusiform thick filaments, whose thin portion is approximately equal in thickness and indistinguishable (in section) from actin filaments. In one of the earliest studies (471) of vascular smooth muscle, denser appearing filaments up to 100 Å thick within dense bodies were described. Large numbers of apparently thick filaments are illustrated in a study of lymphatic vessels of the guinea pig (530).

One of the most recent and interesting ultrastructural studies is that of Kelly and Rice (330) on the chicken gizzard. They could demonstrate distinct thick

(150 Å) filaments and thin filaments, provided a pH 6.6 or lower was maintained during the preparatory procedures. In some areas of cross sections through the filaments there were distinct rosettes, consisting of one thick and several thin filaments. At pH 7 or higher, usually only one type of filament was observed. This pH dependence of filament size appears to be a somewhat general property of myosin: artificial filaments prepared from rabbit skeletal myosin (316) or from uterine myosin (446) also tend to be longer at lower pH. Smooth muscle actomyosin is soluble at pH 7 in solutions of low ionic strength, but at pH 6 it is insoluble or, if extracted, superprecipitates (531 and section V A). The chemical evidence thus favors the possibility that the destruction of myosin filaments by the usual preparatory techniques at pH 7 is responsible for the apparent absence of thick filaments in smooth muscle. These recent findings, however, raise the questions whether a normally low intracellular pH, denied on the basis of extraction experiments (531), maintains the stability of thick filaments *in vivo* and if so, why there is no crystallographic evidence of their presence.

In summary, the balance of as yet somewhat inconclusive evidence suggests that the use of special techniques will reveal two kinds of filament in mammalian smooth muscle. The probability of a sliding mechanism of contraction is also suggested by the length-tension relationship (section V A) of vascular smooth muscle. It must be reemphasized, however, that although we have drawn on ultrastructural evidence obtained with avian and mammalian smooth muscles, there is no reason to expect that the myosin filaments of different smooth muscles have the same morphology. Antibodies to bovine arterial contractile proteins do not cross-react with bovine myometrium or gastric smooth muscle (341) and, unlike those of actin, the properties of the myosins obtained from different sources are quite variable (section IV A).

C. Formed intracytoplasmic elements: the morphogenetic role of vascular smooth muscle

A central, elongated, ellipsoid nucleus, frequently containing one or two nucleoli, and surrounded by a double nuclear envelope, is characteristic of relaxed cells. It becomes highly compressed and convoluted in contracted muscle (493). The Golgi complex is located near the nucleus and is often related to smooth-surfaced endoplasmic reticulum (493). We have also noted in some of the published photographs (figs. 10 and 12 of 493) an association between mitochondria and the smooth-surfaced endoplasmic reticulum (for further discussion of smooth reticulum, see section VI G). Mitochondria are frequently located at the nuclear poles (238, 511, 619). Microtubules, 200 to 400 Å in diameter, arranged mainly parallel to the longitudinal axis of the cell, have been described recently in glutaraldehyde-fixed vascular smooth muscle postfixed in osmium tetroxide (512, 516) but their function is still unknown.

The rough-surfaced endoplasmic reticulum consists of tubular structures coated with ribosome particles. Ribosomes are thought to contain ribonucleoprotein and hence suggest intracellular protein synthesis.

An important result of the electronmicroscopic studies is that the *smooth*

muscle cells are the only cell-type found within the aortic media of mammals (62, 114, 320, 468, 472, 536) and are responsible for the production of collagen and elastin. The morphogenetic role of mammalian vascular smooth muscle correlates well with the presence of a well-developed rough endoplasmic reticulum, which is especially prominent in embryonic, young, and injured vascular smooth muscle (section I F). In the aortas of the parakeet and the turkey, two cell types have been found: one smooth muscle, the other fibroblastlike (267, 546).

An obvious implication of the fact that smooth muscle is the only cell present in mammalian aorta is that biochemical studies (*e.g.*, respiration, glycolysis) of aortic media do in fact sample the properties of smooth muscle. It also follows that certain vascular disorders characterized by disturbances of the fibrous elements of the vessel wall (*e.g.*, lathyrism, aging) may be considered secondary to disturbances of vascular smooth muscle metabolism.

D. Intercellular connections: myo-myo and myoendothelial junctions

Intercellular connections provide a means of direct transfer of information from one member of a given cell population to another of the same or to a different cell population. Electrical transmission is impossible over large intercellular distances because of short circuiting by extracellular fluid (156). Dewey and Barr suggested (42, 141) that in smooth muscle specialized areas of close membrane apposition (nexuses) provide the low-resistance pathway for conducted action potentials. Their description of the nexus, based upon permanganate-fixed material, suggests that in the area of the nexus the sarcolemma of each of two adjacent smooth muscle cells loses its covering of basement membrane, and the now closely apposed plasma membranes appear fused. In their material fusion of the dark, outer lamellae of two (triple lamellar) unit membranes leads to the appearance of a single quintuple-layered membrane. The following discussion on vascular smooth muscle is based mostly upon other than permanganate-fixed material. It is generally implied that in material fixed in glutaraldehyde or osmium, or both, the equivalent of the nexus is a close apposition of two adjacent plasma membranes without intervening basement membrane or intercellular substance. Triple lamellar areas of fusion have been demonstrated in immature rat aortas fixed in osmium tetroxide and uranyl acetate (114).

In the large elastic arteries of adult animals there are generally no nexuses (320, 329). In the aorta of newborn rats and with decreasing frequency up to 3 months of age, nexuses may be present (114). Aortic smooth muscle cells are further separated by the production of connective tissue with aging (114, 320, 329). Occasional areas of close intercellular contact have been described in rabbit aorta (62, 536), but there is no evidence that these areas are specialized nexuses.

The nexus occurs with some frequency in human umbilical arteries (570), and in rabbit (282) and canine (140) mesenteric veins. Rhodin (494), in comparing arterioles (50 to 100 μ), terminal arterioles (10 to 50 μ, only one smooth muscle cell layer), and precapillary sphincters, (7 to 15 μ) observed the nexus in each type of vessel. There was, however, a distinct increase in both number and length of nexal contacts with decreasing vessel size, and in terminal arterioles a nexus joined each muscle cell with its neighbors.

Peg and socket junctions, invaginations of a smooth muscle cell into another one, also occur in vascular smooth muscle (403, 637). Although Zelander *et al.* (637) described a 70 Å interspace between adjacent plasma membranes of the invaginated areas, intercellular spaces of this magnitude can be caused by preparatory techniques and may in reality represent fused membranes (494).

The correlation with functional evidence (section VI E) supports the view (42, 141, 165) that intercellular connections serve as low resistance pathways for conducted electrical activity in smooth muscle.

The myoendothelial junctions between endothelial protrusions (424) and smooth muscle form tight junctional (nexus) connections (494). Rhodin (494) suggested that myoendothelial junctions provide a relatively direct path for the entry of blood-borne transmitters into smooth muscle cells. It seems more likely to us that local metabolites, hydrogen ion, and osmotically active substances (rather than biogenic amines or peptides) reach and exert their effects on vascular smooth muscle through myoendothelial junctions. Rhodin (494) also suggested that depolarization may be propagated from endothelial cells to smooth muscle; but others have found that endothelial cells do not generate action potentials (197). Electrotonic spread of a graded change in endothelial potential cannot be ruled out, but a region of high myoendothelial electrical conductance, in the absence of rectifying effects, could also create an electrical sink from smooth muscle to endothelium.

E. Nerve-muscle relationships

A relatively long distance between axons and smooth muscle cells is the most characteristic feature of the neuroeffector organization of most, but not all, blood vessels. Nerves frequently do not enter the media (for exceptions see below and sections I G and II D) but are limited to the adventitio-medial junction (14, 18, 62, 127, 241, 362, 461, 471, 472, 490, 603; see also section II D). Although the basement membranes of smooth muscle and the axon-Schwann cell complex may fuse (35, 82), the *shortest* distances recorded between the plasma membranes of unmyelinated axons and smooth muscle are 600 Å in the auricular (18) and the bronchial (14) artery of the rat, 700 to 800 Å in pancreatic arterioles of the rat (637) and 830 Å in those of the guinea pig (362), 950 Å in sheep renal arterioles (548), 1000 Å in the juxtaglomerular vasculature of the monkey (35), 1000 Å in parathyroid arterioles (81, 82) and in mesenteric arteries of the rat (403), 2000 Å in the coronary arterioles (361) and 4000 Å in the main pulmonary artery of the rabbit (603). These values should be compared with the 500 Å synaptic cleft of the motor end-plate of striated muscle (156). Furthermore, the above cited values represent minimal distances: since nerves do not enter the aortic media of any species observed, the maximal axon-smooth muscle separation is of the order of the entire thickness of the aortic media.

There appears to be a more intimate neuromuscular relationship with decreasing vessel size. In terminal arterioles and precapillary sphincters, increasing numbers of axons are seen with decreasing vascular caliber, and *close membranous* contacts between axons and smooth muscle are also present (494).

The majority of nerves discussed are unmyelinated axons enwrapped by Schwann cell processes: the structural equivalent of an adrenergic neuroeffector junction is represented by the region of an axon containing mitochondria and microvesicles of variable size (300 to 1000 Å), denuded of its Schwann cell covering, and facing *one or more* adjacent smooth muscle cells (361, 362, 403, 494, 603, 604, 637). Smooth muscle cells are sometimes seen to send vesicle-containing processes towards nerve bundles (18, 81, 548) and mitochondria are also present in these processes in material fixed in osmium rather than in glutaraldehyde (403). The absence of adrenergic nerve endings from the media of large vessels implies that the processes related to adrenergic release and uptake mechanisms are more predominant in the smooth muscle near to the adventitiomedial junction (see Part II).

The cholinergic fibers of blood vessels have not been studied in as much detail as adrenergic fibers. Naked axons containing vesicles not having granulation or an electron-dense core, in conjunction with the presence of acetylcholinesterase and the absence of demonstrable catecholamines, are considered to represent cholinergic nerves (224, 419). Cholinesterase activity and the uptake of tritiated norepinephrine do not occur together in a single axon, but cholinergic and adrenergic axons can run together (224). The juxtamedullary efferent arterioles and proximal vasa recta in the outer stripe of the kidney medulla are innervated by cholinergic endings at an axon-muscle distance of 500 to 1000 Å (419).

Unusual and probably specialized neuromuscular junctions have been found in the microarteriolar tree (494). These are pale, clublike (0.5 to 2 μ) invaginations of axons into smooth muscle, which are most often seen in the vicinity of precapillary sphincters. The most noteworthy characteristics of these structures are the paucity of vesicles within the pale cytoplasm and the very close apposition (45 Å) between the plasma membranes of the axonal club and the adjacent smooth muscle. If these areas represent fusion of plasma membranes (nexus) they provide the ultrastructural requirement for an electrical synapse: a highly unusual occurrence in vertebrate neuroeffector organization (156). Functional evidence for this speculation could be provided by demonstration of a very short synaptic delay of action potentials (excitatory synapse) or hyperpolarization (inhibitory synapse) transmitted between nerve and muscle. If, in addition, the clublike structures are motor, rather than sensory endings, then adrenergic or cholinergic blocking agents would not abolish the effect of nerve stimulation. As pointed out by Rhodin (494), the connections to these clublike endings have not yet been followed from adventitia to media, and we cannot be certain whether they originate from adrenergic or cholinergic, or even afferent or efferent nerve fibers.

F. Ultrastructural variations: typical, atypical, and specialized vascular smooth muscle; striated muscle in blood vessels

The generalized ultrastructural appearance of vascular smooth muscle, described in the preceding sections, is subject to individual variations. The latter are influenced by three interrelated factors: first, the localization of smooth

muscle to a given segment of the vascular tree and whether, within a given segment, it is subendothelial or near the adventitia; second, the developmental or functional (contractile or morphogenetic, or both) state of smooth muscle; and third, the adaptation of smooth muscle for specialized function.

Aortic smooth muscle cells are distinguished by greater length (90 to 130 μ as compared with 30 to 40 μ in arterioles) and poor development of myofilaments and dense bodies (62, 320, 468, 472). In developing chick embryo aorta (318) and in the aorta of young mice (320) and growing rats (114), the transition can be detected between cells possessing few filaments, many intracellular organelles and a marked rough-surfaced endoplasmic reticulum and the clearly identifiable smooth muscle cells containing parallel myofilaments. A reverse transformation of subendothelial smooth muscle cells into vesiculated and lipid-containing "Langhans" cells (230, 490), also occurs and may be related to atherogenesis (see section III B). In the inner media of the bovine aorta smooth muscle cells with a prominent rough endoplasmic reticulum are associated with elastic lamellae, while "typical" smooth muscle cells and no elastic lamellae characterize the outer media (340). An unusually prominent rough endoplasmic reticulum is present in the smooth muscle of the spiral arterioles of human endometrium (16).

There is a gradual transition from typical smooth muscle cells to pericytes (Rouget cells) between arterioles and metarterioles, and small veins and venules. Pericytes possess numerous cytoplasmic processes, scanty myofilaments, and no dense bodies (174, 427). The presence of a basement membrane, pinocytic vesicles and cytoplasmic filaments indicates that pericytes are related to smooth muscle (142, 280, 405), although the question whether they are contractile is still controversial (for review, see 225). Filament-containing pericytes are found surrounding the lung capillaries of cattle and swine (163), the renal vasa recta of rats (142, 419), and human retinal metarterioles and veins (279, 280). The extensive rough endoplasmic reticulum, free ribosomes, and mitochondria in the pericytes of regenerating tissues (175) and the estrogen-stimulated uterus (190) indicate the morphogenetic role of these cells.

The ultrastructure of the descending vasa recta in the inner stripe of the outer renal medulla is interesting and unusual. Moffat (419) described irregular or stellate perivascular cells surrounding the endothelium of the descending vasa recta. The most characteristic feature of these noninnervated cells is their structural polarization. The filaments, which have the appearance of myofilaments, are localized to an 800 to 1500 Å strip on the side of the cell facing the lumen of the descending vessel. On the opposite side, facing the ascending vessel's lumen, are typical pinocytic vesicles 500 to 600 Å in diameter. There is a gradual transition from the typical vascular smooth muscle of the arterioles, through specialized perivascular cells to the interstitial cells of the renal medulla (419). The ingenious suggestion has been made (419) that the specialized perivascular cells form a feedback mechanism, sampling the fluid domain of the ascending vasa recta and regulating the blood flow to the medulla accordingly.

The granular cells of the renal juxtaglomerular apparatus are a form of specialized vascular smooth muscle that contains secretory granules thought to be

renin (35, 36, 103, 357, 548). The presence of filaments, pinocytic vesicles, and a basement membrane indicate that granular cells are a modified form of smooth muscle.

The striated muscle of thoracic and pulmonary veins is similar to cardiac muscle (319), but, by definition, is outside the scope of this review.

G. Ultrastructure of invertebrate vessels

The lowly earthworm (Phylum Annelida) has a closed circulatory system, lined by myoendothelial cells of varying specialization (243). The myofilaments are 250 Å thick and have the same appearance whether organized in a striated pattern in the ventral vessel or scattered in small numbers within the capillary endothelium. Vesicular infoldings of the plasma membrane of the myoendothelial cells link them with both mammalian endothelium and vascular smooth muscle, while their intercellular attachment *via* desmosomes is a more endothelial characteristic. In the cells of the ventral vessel, myofilaments can be seen running both longitudinally, in the luminal side of the cell, and circularly, within the opposite (outer) part of the cytoplasm. This observation raises a rather interesting question about the nature of the intracellular feedback mechanism that supplies compensatory inhibition between mechanically antagonistic fibrils of the same cell. The blood vessels of the horseshoe crab, *Limulus* (Phylum Arthropoda), contain, in addition to fibrocytes, striated muscle containing the usual two sets of filaments organized into sarcomeres (149).

The blood vessels of cephalopods (Phylum Mollusca) are again not lacking in thick filaments. The following description is based on the studies of Barber and Graziadei (39, 40) on *Octopus* and *Sepia*. The contractile elements are represented by pericytes, which contain thick (150 to 200 Å) filaments and an endoplasmic reticulum. Thick filaments are present in both the outer longitudinal and the inner, circular muscle layer. As in some mammalian vessels, there is a gradual decrease in the number of myofilaments between 15 to 20 μ arterioles and the smaller (type II, III) vessels, which are surrounded by pericytes lacking myofilaments.

The two layers of *Octopus* aortic muscle receive structurally different innervation (40). In the circular muscle the synaptic gap between club-shaped projections of muscle and vesicle-containing axon profiles is narrow (less than 100 Å) and may form tight junctions. The second type of synapse is found in longitudinal muscle and consists of a vesiculated axon sunk into a depression in the muscle: the cleft between pre- and postsynaptic membrane is 80 to 100 Å. The available evidence therefore suggests that both the thick filaments of vascular smooth muscle and the specialized neurovascular connections are relatively early phylogenetic acquisitions, and their absence in mammalian material cannot be regarded as evidence of arrested evolution.

II. LIGHT MICROSCOPY

In addition to extension of the classical light-microscopic literature reviewed by Furchgott (200), new approaches and techniques have been introduced. These include the morphological study of blood vessels fixed under different

degrees of distention and vasoconstriction, the use of histochemical techniques for the localization of enzymatic activity, and fluorescence-microscopic (169) studies of the adrenergic innervation of vascular smooth muscle.

A. Orientation of smooth muscle: development of smooth muscle in response to motion, age, and gravitational forces

The organ- or species-related variations in vascular architecture were recognized by earlier microscopists (for review, see 200). Interest in longitudinal vascular smooth muscle has been increased by electrophysiological studies of the adventitial longitudinal smooth muscle of portal-mesenteric veins (section VI E), and the apparent role of intimal longitudinal muscle in atherogenesis (section III B).

On the basis of an extensive review and his own observations of human blood vessels, Bucciante (87) supported the view that the adventitial, longitudinal smooth muscle of veins develops with age in response to dependent venous pressure. With some individual variations, longitudinal muscle is better developed in the veins of the lower than in the upper extremities. It is very well developed in the spermatic veins and in adult portal veins, and it is the sole muscular coat of the coronary veins and of the adult inferior vena cava. That the development of longitudinal muscle cannot be ascribed solely to postnatal gravitational forces, is illustrated by the predominantly longitudinal muscle of human renal (87, 594) and adrenal veins (595).

The spiral valve of the portal vein contains each layer of the venous wall, with the smooth muscle taking a more circular orientation towards the free edge, and the active contraction of venous muscle imparting a wringing, propulsive motion to the blood (72). It is particularly well-developed in some rodents but, among laboratory animals, absent in dogs (72). Longitudinal smooth muscle is present in both the umbilical vein and the artery in man (574).

Arterial longitudinal muscle, with some exceptions, develops as an intimal coat, in contrast to the medio-adventitial longitudinal muscle of veins. A well-developed adventitial layer of longitudinal muscle is characteristic in the anterior mesenteric artery of the fowl and the turkey (34). In addition to the intimal longitudinal arterial muscle associated with vascular pathology (section III B), longitudinal muscle also appears more prominent in arteries subjected to longitudinal traction, such as the bronchial and cardioaortic arteries, the arteries of the bladder, stomach, esophagus, and penis (353, 614), and the arteries of benign cysts and adenomas (100). Experimental production of longitudinal muscle in mesenteric arteries of the rat has been accomplished by connecting these with a thread to the diaphragm (100, 614), thus subjecting the vessels to the traction of respiratory movement. The development of longitudinal muscle was accompanied by mitotic figures in the media, where smooth muscle cells had rounded up and were migrating to the intima (614). Similar changes are also seen after other forms of injury to the vessel wall (section III B) and therefore cannot be considered entirely specific responses to the diaphragmatic movement.

In the giraffe the enormously thick muscular coat of the metatarsal arteries

and the relatively thin wall of the carotid artery at the angle of the jaw are good examples of the different development of muscular arteries in response to functional conditions: the pedal hydrostatic pressure alone may be close to 500 mm Hg in this animal (217).

Elastic tissue production by the smooth muscle of the arterial Windkessel appears to be stimulated by high pulse pressures: the aorta of an acardiac twin parasite, which does not receive the stroke volume of cardiac systole, has the structure of a muscular artery (21). Postnatal regression of the elastic lamellar structure occurs normally in the main pulmonary artery, but the elastic Windkessel persists in the presence of pulmonary hypertension (259, 500). Progress of elastogenesis has been observed in tissue culture, however, and is reportedly improved by the addition of Factor P (O-β-hydroxyethylrutosidea) to the growth medium (101).

In the so-called hybrid vessels, such as the major branches of the aorta, the outer portion of the media has the appearance of an elastic vessel and the inner portion, that of a muscular vessel (404). It seems reasonable to propose that in these vessels the smooth muscle fibers of the two areas are also functionally differentiated: the outer layer for a morphogenetic, the inner for a contractile role.

The variable morphology of the vascular wall may be further illustrated by reference to the vertebral aspect of the dorsal aorta of the carp, where a fibrous raphe completely replaces the scanty smooth muscle (146), and to the bovine pulmonary veins, which have a considerably thicker media than the corresponding arteries (9).

B. Structural effects of vascular distention, collapse, and constriction

The morphology of arteries fixed under physiological distending pressures differs from that of collapsed arteries (93, 183, 413, 631). In the collapsed elastic vessel the elastic lamellae are wrinkled, the smooth muscle runs obliquely between adjacent lamellae, and the radius/wall thickness ratio is relatively low. In the distended aorta the lamellae are straight (93, 631), smooth muscle cells run a more circular course (632), and the wall thickness is reduced to less than one half of that of the collapsed vessel (631). These changes in turn affect the elastic properties of the vascular wall and the contribution of smooth muscle to wall elasticity (see section V D).

Distention of muscular arteries converts an irregular cross section of muscular polygons outlined by elastic fibrils to a regular array outlined by parallel elastic fibrils, resembling in miniature the arrangement of the elastic lamellae of major elastic arteries (183). The suggestion (258) that "passively" collapsed small arteries cannot be distinguished from those actively contracted by epinephrine seems to hinge upon the definition of passive collapse. If a vessel has active tone, allowing it to "collapse" by removing its contents is analogous to unloading an isometrically contracting muscle. This procedure permits active shortening rather than passive collapse due to an external restoring force. This is also apparent from the fact that collapsed vascular segments excised from living animals contract more than similar vessels obtained post mortem (93).

Quick-freezing of small mesenteric arteries that have been constricted with topically applied epinephrine has been employed by Van Citters *et al.* (599–601) to define some of the architectural changes occurring during vasoconstriction. Complete closure of vessels whose constricted outer diameter was 50 μ or less could be produced by intense vasoconstriction, closure itself being due to endothelial infolding. Asymmetrical vasoconstriction of small arteries could be limited to the side of application of epinephrine without being propagated to the opposite side within the same cross section (600). Vasoconstriction changed the radius/wall thickness ratios of dilated small arteries from 10/1 to 1.3/1 and of dilated arterioles from 5/1 to 0/25 (occluded). These findings raise the question whether, in the theoretical treatment of vascular elasticity, small arteries can be arbitrarily classified as either thin- or thick-walled vessels.

A valuable light-microscopic index of the individual smooth muscle cell's state of shortening is provided by the appearance of the nucleus. The nuclear length/diameter ratio of relaxed muscle (6/1 to 8/1) decreases to 1/1 (rounded and convoluted nuclei) in fully contracted vascular smooth muscle (601). The authors have also described the gradual transition of vascular calibers from the constriction at the point of application of epinephrine to the dilated vessel beyond the range of drug diffusion (600). A significant finding, not stated but apparent from the illustrations published, is the fact that graded vasoconstriction is associated with a continuous spectrum of nuclear length/diameter ratios, rather than a varying admixture of completely relaxed and fully contracted nuclear profiles. This finding suggests to us that the contractions of individual smooth muscle cells are graded.

C. Histochemistry and tissue culture

The histochemical approach permits localization of enzyme activity, not attainable by conventional chemical studies of minced tissues (see section IV F). Those of us advocating the view that the vascular smooth muscle of a single vessel may be heterogeneous (554, 557) receive support from differences in the ultrastructural (section I F) and histochemical appearance between smooth muscle cells of the same artery (see below), although there is no evidence at this moment that histochemical differences correlate with pharmacological responsiveness. It is also possible that these differences are not genetically coded but the expressions of different functional influences such as the adventitiomedial location of adrenergic nerves and the high pulse pressures within the thoracic aorta.

The ATPase activity of beagle aortas (269, 270) reveals a radial and an axial gradient of activity. Enzyme activity is greater in the outer than in the inner media at any given level and it also decreases caudally. There is very little histochemically demonstrable ATPase in the aorta below the level of the renal arteries, although muscular branches of the abdominal aorta show strong activity. There are species differences in the aortic ATPase gradients. There is a uniform distribution of ATPase along the entire aorta in children while the enzyme is reported to be completely absent from the feline subdiaphragmatic aorta (518). In view of the universal functional importance of membrane ATPase,

it is very probable that different reactivities of the ATPase, rather than true enzyme deficiency, is responsible for some failures to demonstrate its presence histochemically. Ultrastructural localization of ATPase activity to the vicinity of the pinocytic vesicles and the basement membrane of smooth muscle (278, 521) is compatible with the notion that the enzyme visualized is a membrane ATPase. The extracellular localization asserted in these studies (278, 521) has to be reevaluated in view of the recent questions raised about localizing ATPase activity with lead precipitation methods (216). The extracellular localization of vascular ATPase may have been due to preferential binding of lead to the mucopolysaccharides of ground substance and to elastic laminae.

Alkaline phosphatase activity is generally absent from the muscular coat of blood vessels. It is present in the adventitia, where it persists during prolonged incubation in tissue culture (168). The alkaline phosphatase activity of the adventitio-medial junction has been attributed to the net of vasa vasorum, to lymphatics, or to active fibrillogenesis (562, 579). Alkaline phosphatase activity is demonstrable in lathyritic smooth muscle cells (section III C). Acid phosphatase has been demonstrated in smooth muscle cells of the rabbit aorta (365).

Succinic dehydrogenase was reported to be absent in one study (508) but other workers demonstrated it in vascular smooth muscle (297, 562). The reported variations may be real or caused by differences in technique: in one study succinic dehydrogenase activity was present in block-incubated sections but not in frozen sections of rat aorta (562). There is an increase in the succinic dehydrogenase activity of myometrial blood vessels during pregnancy (296).

The enzymes concerned with glycogen synthesis and breakdown, uridine diphosphate glucose (UDPG)-glycogen transferase and phosphorylase, have not been investigated in sufficient detail to permit generalization of their occurrence in vascular smooth muscle. In blood vessels of the rabbit tongue, phosphorylase activity is said to be moderate and UDPG-glycogen transferase difficult to demonstrate, whereas in pulmonary blood vessels only phosphorylase is histochemically demonstrable (587). In the rat aorta phosphorylase activity is said to be absent (562) while both this enzyme and UDPG-glycogen transferase may be detected in uterine arteries of mice (240). At present, there are insufficient data to indicate whether the UDPG-glycogen transferase system is the major pathway of glycogen synthesis in all types of vascular smooth muscle, as it is in the liver. There is some evidence that the physicochemical state of glycogen itself is variable among different blood vessels: it is stained by acid carmine in veins, but not in the arteries of the guinea pig (196). The absence of glycogen granules from modified smooth muscle cells will be discussed later (sections III B and IV C). The regulation of glycogen synthesis by catecholamines has been investigated by biochemical methods (section IV C). Histochemical evidence of increased glycogen production in vascular smooth muscle of estrogen-treated uteri has been reported by some (550) and denied by others (240).

Cholinesterase is absent from vascular smooth muscle of fetal ductus arteriosus

of rodents and man, whereas the media of the adjacent aorta and main pulmonary artery stains for this enzyme (284). The cholinesterase activity of rabbit aorta is due to both true and a nonspecific cholinesterase (639).

Lipase, cytochrome oxidase, 5-nucleotidase, aldolase, and amino peptidase are some of the other enzymes that have been demonstrated histochemically in blood vessels (365, 562, 579). The carboxyl esterase activity of the rabbit aorta increases rapidly towards the adventitia (365).

In tissue cultures of rabbit mesenteric arterioles, which may undergo spontaneous contractions for up to 5 days in culture, glucose-6-phosphate dehydrogenase activity disappears in 3 days (168). Malic and lactic dehydrogenase persist, at diminished levels, for at least 7 days (168). The outgrowth of aortic smooth muscle cells in culture has been studied by Pollak and Kasai (479), who found this to be present from the 10th day of culture. The integrity of the smooth muscle of chick embryo blood vessels in culture is reported to require Factor P (101, 102).

It seems to us that further application of histochemical and tissue culture techniques to studies of vascular smooth muscle would be most desirable. Determination of the effect of drugs on vascular enzyme activity appears to be a productive line of inquiry. It would also be of considerable interest to determine whether smooth muscle cells in tissue culture retain their sensitivity to vasoactive agents: logical extension of these studies could yield further insights into the nature of receptors and the means whereby genetic information is conveyed for the coding of receptors.

D. Nerve plexuses and vasa vasorum

The only valid generalization regarding the distribution of adrenergic innervation to blood vessels is that nerve fibers enter from the adventitia, whence they proceed to penetrate to a variable distance towards the lumen. The nerve ground plexus is located within the adventitia. Nerve endings in several large vessels do not penetrate further than the outer surface of the media: this preferential distribution of adrenergic storage and release sites is of considerable functional significance (see Part II). Considerable variations exist not only in the innervation among blood vessels of different species (159, 184), but even within different segments of the same vessel. In elastic arteries adrenergic nerves are usually limited to the outer one third of the vessel; in muscular arteries they may penetrate for a short distance into the media; and the media of some of the thick-walled cutaneous veins contains many adrenergic fibers (159). In the proximal part of the main pulmonary artery of the rabbit, fluorescent nerve endings terminate on the outer surface of the media, while in the distal part of the same blood vessel nerves may penetrate close to the intima (107). The fluorescence studies also support the functional evidence for the paucity of adrenergic innervation of the veins in skeletal muscles (159, 203).

Injected norepinephrine has been visualized in rat femoral arteries, with the aid of inhibitors of monoamine oxidase and catechol-O-methyltransferase,

as a diffusely fluorescent material (145). A diffuse fluorescence, indicating medial diffusion of norepinephrine, has also been shown after stimulation of adrenergic nerves *in vivo* (213).

Cholinergic vasodilator fibers have been demonstrated with cholinesterase stains in blood vessels supplying muscles (68). An extensive compilation of the earlier, particularly the Russian, literature can be found in Grigor'eva's (225) monograph on the innervation of blood vessels.

The existence of vasa vasorum in the aorta has been correlated with body weight and aortic wall thickness in different species (633). In species whose adult body weight does not exceed 5 to 6 Kg, no aortic vasa are present. In larger animals, including man, the avascular zone is relatively constant at 0.4 to 0.5 mm or about the inner 29 medial lamellar units (633). In keeping with other general rules, exceptions have been reported, such as the extension of vasa to the intimo-medial junction of the cow aorta (534). The thickness of the avascular layer, which has to be supplied with O_2 by diffusion (primarily from the endothelial surface), and the rate of O_2 consumption determine the critical Warburg distance in blood vessels (413). Ligation of several canine intercostal arteries, which supply a large proportion of the vasa vasorum, results in necrosis of the middle one third of the aortic media (629). The functional avascular zone (about 0.3 mm; see figs. 4 and 10 of 629) illustrated in this study (629), is somewhat narrower than estimated by inspection of normal arteries (633).

III. PATHOLOGICAL STRUCTURE

The recognition of the role of smooth muscle in vascular morphogenesis (section I C) made it apparent that disorders of vascular connective tissue may be secondary to disturbances of vascular smooth muscle. This, and the ultrastructural evidence of very early involvement of smooth muscle in the atherosclerotic process (section III B), are recent discoveries of practical importance, suggesting new avenues of pharmacological inquiry. In presenting the following brief review, our major purpose is to call the attention of pharmacologists to the chronic pharmacology of vascular smooth muscle. A more complete treatment of the vascular wall and its diseases, including atherosclerosis, may be found in the several monographs and symposia published in recent years (1, 315, 354, 457, 499, 519).

A. Aging

There are three major aspects of the effect of aging upon vascular smooth muscle: the appearance of degenerative changes within smooth muscle itself, the alterations in the connective tissue components that are normally produced by vascular smooth muscle, and the migratory and proliferative changes of smooth muscle within the intima.

In the human aorta, smooth muscle cells become vacuolated and more deeply stained with increasing age (5). Those smooth muscle cells which have irregular nuclei give a strong histochemical reaction for choline-containing phospholipids (64). In the stretched aorta of older guinea pigs the nuclei are thinner within

the adventitial than in the inner part of the media (572), and in unstretched aorta the medial smooth muscle is more irregular and the nuclei more tortuous (572, 573). The histochemically demonstrable lactic dehydrogenase increases and the $NADH_2$-tetrazolium reductase activity decreases in the aging human aorta (4).

The impairment of the morphogenetic function of vascular smooth muscle by aging is manifested by an increase of connective tissue elements, particularly collagen, relative to smooth muscle (186, 573). Other changes include a decrease in the ratio of hyaluronic acid/chondroitin sulfate of the ground substance (45, 52–54) and fragmentation of the elastic lamellae (54, 573). The functional result of these alterations is the decrease in vascular elasticity with age (425, 498). A specialized example of hormonal influences on aging is the phagocytosis of the elastic elements of mesometrial arteries by smooth muscle during gestation (6, 85) and the postpartum formation of new elastic lamellae, increasing in numbers with increasing parity (7).

The third major manifestation of aging is the subendothelial proliferation of smooth muscle leading to intimal thickening. It is still argued, and with some justification, that at branching sites the valve-like intimal cushions containing smooth muscle are normal, rather than pathological, structures (411). In view of the vulnerability of both localized and generalized intimal thickenings to atherosclerosis, however, these changes will be discussed as part of the atherosclerotic process.

B. Atherosclerosis

According to a number of investigators, atherosclerosis, particularly coronary atherosclerosis, is a disease beginning in infancy, progressing rapidly during the first and second decade of life, and becoming symptomatic in the adult. The evidence for this point of view has been reviewed in detail (409). Modified smooth muscle cells (132) infiltrate the coronary arterial intima as early as the first month of life. Intimal thickening, accompanied by hyperelastosis and splitting of elastic lamellae, involves the entire vascular circumference after the first decade (63). Intimal proliferation is greatest in regions of greatest motion (74; section II A), at vascular branchings (409, 577), and appears to be a precursor of atherosclerosis. The intimal thickness that is accompanied by smooth muscle necrosis is variable: in one series necrosis was always observed when the intimal thickness of (formalin-fixed) coronary arteries reached 0.83 mm (132).

Electronmicroscopy of spontaneous and induced lesions has proved the involvement of smooth muscle in atherosclerosis. Ultrastructural criteria identifying atypical intimal cells as smooth muscle are the presence of myofilaments, dense bodies, pinocytic vesicles, and a basement membrane (33, 89, 90, 119, 132, 255, 266, 301, 340, 409, 462–464, 581). The modification of typical smooth muscle cells involves progressive dilatation of the endoplasmic reticulum and an accompanying increase of ribosomelike particles, vacuolation and increase in the number of mitochondria, a prominent Golgi apparatus, and crowding of

myofilaments towards the periphery of the cell (89, 90, 132, 266, 301, 340, 463, 464, 581). The modified smooth muscle cells contain no glycogen granules (301). Lipid droplets appear within smooth muscle cells before the appearance of extracellular lipids and the increasing lipid infiltration with progressive loss of myofilaments may transform smooth muscle into foam cells, although the latter may also arise from other cell lines (33, 90, 209, 301, 340, 368, 463, 464, 581). Migration of medial smooth muscle cells through the internal elastica has also been observed (301, 464, 515). Even in the turkey aorta, which contains a dual population of cells, fibroblasts and smooth muscle, the latter predominate in atherosclerotic plaques (546).

It must be emphasized that the early modifications of smooth muscle cells are not specific manifestations of the prelipid stage of atheromatosis. Prominence of the dilated endoplasmic reticulum, ribosomes, mitochondria, and Golgi apparatus, at the expense of peripherally crowded myofilaments, are common ultrastructural features of smooth muscle in vessels injured by freezing, ligation, proteolytic enzymes, incision, abrasion, Dacron prosthesis, and irradiation (89, 133, 434, 435, 456, 582, 589). The presence of similar cells in normal embryo aortas (section I C) suggests that these modifications of smooth muscle cells can also reflect their participation in the healing process.

Lipid-induced changes have also been demonstrated in tissue cultures of vascular smooth muscle. Recognizable smooth muscle cells from explants of atheromatous plaques have poorly staining myofilaments and migrate later into the culture medium, as compared to normal aortic cultures (321, 479). The outgrowth of other cells appears earlier in atheromatous cultures (345, 436). The possibility cannot be ruled out that the early migrating cells of plaques are modified smooth muscle cells no longer recognizable under the light microscope. Lipemic sera reportedly produce degenerative changes in rabbit (436) but not in human aortic cultures (437). However, the cholesterol concentration of the culture medium used for rabbit was considerably higher than the one used for human cultures.

The fragmentation of the elastica and the histochemical alterations of the elastic and ground substance in atherosclerotic vessels (2, 20, 79, 464) can now be understood as additional manifestations of a chronic disturbance of the smooth muscle responsible for their production. The accumulation of sphyngomyelin upon elastic lamellae of atherosclerotic vessels (2), for example, is associated with an increase in the choline-containing phospholipids of smooth muscle (64). Within this context we may regard the low ratios of hyaluronic acid/chondroitin sulfate in vessels that are particularly prone to atherosclerosis (396) as expressions of differences in the synthetic function of smooth muscle.

Extensive histochemical studies of atherosclerosis have been conducted and reviewed by Sandler and Bourne (519) and by Zemplenyi and Lojda (365, 639) and their co-workers. There is no clear-cut pattern detectable in smooth muscle, perhaps because of species variations and the difficulties inherent in quantitative evaluation by visual methods. Thus, while a patchy decrease in ATPase and

5-nucleotidase activity of otherwise normal-appearing smooth muscle has been described in atheromatous human aortas (517, 519) and in aortas of cholesterol-fed rats (266, 519) and dogs (519), there is no clear-cut decrease in the activity of atheromatous rabbit aorta (639). The lipolytic activity of the aorta, subject to aging and atherosclerotic changes, has been studied by chemical methods (section IV E).

The comparative aspects of atherosclerosis have been discussed recently in the proceedings of an extensive symposium (499). It is probable that at least some of the vascular disorders of different species are due to more than a single etiological entity. For example, the lesions of intramyocardial coronary arteries of chickens, accompanied by proliferative changes and periodic acid-Schiff positive vacuolation of smooth muscle (489) bear some resemblance to the hereditary medial necrosis of small coronary arteries (305) of man.

Because of the well-established (but not universal) correlation between hypercholesterolemia and human atherosclerosis (88), therapy has been generally directed towards controlling serum cholesterol levels. The importance of local vascular factors is indicated by such findings as the increase in atherosclerosis associated with triparanol-induced lowering of plasma cholesterol (384) or the inhibition by insulin of the regression of atherosclerotic lesions (575). Two points of pharmacological intervention are suggested by the nature of the atherosclerotic changes in smooth muscle. First, the stimulus for intimal migration of smooth muscle cells during the first two decades of life should be determined in order to prevent or perhaps reverse intimal thickening. This suggestion rests upon the assumptions that the intimal thickenings are not unavoidable results of maturation and that they are sites of predilection for the development of atherosclerosis. The second therapeutic approach suggested is the development of compounds that can create a more advantageous local balance of vascular lipid metabolism. This could be accomplished either through decreasing lipid uptake or synthesis by vascular smooth muscle or through the stimulation of vascular lipolytic enzymes. This approach lends itself to screening methods *in vitro* and deserves exploration in view of the apparent reversibility of the early fatty lesions of the vascular wall (322).

C. Disorders of morphogenetic function: lathyrism, idiopathic (human) medionecrosis, copper deficiency

The feeding of sweet peas, or their active ingredient, *beta* aminoproprionitrile, induces marked abnormalities in the connective tissue elements of the rat aorta. The production of abnormal elastin and collagen and accumulation of mucinous material are indirect evidence of vascular smooth muscle dysfunction (242, 286, 328, 397). The ATPase and AMPase activity of aortic smooth muscle and the vascular uptake of S^{35} and tritiated L-proline are increased, but these changes are probably not specific (112, 455). The medial smooth muscle cells of lathyritic aorta assume a radial orientation and become alkaline phosphatase positive (286). Ultrastructural abnormalities include separation of muscle from the elastic

lamellae and the appearance of smooth muscle cells that contain a widely dilated endoplasmic reticulum with an increased number of ribosomes, mitochondria, and nucleoli and a prominent Golgi apparatus in conjunction with a decrease in myofilaments (242, 328). These changes, suggestive of the morphogenetically active embryonic smooth muscle (section I C) are followed by the appearance of rounded cells suggestive of degenerative change (242). Rounded up smooth muscle cells and some changes in histochemically active ATPase have also been described in human cystic medial necrosis (395). A spontaneous dissecting aneurysm occurs in turkeys and its incidence is markedly increased by high doses of estrogen (347, 545).

Copper deficiency leads to abnormal ultrastructure of the myoblastic cells of chick embryo aortas, associated with marked abnormalities in aortic elastogenesis (547). Copper deficiency in young swine results in a decrease in total, and a marked increase in soluble aortic elastin (615). It has been suggested that the common defect in lathyrism and Cu deficiency is an inability to incorporate lysine into desmosines, with the resultant failure of cross-link formation in elastin (418).

D. Hypertension

True hypertrophy of smooth muscle, as manifested by increased incorporation of tritiated thymidine, has been demonstrated in adult rats made hypertensive with desoxycorticosterone and salt (122). Increased DNA synthesis was evident in nuclei of arterial and arteriolar smooth muscle but not in venous smooth muscle or in the aorta. Similarly, the increase in vascular smooth muscle mass encountered in experimental renal hypertension is detectable in mesenteric and coronary arteries but not in the aorta (181). Quantitative methods suggest that hypertrophy of pulmonary arterial muscle occurs in the pulmonary hypertension of cattle induced at high altitude (10, 11), and that pulmonary vascular muscle mass decreases during the neonatal period (438). The ultrastructure of pulmonary arterial smooth muscle, in experimental pulmonary hypertension, undergoes a transformation suggestive of morphogenetic activity and associated with an increase in collagen (164). These findings confirm the classical views regarding the development of hypertrophy in at least some types of pulmonary and systemic hypertension. Mitotic activity in adult, normotensive animals is rare, but can be demonstrated after infusion of tritiated thymidine (563, 571).

The most distinct, although not specific, histochemical alteration of vascular smooth muscle in experimental hypertension is the increase in AMPase activity, which precedes fibrinoid changes (453, 454). AMPase activity is increased not only in arteries and arterioles, but also in the aortic smooth muscle of young hypertensive animals (454). The previously mentioned studies (122) showing no increase of thymidine incorporation into the hypertensive aorta were conducted on adult animals. Therefore, dissociation of AMPase activity and DNA synthesis in the aortic smooth muscle of hypertensive animal should not be inferred.

Fibrinoid necrosis of uterine (basal and spiral) arteries occurs in pre-eclampsia and eclampsia, unaccompanied by similar changes in other vascular beds (502).

E. Spontaneous aortic lesions in rabbits and vascular lesions induced by chemical agents

The incidence of spontaneous medial sclerotic lesions of the aorta varies among different breeds and may be present in up to 50% of "normal" adult rabbits (528, 561). The spontaneous arteriosclerotic lesions of the thoracic aorta are morphologically indistinguishable from the late sequelae of the hyalin necrosis of medial musculo-elastic layers produced by epinephrine or nicotine (51, 227) or by the injection of heterologous aortic homogenates (525). Electronmicroscopic studies of the nicotine-induced lesions revealed early abnormalities of smooth muscle, which become rounded and develop a dilated endoplasmic reticulum and cytoplasmic diverticula in later lesions (227).

Necrotizing or hyalinizing lesions of blood vessels have been induced by cortisone and estrogen, allylamine, monocrotaline, and methylcellulose (22, 117, 412, 578). Medial arteriosclerotic lesions can be produced in the rat aorta by dietary deficiencies and by certain compounds (148).

IV. BIOCHEMISTRY

A. Muscle proteins

The contractile proteins of vascular smooth muscle are closely related to those of other mammalian smooth muscles and, in spite of some differences to be described below, to those of skeletal muscle. The general biochemistry of muscle proteins has been the subject of recent symposia and reviews (147, 210, 211, 256). Reference to these will place the narrower field of vascular smooth muscle proteins within the context of the extensive work conducted on skeletal muscle.

The observation of Ivanov *et al.* (303) and of Laszt and Hamoir (356) that the major contractile protein of smooth muscles can be extracted with solutions of low ionic strength ($I < 0.2$) has been repeatedly confirmed (180, 206, 244–246, 249, 291–293, 300, 446, 510, 531). Although skeletal actomyosin can be extracted only by solutions of high ionic strength, there is now sufficient reason to conclude that the protein extracted from vascular and other smooth muscles, at low ionic strength and pH 7 and in the presence of traces of ATP, contains an actomyosin, and furthermore the only actomyosin present in smooth muscle. The evidence for this is the following: 1) thorough and repeated extraction with solutions of low ionic strength produces all the extractable contractile proteins; further extraction of the residue with solutions of high ionic strength produces no additional actomyosin; and the quantitative yield of the two methods is identical (510); 2) the superprecipitation reaction, viscosity, ATP sensitivity, and ATPase activity of *purified* low- and high-ionic-strength extracts are identical (180, 293, 510, 531). The designations tonoactomyosin and tonomyosin, originally applied to low-ionic-strength extracts of smooth muscle (356) are therefore no longer employed (292). The relative amounts of actomyosin and myosin obtained may vary with the ionic strength of the extracting solution (390).

The total actomyosin content of smooth muscle is relatively small, usually

between 2 and 10 mg/g wet weight, depending upon the type of smooth muscle and the purification of protein (38, 66, 116, 180, 391, 446, 510, 531), compared with about 70 mg/g in skeletal muscle (446). The fact that vascular smooth muscle can develop considerably more than one seventh of the tension developed by striated muscle (section V A) suggests a greater efficiency of the contractile mechanism. This suspicion is also supported by the finding (526) that the tension developed/ATP split in glycerinated hog carotids is considerably greater than found in a number of other glycerinated muscle preparations.

The extraction of the smooth muscle actomyosin at low ionic strength is facilitated by trace amounts of ATP (244, 246, 248, 293, 300, 510). Unlike skeletal muscle actomyosin, which in solutions of low-ionic strength exists as an associated, Mg-activated ATPase (211), smooth muscle actomyosin is dissociated by ATP and exhibits predominantly Ca-activated myosin ATPase activity (246, 391, 531).

Lowering of the pH to 6.0 leads to association of vascular actomyosin in solution, with consequent Mg-activated ATPase activity, and to a marked decrease in the extractability of the protein from glycerinated vessels (531). The high solubility of smooth muscle actomyosin and myosin at pH 7 may have been responsible for the difficulties in electronmicroscopic visualization of thick filaments in smooth muscle (section I B).

Vascular myosin ATPase is activated by Ca and this Ca-activated enzyme is inhibited by Mg (206), as is skeletal muscle myosin. Vascular myosin is more thermostable than the skeletal myosin (206, 245). At pH 6.2 (I 0.35) vascular myosin precipitates, while rabbit skeletal myosin remains in solution (293).

The amino acid compositions of the myosins of various smooth muscles differ from each other and from those of striated muscle myosin (38, 248, 292, 293). Tryptic digestion of skeletal myosin produces two fragments, L-meromyosin and H-meromyosin, the latter containing the ATPase activity (211). Tryptic hydrolysis of smooth muscle myosins is slower, but also yields the two meromyosin fragments (116, 291, 292). The comparatively low ATPase activity of (uterine) smooth muscle myosin increases markedly during tryptic digestion, and the uterine acto-H-meromyosin ATPase activity is of the same order of magnitude as that of skeletal muscle acto-H-meromyosin (446).

Vascular F-actin can be obtained either through dissociation of the actomyosin, with Mg-ATP, into myosin and F-actin combined with tropomyosin B (207a, 531) or by the polymerization of an acetone-dried powder of G-actin, in the presence of Mg, to F-actin (510). The transformation G-ATP $\rightarrow$ F-ADP is similar to that observed with skeletal muscle actin (211, 256), but polymerization and depolymerization proceed more slowly (207). The depolymerization of F- to G-actin is probably incomplete (207a). Vascular actin combines with skeletal muscle myosin to form a hybrid actomyosin, with the usual properties of a Mg-activated ATPase that superprecipitates at low ionic strength (510, 531). Gaspar-Godfroid *et al.* (207) found that vascular actin, prepared by methods suitable for skeletal muscle actin, is heavily contaminated with tropomyosin B. Using Carsten's method, they obtained a yield of 0.6 to 1.2 mg actin/g arterial

tissue, containing at most 20% tropomyosin B. Any of the current methods of isolation yields an F-actin that remains closely associated with tropomyosin B, although G-actin can be isolated without tropomyosin B (207a).

The functional significance of tropomyosin (342) has been only recently recognized, primarily through the efforts of Ebashi *et al.* (153–155). Native tropomyosin, a complex of troponin and tropomyosin B, is intimately associated with actin filaments and regulates the effect of Ca upon actomyosin, conferring the Ca requirement for superprecipitation upon purified reconstituted actomyosin. Troponin in fact appears to be the receptive site which combines with Ca, 4 moles of Ca combining with 100,000 g troponin obtained from skeletal muscle (153a, 154). Ebashi's group also produced evidence that the native tropomyosin isolated from smooth muscle of the gizzard has essentially the same activity as that isolated from skeletal muscle (155). The chemical structures of the tropomyosins of smooth and skeletal muscle are somewhat similar, both having two free-SH groups per molecule, but their amino acid compositions and crystalline forms are different (105, 342). A third of the tropomyosin B content of cow carotids can be extracted with a solution of low ionic strength that would yield only a few percent of skeletal muscle tropomyosin (247). The tropomyosin B/actomyosin ratio is much higher in smooth than in striated muscle (155, 247, 446). This may contribute to the wider range of the Ca "dose-response curves" of glycerinated smooth muscles, compared to striated muscle (526). The Mg requirements for contraction of glycerinated strips (179) and for superprecipitation of recombined vascular actomyosin are also higher than those of the skeletal muscle protein (531). The high Mg requirement may be inherent in vascular actomyosin or conferred to it by an auxiliary protein.

Another protein closely associated with actin is *alpha* actinin; this protein stimulates network formation of F-actin and superprecipitation of actomyosin solutions (153, 256). *Alpha* actinin has been isolated from chicken gizzard (155). A related protein, *beta* actinin inhibits network formation by F-actin and keeps the length of actin filaments between 1 and 2 μ (153, 400), but we are not aware of any reports regarding the isolation of *beta* actinin from smooth muscle.

Tropomyosin A is a protein extracted from some molluscan muscles, and it has been suggested that this protein is responsible for the very high tension supported by molluscan catch muscles. Several unsuccessful attempts to isolate tropomyosin A from mammalian smooth muscle indicate that it is absent (105, 446, 510) and therefore cannot be implicated in the maintenance of tone.

There is some incomplete evidence for a difference in the extractable protein composition of the different vascular beds. The electrophoretic behavior of an extract of bovine coronary arteries is somewhat different from that of the aortic and femoral artery extracts (249). Raising the potassium concentration (to 0.5 to 0.6 M) does not increase the ATPase activity of actomyosin obtained from human umbilical artery, although this effect is observed with a number of other smooth muscle actomyosins (392). Furthermore, while Ca, Ba, and Sr stimulate the ATPase of bovine aorta and carotid artery, Ba and Sr do not increase the ATPase activity of umbilical artery protein (392). In view of Ebashi's more

recent work (154) low Sr sensitivity may be due to differences in troponin rather than in actomyosin structure. In the presence of small amounts of ATP, bovine venous protein is more easily extractable than arterial (244). After hydrolysis of ATP the vein actomyosin becomes much more inextractable. In the light of the recent findings of Kushmerick and Davies (350), that the appearance of rigor mortis in skeletal muscle requires the absence of both ATP and ADP, analysis of the latter nucleotide in vascular smooth muscle would be of interest. It may well be that the difficult extractability of venous smooth muscle actomyosin is due to the formation of rigor links. A related question to be explored is whether the solubilizing action of ATP upon vascular smooth muscle in media of low ionic strength is specific or also exhibited by ADP and other polyphosphates (see also section IV D).

As noted earlier (section I A), bovine uterine and vascular myosins are antigenically different. Further evidence is required to determine whether there is more than one vascular smooth muscle myosin in a single species. According to Bárány (37), the myosin ATPase activity of different muscles is directly proportional to their speed of shortening. Since two types of vascular smooth muscle, one spike-activated, the other gradedly responsive, have been demonstrated in different vessels within the same species (557), a comparison of their respective ATPase activities would be of interest.

B. Oxygen consumption

The reported values of oxygen consumption by isolated vascular segments vary considerably. It has been suggested (358) that metabolic activity should be expressed on the basis of DNA because the usual manner of expressing oxygen consumption (Q_{O_2} as μl O_2/mg dry wt/hr) is influenced by variations of extracellular solids. The functional state of vascular smooth muscle, whether contracted or relaxed, would be another obvious determinant of O_2 consumption. The ability of nitrites to lower the Q_{O_2} in some vascular strips but not in others (78) may merely reflect the increased Q_{O_2} associated with pre-existent tone in the former. Under these circumstances vasodilators would, by abolishing tone, establish a more truly resting state. A less obvious source of error due to metabolic activity suggests itself in surveying the experimental techniques employed for measurement of Q_{O_2}. In a number of studies reviewed, the adventitia was stripped from vascular segments that were dissected in "ice-cold saline" or kept for several hours under refrigeration. Under these conditions, vascular smooth muscle accumulates large amounts of sodium (237), which it subsequently extrudes, at higher temperatures, against an electrochemical gradient (section VI A). Operation of this sodium pump is an active, energy-requiring process, and would be expected to be associated with an oxygen consumption above basal levels. This interpretation is consistent with earlier observations (334, 621) that if refrigerated aortas were allowed to warm before being placed in the Warburg apparatus, the oxygen consumption dropped precipitously. We would therefore recommend gentle dissection and abandonment of cold solutions in preparing vascular strips for metabolic studies.

The earlier reports of Q_{O_2} values of several vessels of different species ranging from 0.31 to 1.14, have been summarized in Lehninger's review (358). In a recent study, the Q_{O_2} of rabbit aorta and rabbit liver were found to be, respectively, 0.72 and 5.58 (264). In view of the absence of significant tone in rabbit aortic strips, already emphasized by Furchgott (200), the Q_{O_2} (0.5 to 0.9) of this preparation (108, 264) is probably close to a true resting value. Aortic oxygen consumption tends to be smaller with increasing size of animal species (262, 358) and with increasing age (113, 262, 420, 617).

The oxygen consumption of the different aortic segments is unequal: it is lower in the abdominal than in the thoracic aorta of the rat (113, 307, 483), dog (379), and rabbit (108). The differences in the oxygen consumption of different rabbit aortic segments are accentuated when succinate is the substrate (108), and succinic dehydrogenase activity also parallels the segmental Q_{O_2} of the canine aorta (379). Unlike the whole segments, the intimal layers isolated from different aortic segments consume similar amounts of oxygen (621).

Endogenous respiration may proceed without added substrate and without being augmented by addition of glucose to the incubation media; this probably reflects utilization of endogenous glycogen (108, 264). Thin layers of rabbit aortic intima, however, consume no or very little oxygen without added substrate (621, 622); this may be due to a lack of glycogen in intimal smooth muscle (section III B). Aortas removed from fasting rats also show no spontaneous respiration without added substrate (607).

Among the various Krebs' intermediate metabolites tested, succinate seems to produce the greatest increase in oxygen consumption of vascular smooth muscle: about 2- to 7-fold of the resting values (78, 108, 264, 621). The stimulation of respiration by succinate has also been observed in a particulate (probably mitochondrial) fraction of pig aorta (306).

Norepinephrine increases the oxygen consumption of rat aorta (617). In dog femoral arteries the contractile response to catecholamines and oxygen consumption show parallel changes (346). Kosan and Burton (346) suggested that initial, passive stretch has no effect on oxygen consumption, but all of their preparations were stretched longitudinally. The basal Q_{O_2} of 2.26 reported in this study seems high.

The Q_{O_2} of small arteries (less than 150 μ in diameter) is significantly greater than that of larger arteries (250 to 400 μ): 1.05 compared with 0.58 (287). This difference could be due to more extensive spontaneous spike activity, and therefore tone, in smaller vessels. The very high Q_{O_2} values reported for retinal vessels, up to 28, may have been influenced by the extensive dissection in ice-cold solution (288). Respiration of vascular smooth muscle can be completely abolished with 10^{-4} M KCN (288).

Depression of the oxygen consumption of rat aorta by reserpine (215, 337), hydralazine (337) and by acetylcholine (617) have been reported. Alloxan diabetes decreases, and insulin increases the Q_{O_2} of the aorta (617). Hypercholesterolemia has been reported to increase oxygen consumption of rat or rabbit aorta, but not of cockerel aorta (366, 431, 621, 622). The effect on oxygen con-

sumption may also depend on the stage of atherosclerosis and the degree of involvement (382). The effects of gonadectomy and sex steroids have been the subject of conflicting reports by two groups of investigators (385–387, 495). According to Malinow's group (387, 388), aortic oxygen consumption and succinic dehydrogenase activity increase in rats after gonadectomy. Aldosterone has been reported to increase and hydrocortisone to decrease the respiration of rat aorta (272).

C. Carbohydrate metabolism

Carbohydrates are the major, though not the immediate, source of energy in vascular smooth muscle. The predominant utilization of carbohydrates is indicated by a respiratory quotient of nearly 1 (346, 616). It is probable that, as in skeletal muscle, preformed glycogen is utilized preferentially by vascular smooth muscle. This is indicated by the absence of respiratory stimulation of whole vascular segments by glucose added to incubation media (108, 264). The respiration of a particulate, presumably mitochondrial, fraction is augmented by glucose in the medium (306).

The amount of vascular glycogen varies among different blood vessels of the same species: it is several times greater in the bovine aorta than in the inferior vena cava (460). The glycogen metabolism of the bovine mesenteric artery has been extensively investigated by the Lundholms: its average glycogen content is about 160 to 250 mg/100 g wet weight (372). This value, comparable to the one found in the bovine aorta (460), is 8 to 10 times higher than the glycogen content in the rat aorta (616). The different histochemical reactivity of arterial and venous glycogen (section II C) and the absence of glycogen granules in modified smooth muscle cells (section III B) have been mentioned.

The spontaneous tone developed by the bovine mesenteric artery as well as its contractile response to drugs can be maintained by endogenous glycogen for at least 8 hr under aerobic conditions and for about 1 hr under anaerobic conditions (369, 370). Both the contractile effect and the increase in lactic acid production elicited by epinephrine are lost during severe substrate depletion, while the muscle is still somewhat responsive to histamine (370). In these depleted preparations the persistence of the contractile response to potassium is even longer than to histamine (373). It is probable that epinephrine and, to a lesser extent, histamine stimulate metabolic processes that compete with the contractile system for the limited ATP supply.

Glycogen can be synthetized by vascular smooth muscle, *in vitro*, from glucose added to the medium. Glycogen synthesis is increased by norepinephrine (617) and by insulin (374, 429). Glucose uptake is decreased in aortic smooth muscle of alloxan-diabetic rabbits and rats (429, 616–618), rats maintained on a high fat diet (617), or hypothyroid rats (618). Reports are conflicting whether insulin added *in vitro* corrects the defect in diabetic vascular glucose metabolism (596, 635) but this may depend on the nature of the incubation medium (616). Because insulin stimulates the uptake of D-glucose, D-galactose, D-xylose, and L-arabinose, but not that of D-arabinose and D-fructose, it has been suggested that a selective sugar transport mechanism operates in the rat aorta (618).

Glycolysis can be demonstrated under aerobic, as well as anaerobic, conditions in vascular smooth muscle. Lehninger (358) pointed out the functional significance of the failure of oxygen to suppress lactic acid production, in terms of the low energy yield of aerobic glycolysis and the potential effect of the lactate produced on tissue pH. The extent of aerobic glycolysis is variable and may be influenced by the experimental techniques employed (315; see Malinow in "Discussion," p. 167). The rate of anaerobic glycolysis and the development of tone by bovine mesenteric arteries were found to be higher in January than in August (369). This finding indicates that vascular smooth muscle function may be subject to seasonal variations. In the bovine inferior vena cava, obtained from young animals, 43 % of carbohydrate is metabolized by oxidation and 57 % by glycolysis: the respective figures for the aorta are 26 and 74 % (460). In bovine mesenteric arteries the Pasteur effect, inhibition of lactic acid production by oxygen, is clearly demonstrable: aerobic lactic acid production is about 20 % of the anaerobic value (369, 370). The proportion of glycolysis, as compared with oxidative carbohydrate metabolism, has been reported to increase with age and arteriosclerosis (187, 460). On the other hand, the total aerobic glycolysis of the rabbit aorta is reportedly either unchanged (109) or decreased by feeding cholesterol (449). Anaerobic glycolysis can supply the energy requirements of a 70 % maximal contraction of rabbit aorta (201).

A certain amount of lactic acid production is closely associated with the energy requirements of the contractile process, but the contractile effects and lactic-acid-producing effects of drugs can be dissociated (373). *Alpha* adrenergic blocking agents, in concentrations sufficient to block the contractile response to epinephrine, only partially block its lactic-acid-stimulating effect. Histamine and epinephrine stimulate lactic acid production, without a contractile effect, in bovine mesenteric arteries suspended in ion-free dextran solution, while potassium contractures are associated with decreased lactic acid production (373). *Beta* adrenergic blocking agents abolish the stimulation of glycolysis by adrenergic agents (377, 378, 423). An unusual feature of the *beta* adrenergic metabolic response of bovine mesenteric arteries, compared with other tissues, is the reported absence of phosphorylase activation (377, 422). Lactic acid production may proceed without demonstrable glycogenolysis from intermediates other than glycogen (372). Lactic acid does not diffuse out of bovine mesenteric arterial smooth muscle until it reaches a threshold value, and under certain conditions the ratio of lactic acid in tissue over that in the extracellular fluid may be as high as 78 (369).

The hexose monophosphate pathway is also available for glucose utilization in vascular smooth muscle. The enzymes catalyzing the oxidative shunt, glucose-6-phosphate dehydrogenase and 6-phosphogluconate dehydrogenase, were first demonstrated in human blood vessels by Kirk *et al.* (336). The preferential incorporation of C^{14} into CO_2 from carbon 1 of glucose over that from carbon 6, by the aorta of guinea pig (524), ox (394), and rat (618) provided further evidence of a functioning hexose monophosphate shunt. In one study (47) the shunt was absent in venous, but present in arterial smooth muscle. Insulin increases the carbon 1/carbon 6 incorporation ratio of rat aorta (618), and

autologous serum increases this ratio even more (524). Unfortunately, in none of the metabolic studies reviewed is there any indication whether the effects attributed to serum are associated with its contractile effect. The NADPH produced by the hexose monophosphate shunt is thought to be used in synthesis (625), and hexose monophosphate may be a source of the lactate produced without glycogenolysis under the influence of catecholamines (58).

The use of substrate-depleted, anaerobic vascular strips was first employed by Furchgott (200–202) to demonstrate that, in addition to glucose, Krebs' intermediate metabolites and fatty acids can provide energy for contraction of vascular smooth muscle. His original (200–202) findings have been confirmed by others (115). The latter authors were unable to demonstrate utilization of fructose by studying the contractile responses of substrate depleted rabbit aortic strips (115), but earlier, biochemical studies (607) have shown the utilization of fructose by rat aortas.

D. High-energy phosphates and the question of rigor

That APT is the immediate energy source of contraction in skeletal muscle has been demonstrated largely through the efforts of Davies *et al.* (134, 135). It gives the student of vascular smooth muscle some satisfaction to point out that the early difficulties in demonstrating ATP breakdown could have been avoided, had these studies employed vascular smooth muscle rather than the frog sartorius. In the latter, the resynthesis of ATP from ADP and creatine phosphate, catalyzed by phosphoryl creatine transferase (Lohmann reaction), prevents the net decrease of ATP during contraction. Furthermore, the small decrease in creatine phosphate itself is obscured by the high levels found in normal skeletal muscle (135). Vascular smooth muscle, in contrast, contains very little creatine phosphate, and vascular ATP breakdown therefore can be demonstrated without undue interference by resynthesis from creatine phosphate.

The ATP content of blood vessels is reported to be between 0.4 and 0.9 μmoles/g wet tissue (57, 59, 125, 263). The lower values observed in bovine aorta may have been caused by the difficulty in extraction (393). Calculated on the basis of vascular smooth muscle, the ATP content would range from 0.5 to 1.8 μmoles/g, not greatly different from the 2.0 to 2.5 μmoles/g normally found in frog sartorius (350, 399). Regional variations in ATP content of the same vessel have been reported (46).

During the first 1 to 7 min of an isometric contraction of bovine artery stimulated with epinephrine, ATP and creatine phosphate are hydrolyzed (57, 125). It is difficult to assess the estimated (57) thermodynamic efficiency of vascular smooth muscle contraction. The internal work performed during an isometric contraction is greater in vascular smooth muscle than in skeletal muscle, because of the much larger series-elastic component of vascular smooth muscle, 15 to 20% compared with 3 to 4% in skeletal muscle (376). Arterial resynthesis of ATP (59, 371) and the production of fructose-1,6-diphosphate (58), which consumes ATP, are stimulated by epinephrine. Epinephrine-induced contractions therefore are not very suitable for estimating the energy cost of contraction. ATP hy-

drolysis at the termination of prolonged (30 min) potassium contractures can be demonstrated only if glycolysis is blocked (125). ATP breakdown (126) and lactic acid production (375) are higher during the development than during the maintenance of tension (see also section VI H). As indicated earlier (section IV A), the ATP cost of contraction is lower in glycerinated preparations of vascular smooth muscle than in those of skeletal muscle. The hydrolysis of ATP by bovine carotid arteries stimulated by potassium in subcontractile (25 mM) concentrations (125) may be compared with the Solandt effect, the metabolic stimulation of skeletal muscle by potassium (602).

In skeletal muscle the creatine phosphate, 10 to 30 μmoles/g wet tissue, is the immediate source for rephosphorylation of ADP (125, 135, 398, 399). In vascular smooth muscle the concentration of creatine phosphate is much lower, about 0.2 to 0.8 μmoles (57, 59, 125), and its contribution to the resynthesis of ATP is much smaller than in skeletal muscle. Monoiodoacetate delays the activation of the Lohmann reaction in bovine carotid arteries, which is rapid in skeletal muscle (126). In vascular smooth muscle much of the ATP required is synthesized directly, through glycolytic and oxidative phosphorylation, during contraction (125). This is one of the more important, quantitative differences between the energy metabolisms of skeletal muscle and vascular smooth muscle, undoubtedly facilitated by the latter's slower speed of contraction.

Vascular smooth muscle also contains other high-energy phosphates, guanidine triphosphate (GTP) and uridine triphosphate (UTP), that are not readily demonstrable in skeletal muscle (125). These compounds are hydrolyzed and hexosediphosphates may be produced during contraction (125). The concentration of the low-energy adenosine monophosphate, relative to ATP, is also much higher in normal vascular than in skeletal muscle (57, 125).

The question whether rigor occurs in vascular smooth muscle has direct bearing upon the applicability of theories of striated muscle contraction to smooth muscle. In striated muscle the development of rigor is preceded by the disappearance of both ATP and ADP (350). Furthermore, according to Bozler's (76) earlier work, a number of phosphate compounds including inorganic pyrophosphates can plasticize glycerinated skeletal muscle in rigor. The Lundholms (376) were unable to produce rigor with 22 mM monoiodoacetic acid in bovine mesenteric arteries maximally contracted with norepinephrine, although Daemers-Lambert (125) observed an "irreversible contracture" of bovine carotid arteries in the presence of 0.6 mM monoiodoacetate. Relaxation of glycerinated bovine carotid arteries by ATP (531) could be interpreted as being due to plasticizing action on rigor links. The possibility that the reported absence of rigor in vascular smooth muscle (376) was due to accumulation of "plasticizing" phosphates (other than ATP or ADP) has to be considered.

Oxidative phosphorylation by homogenates of rat (634) and guinea pig aorta (496) is efficient: the P/O ratio, 1.5 to 2.0 is only slightly lower than in liver and brain. The relatively large contribution by glycolytic phosphorylation in intact vascular smooth muscle is presumably due to the paucity of mitochondria, rather than deficient function of the mitochondrial enzyme chain.

E. Lipids and atherosclerosis

Fatty acid synthesis from C^{14}-labeled acetate has been demonstrated in the aorta of dog, rat, rabbit, and baboon (172, 367, 580, 622–625). Cholesterol feeding decreases fatty acid synthesis in the liver but not the aorta (172) and in fact increases incorporation of C^{14}-acetate into the intimal layer (622). The ATP utilized for increased fatty acid and phospholipid synthesis (see below) may have been responsible for the apparently decreased efficiency of oxidative phosphorylation by aortic mitochondria of cholesterol-fed rabbits (624, 625). Fatty acid synthesis is markedly stimulated by succinate, and rabbit aortic mitochondria incorporate tritium from succinate-2, 3-H^3 into long-chain fatty acids (622, 623). Fatty acid synthesis appears to occur in mitochondria *via* a chain elongation pathway that adds acetate to preformed C-16 and C-18 fatty acids with the production of C-20 and C-22 chains (623–625). The NADH/NAD ratio correlates well with, and may control, vascular synthesis of fatty acids (624, 625). It has been suggested by Whereat (625) that the fundamental defect in atherosclerosis is the impaired oxidation of NADH with a resultant increase of fatty acid synthesis in vascular mitochondria.

Phospholipid synthesis has been demonstrated in aortas incubated with either P^{32}-labeled phosphate or C^{14}-linoleic acid, glucose, or acetate (367, 441, 447, 465, 480, 580). Cholesterol feeding increases synthesis of phospholipids, particularly of phosphatidylcholine (447). Parker *et al.* (465) correlated increased phospholipid synthesis with the ultrastructural appearance of membranous organelles in the myointimal cells of atheromatous lesions. It is probable that, as they suggested, the increase in phospholipid-containing internal membranes is responsible for the increased phospholipid synthesis. Phosphatidylcholine (lecithin), which is associated with internal membranes, is present in larger amounts in fetal rhesus and young rat aortas, while the relative proportion of sphyngomyelin, associated with external membranes, increases with age (442, 481, 482). The incorporation of C^{14}-linoleic acid into a cell-free preparation of squirrel monkey aorta takes place predominantly within the microsomal fraction, which consists of smooth or denuded rough endoplasmic reticulum and possibly of plasmalemmal pinocytic vesicles (480, 482). In cell-free preparations of atherosclerotic squirrel monkey aorta phospholipid synthesis, without added lysophosphatidylcholine, is increased (480). Lysophosphatidylcholine increases the incorporation of C^{14}-linoleic acid into phosphatidylcholine and abolishes the difference between normal and atherosclerotic material (480). Human umbilical artery extracts lysophosphatidylcholine (lysolecithin) preferentially and acylates it to phosphatidylcholine (160). Epinephrine and acetylcholine increase the rate of incorporation of P^{32} into aortic phospholipids (151, 442).

Phospholipase A (phosphatide acylhydrolase), the enzyme that catalyzes the conversion of phosphatidylcholine to lysophosphatidylcholine, has been demonstrated in pig aorta (460). If, as had been suggested, the level of lysophosphatidylcholine regulates atherosclerotic vascular phosphatidylcholine synthesis (480), it would be of interest to determine whether this system is controlled by varia-

tions in phospholipase activity. Phospholipase C (sphingomyelin choline phosphohydrolase) is present in the aorta of rabbit, guinea pig, and dog and in human umbilical artery, its concentration increasing in that order (487).

Cholesterol is synthesized by the aorta of a number of species, but C^{14}-acetate may also be incorporated into digitonin-precipitable sterols other than cholesterol (27, 161, 188, 363, 514). Squalene, a precursor of cholesterol, is synthesized by human and pigeon aorta, and the amount produced by the latter is proportional to the extent of atherosclerosis (514). Cholesterol synthesis by human aorta *in vitro* occurs (110, 514), but the amount produced is small. Cholestanol synthesis by human aortic intima is more extensive (110). The experiments *in vitro* are generally of short duration, and for this and other technical reasons (514) one cannot rule out the occurrence of more extensive cholesterol synthesis *in vivo*. Nevertheless, the cautious consensus is that most of the cholesterol in atheromata is derived from plasma rather than from local synthesis (3, 138, 139, 176, 448, 486; for review see 315, 514, 519, 625). The local synthetic role of vascular smooth muscle is suggested by the observations that thyroxine stimulates vascular synthesis of sterols (401) and can be atherogenic in spite of lowering serum cholesterol (475). Transplanted segments of canine abdominal aorta retain their high susceptibility, and those of thoracic aortas their low susceptibility for atherosclerosis (239). Total lipid and sterol synthesis of cultured chick aortic cells is increased by incubation with histamine, and this effect is blocked by diphenhydramine (433). The lipid synthetic activity of this preparation is blocked by puromycin (432).

Lipolytic activity in the aorta has been reported to be higher and activated by heparin in young rats and lower and not influenced by heparin in old animals (152). No lipase activity was detected in aortic homogenates of man, rabbit, and dog, although esterase activity was present, and even increased in atherosclerotic areas (359, 380, 381). Lipolytic activity of perfused rabbit aortas has been ascribed to an endothelial factor (642). Cholesterol esterase activity is similar in normal and atheromatous rabbit aortas (137).

In summary, vascular smooth muscle has an active lipid metabolism. The multiple etiologic factors responsible for the atherosclerotic process need not be belabored here. The available evidence unequivocally indicates that vascular smooth muscle (section III B) has a key role in the development of atheromata. It remains to be determined whether the morphological and biochemical alterations seen in atherosclerotic vascular smooth muscle are always secondary to hyperlipemia or, in certain forms of atherosclerosis, may be expressions of a primary metabolic disorder of vascular smooth muscle. Since the early ultrastructural appearance of atherosclerotic myointimal cells is similar to that found in a variety of other vascular disorders (section III B), some of the metabolic changes associated with myointimal proliferation may also be nonspecific. The possibility of developing antiatherogenic agents that act by modifying vascular lipid metabolism has been mentioned (section III B). Most certainly, evaluation of the cholesterol-lowering agents cannot be considered complete unless their possible effects on vascular lipid metabolism are also determined.

F. Enzymes

The metabolic activity of vascular smooth muscle implies the existence of the necessary enzyme systems, some of which have been discussed earlier. Detailed reviews of the specific studies of vascular enzymes, and their modifications in atherosclerotic vessels, have been published by workers active in this field (272, 331, 332, 519, 638, 639). Our discussion will be limited to some general comments and work published since the appearance of these reviews. In all the literature surveyed, species-specific variations in enzyme activity are apparent: of the species investigated the aorta of the rat seems to have the highest metabolic activity. The methods of extracting tissues and the amount of vascular connective tissue may also have a significant effect upon enzyme yield (523).

There are differences in the enzymatic activity of the various blood vessels of a given species. The *alpha* glycerophosphate dehydrogenase activity of the human coronary artery is higher than that of the aorta, pulmonary artery, or vena cava (335). There is an estrogen-inactivating enzyme in the coronary artery of cockerels that is absent from the aorta and the carotid artery (389) The sorbitol dehydrogenase activity of the human vena cava is lower than that of pulmonary and coronary arteries (497). Glutamic dehydrogenase and glutathione reductase are higher (333) and *beta* hydroxyacyl dehydrogenase is much lower in the human vena cava than in the aorta (522). The succinic dehydrogenase activity is higher in the canine pulmonary artery than in the aorta, and decreases peripherally in both vessels (231). Alkaline phosphatase activity is higher in the cerebral vessels of the rat, rabbit, and chicken than in the aortas of the same species (383). Alkaline phosphatase is demonstrable in aortic homogenates of species (*e.g.*, rabbit) in which no histochemically demonstrable enzyme is normally present in smooth muscle (383, 640). Reserpine and guanethidine, administered to rats *in vivo*, have been reported to decrease, and epinephrine to increase the phosphomonoesterase I (alkaline phosphatase) activity of aortic homogenates (8). This enzyme is also increased by vitamin D feeding (641) and is normally higher in male than in female rats (428). Whether the variations in alkaline phosphatase reflect enzyme activity in smooth muscle or in endothelium cannot be determined without histochemical evidence.

There is a variable distribution of lactic dehydrogenase (LDH) isoenzymes among different species (364). The LDH isoenzyme pattern also varies in different layers of the aorta and is clearly distinct from the pattern of intestinal smooth muscle. Alterations of thyroid state induce shifts in the aortic LDH isoenzyme pattern.

The vascular enzymes that attack mucopolysaccharides and glycoproteins (91, 261, 261a, 346a, 491, 620) presumably reflect the morphogenetic activity of smooth muscle. Norepinephrine, *in vitro*, decreases aortic mucopolysaccharide synthesis by the dog aorta and this effect can be blocked by phentolamine (281). The inhibition of synthesis may be due to diversion of the high-energy phosphates of vascular smooth muscle from the synthetic to the contractile process. A vascular ribonuclease, similar to but not identical with the pancreatic enzyme, has been isolated from bovine aorta (204).

V. MUSCLE MECHANICS AND ELASTICITY

A. Length-tension and length-shortening relationships and the maximal active tension

The force developed by skeletal muscle in response to a supramaximal stimulus depends upon its initial length. The maximal active tension P_0 is developed at a length often arbitrarily designated L_0, which is also the approximate resting body length of a given muscle. The passive tension of skeletal muscle at L_0 is negligible, while cardiac muscle develops a significant passive tension when stretched to the length of optimal force development (226). The length-active tension curve of vascular smooth muscle also passes through an optimum L_0, where, as in cardiac muscle, an appreciable passive tension is developed (565, 567). The passive tension developed at length below L_0 is probably a property of smooth muscle rather than of connective tissue, because it is abolished by metabolic inhibitors (376). There is considerable evidence (221, 222, 257) that the length-active tension curve of striated muscle is regulated by the degree of overlap between actin and myosin filaments. It is reasonable to suggest that the length-active tension curve of vascular smooth muscle is also related to a contractile mechanism of sliding filaments.

The isotonic shortening of vascular smooth muscle is also a function of initial length (555) presumably related to it by the same mechanism as force development. Below a minimal length, 25 to 30% of L_0 in bovine mesenteric artery (376), muscle does not contract any further. We therefore concur with the argument (313, 476) that *critical closure* (98) due to tension development unopposed by tangential stress is not a logical necessity in blood vessels. Although the tangential stress against which vascular smooth muscle contracts decreases with decreasing radius, so does the active tension developed by muscle as its length is decreased below L_0. Total closure of constricted small vessels by endothelial protrusions is a different and experimentally demonstrable phenomenon (599).

The maximal isometric tension developed by bovine mesenteric arteries stimulated with epinephrine, at L_0, is about 2 kg/cm^2 (376). We have reported earlier somewhat lower values in three types of canine blood vessel stimulated electrically (551). More recently, in rabbit iliac artery strips adjusted to L_0 and maximally stimulated with norepinephrine we found an active tension of 1.6 ($\pm$0.55 S.D.) kg/cm^2 cross section. The differences among these values could be due to the different modes of stimulation and proportion of smooth muscle. The tensions developed by vascular smooth muscle and by skeletal muscle are fairly comparable (260, 488), particularly considering that the smooth muscle content is probably only 50 to 75% of the vessel wall. The inverse relationship between load and shortening velocity, the force-velocity relationship of vascular smooth muscle, is qualitatively similar to that of skeletal muscle (254). The reported difference between the force-velocity relationships of the electrically stimulated and the K-induced contractions of arterial smooth muscle (254) could be caused by the different levels of the respective active states: one curve

was obtained immediately after electrical stimulation and the other 10 min after initiation of the K contracture.

B. Stress relaxation and myogenic response

Rapid stretch of a vascular preparation causes a sudden increase in tension followed by its exponential decay (223, 567). This stress relaxation is a characteristic of viscoelastic bodies (for review, see 29, 492). Stress relaxation is increased in contracted, as compared with relaxed, strips of canine peripheral veins (360). This finding supports Bozler's (76) opinion that the same mechanism is responsible for isometric relaxation and for stress relaxation of smooth muscle. In modern terminology, which for smooth muscle is only presumptive, the major part of stress relaxation is probably due to the slow breaking of contractile links. Creep, continued extension under constant load, is the isotonic equivalent of stress relaxation.

In some, but not all, types of vascular smooth muscle, stretch elicits a delayed, active rise in tension (136, 310, 559, 564). This myogenic response increases with the rate of stretch (136, 564), and may have a significant effect on the elastic properties of the vascular bed. Although stretch sensitivity can be induced in normally nonresponsive bovine mesenteric arteries by withdrawal of Ca (376), it is generally observed in blood vessels that normally exhibit spontaneous rhythmic activity (section VI E).

C. Stress-strain diagram and elastic modulus

When the vessel wall is subjected to stepwise extensions (strains) and the resultant passive tension (stress) is plotted as the function of length, the resultant plot is the stress-strain diagram or passive tension-length curve of the vessel wall. The elastic modulus relates the change in stress to the change in strain, and is therefore a measure of the extensibility of a material (29, 492). The larger the elastic modulus the lower (the stiffer) is the extensibility of the material under consideration. The generally accepted value of Poisson's ratio of transverse to longitudinal strain is 0.5 (406), but recent studies suggest values of 0.33 (351), or even less, at low longitudinal strains (330a).

The elastic modulus of blood vessels is not constant but increases with increasing stress. The breaking point and the steep portion of the stress-strain curve, above stresses corresponding to distending pressures *in vivo*, reflect primarily the properties of collagen (50, 86, 99, 223, 498). The initial portion of the length-passive tension curve reflects the contribution of smooth muscle and, to a lesser extent, of elastin.

A complete stress-strain curve includes the ascending limb, obtained while the vessel is stretched, and a descending limb, which is the plot of length against passive tension while the vascular strip (or vessel) is returned to its initial length (or volume). The descending limb does not retrace the ascending limb but is displaced towards the length (or volume) axis, the two limbs forming between them the hysteresis loop. Contraction of smooth muscle increases the resistance to stretch during the ascending limb thereby widening the hysteresis loop (12,

208, 223). The hysteresis loop and stress relaxation are probably expressions of the same process: presumably the breakage of contractile links resulting from stretch. It is clear that if the stress-strain curve is obtained when the smooth muscle is contracted, the width of the hysteresis loop will be affected by tachyphylaxis to the stimulating agent and by the time course of a stimulus or of the contractile process. If during the descending limb the active state of muscle persists at a relatively high level, the loop will be narrow, while if the active state has decayed the hysteresis loop will be wide. Finally, if stretch itself elicits myogenic response, the descending limb may be displaced in the opposite direction, to a lesser volume for a given distending pressure, producing an anti-hysteresis loop (591).

D. Wall stress and the effect of smooth muscle on vascular elastic properties

The contribution of smooth muscle to the elastic properties of the vessel wall has aroused considerable interest in the field of hemodynamics. It has been argued, in particular, that under conditions of distending pressures *in vivo*, smooth muscle makes no contribution to the elastic properties of large vessels. This assumption, generated at a time when the relevant experimental data were not available, is contradicted by all the evidence we have reviewed.

The load against which vascular smooth muscle has to contract *in situ* is the tangential stress within the vessel wall. In thin-walled vessels tangential stress T is related to the distending pressure P through Lamé's approximation (Lamé, 1852, cited by 549a) for a thin-walled cylinder: $T = P\ r/h$, where r and h are, respectively, the radius and wall thickness (O. Frank, cited by 30, 476, 477, 535, 555). Calculated for $r/h = 6$, the tangential stress T equivalent of a 10 mm Hg distending pressure is about 8×10^4 dyn/cm^2. The tangential stress corresponding to 100 mm Hg distending pressure and the same r/h ratio will be 8×10^5 dyn/cm^2.

Reliable estimates of the elastic modulus of relaxed vascular smooth muscle are difficult to obtain. The reviewers are at a loss to evaluate the state of smooth muscle reported in some earlier studies of blood vessels placed in water or in isotonic NaCl solution. Recalculation of the data obtained in viable preparations (274) applying a value of 10^6 dyn/cm^2 for the modulus of pure elastin (406) yields 5×10^5 dyn/cm^2 for the modulus of elasticity of relaxed vascular smooth muscle. Without attaching undue precision to these estimates it appears that 10^5 dyn/cm^2 (628) is the correct order of magnitude for this modulus. The upper limit of 20 to 25% contribution of smooth muscle to vascular elasticity at low strains, obtained by this and similar (628) calculations, may be an underestimate. This would be suggested by the observation cited earlier (section V A) that metabolic inhibitors abolished the passive tension developed at strains below L_0.

The active contractile force of about 1.5 to 2.5×10^6 dyn/cm^2 (section V A) which can be developed by stimulated smooth muscle is quite sufficient to make a major contribution to wall elasticity. Strips of canine main pulmonary arteries shorten up to 30% under an isotonic load of 1.6×10^5 dyn/cm^2, which is in excess of the calculated tangential stress due to physiological distending pressures

(555). The stress-strain loop, and in particular its ascending limb, clearly indicates that vasoconstrictor stimuli stiffen veins (12, 13, 223, 360), arteries (55, 223, 253, 348, 349, 417, 477, 593) and arterioles (627, 628). These findings confirm the view long advocated by Heymans *et al.* (268) that vasoconstrictors decrease and vasodilators increase the distensibility of blood vessels.

The elastic modulus of blood vessels can be increased at least 2- to 5-fold by vasoconstrictors (253, 360, 477, 628). The elastic modulus of constricted blood vessels is of the order of 2 to 6 $\times$ 10^7 dyn/cm^2 (253, 348, 628). Smooth muscle has a dominant effect on the dynamic elastic modulus, which also takes into account the time-dependent, viscous properties of the vessel wall (19, 49, 477). The ratio of dynamic/static elastic modulus shows a positive correlation with the amount of smooth muscle in a given blood vessel (49), indicating the contribution of smooth muscle to the viscous properties of the vessel wall.

Several authors (29, 30, 120, 304, 535) have suggested that vasoconstrictors do not change or even decrease the elastic modulus of blood vessels. This divergence of opinion is due not to a difference in experimental results but, as already pointed out by Bevan *et al.* (55), to the differences in computing elastic moduli from experimental results. Thus, if the experimental conditions or mathematical assumptions allow the vessel to constrict while the distending pressure remains constant, it follows from Lamé's approximation that tangential stress will decrease. If stress is not referred to the absolute (resting) length or radius, the calculated elastic modulus will paradoxically decrease during vasoconstriction. This manner of calculation can be misleading because it does not indicate the increased resistance of contracted smooth muscle to stretch or its correct hemodynamic effects on pulse wave propagation.

E. Some circulatory effects of smooth muscle contraction

We can examine only briefly a few of the recent studies that illustrate the hemodynamic consequences of smooth muscle contraction. The authoritative monograph and review by McDonald (406, 407) and Mellander and Johansson (410) deal in detail with the advances in hemodynamics and circulatory physiology during the period under consideration.

Vascular smooth muscle *in situ* may either shorten (isotonically, afterloaded, or even after-unloaded) or contract isometrically and be even further stretched during contraction. The conditions for isotonic shortening of vascular smooth muscle *in situ* can be defined (555) formally, in terms of pressure p, radius r, and their derivatives, as $dp/p = -dr/r$, but it is unlikely that pressure and radius vary in precisely this inverse fashion *in vivo*. There is now considerable evidence that smooth muscle contraction (shortening or isometric) can affect large as well as small blood vessels. In a series of ingenious experiments on canine femoral arteries *in situ*, the Gerós (212) demonstrated that when intravascular pressure was maintained constant, sympathetic stimulation significantly reduced the radius of the femoral artery. Without a pressure-stabilizing device the increase in distending pressure caused by peripheral vasoconstriction can decrease or abolish the femoral vasoconstrictor response. Under the latter condi-

tions, however, the smooth muscle contracts isometrically and wall stiffness is markedly increased.

The hemodynamic consequence of increased wall stiffness is an increased velocity of pulse wave propagation (41, 49, 167, 406, 408). An increased pulse wave velocity under the influence of vasoconstrictors has been demonstrated in the main pulmonary artery (41), the aorta (408), and the great veins (17). The large pulmonary arteries are also constricted *in vivo* by hypoxia, serotonin, or epinephrine (466, 513); these findings conclusively demonstrate the vasomotor function of smooth muscle (555) in large vessels.

The effect of initial length upon the magnitude of active shortening (vasoconstriction) is also demonstrable in the intact animal. The decrease in femoral artery radius elicited by sympathetic stimulation increases with increasing initial distention of the vessel, until a limiting value of 130 mm Hg is reached (212). Gauer and Thron (208, 591) suggested that the vasoconstrictor response of veins declines with increasing distending pressure. Their studies were conducted at relatively high distending pressures of 20 to 100 mm Hg. Between 0 and 20 mm Hg distending pressures there is a distinct increase in active vasoconstriction (452), indicative of the typical length-contractile response relationship. In fact, one gets a rather strong impression that L_0, the optimal length for vascular smooth muscle contraction, is reached at very nearly the physiological distending pressure for a given blood vessel.

The capacitance function of veins (410), whereby vasoconstriction leads to circulatory redistribution of a significant blood volume (157) has been recently investigated and reviewed by Thron (591). The circulatory studies are in agreement with experiments *in vitro* in showing that the vasoconstrictors decrease and vasodilators increase venous distensibility (591, 610).

Autoregulation of resistance (185, 314, 591) and capacitance (507) vessels is frequently considered to be the equivalent *in vivo* of the myogenic contractile response to stretch. This subject has been extensively reviewed by Mellander and Johansson (410).

VI. ELECTROPHYSIOLOGY AND EXCITATION-CONTRACTION COUPLING

The application of electrophysiologic techniques, the measurement of monovalent ion fluxes, and the establishment of the critical role of Ca in contraction mark some of the more important recent developments in the study of vascular smooth muscle. As the result of these, it has become evident that a valid theory of excitation-contraction coupling in vascular smooth muscle cannot be completely identical with the one applicable to fast striated muscle. The following properties of vascular smooth muscle are absent in fast striated muscles, and have to be accounted for by a satisfactory theory of excitation-contraction coupling: 1) their dose-response curves, which indicate continuous gradation of excitation and are difficult to reconcile with an underlying all-or-none process; 2) the lack of quantitative correlation between the contractile effects of drugs and either their action on the membrane potential or related monovalent ion fluxes, in polarized as well as in depolarized preparations; 3) the absence of

action potentials in certain types of vascular smooth muscle; and 4) the inequality of the maximal contractile effect of different drugs on a given vascular smooth muscle.

The peculiarities of vascular smooth muscle, however, stand out only in comparison with fast striated muscle, but not when viewed within the broader context of phasic and tonic muscles of vertebrates and invertebrates (484). We shall attempt to develop the theme that two major forms of excitation-contraction coupling regulate both striated and smooth muscle, and that mammalian vascular smooth muscle presents examples of each of the two forms, certainly within the same blood vessel, and perhaps within the same smooth muscle fiber. The presentation will be based upon the ionic model of the membrane potential (for reviews, see 99a, 450, 538), since practical limitations prevent us from considering alternative hypotheses. For a general survey of the electrophysiology of mammalian smooth muscle and of the effects of anions on excitable cells, the reader is referred to the excellent reviews by Burnstock *et al.* (96), and by Horowicz (285). Some publications on electrolytes in vascular smooth muscle, previously reviewed by Bohr (65), have not been included in the present review.

Two assumptions, by now almost generally accepted, will be employed to account for a variety of experimental observations. First, it is assumed that drugs may bring about changes in membrane potential either by changing the passive permeability of the membrane to some (one or more) ionic species or by inhibiting or stimulating an electrogenic ion pump, or both. The second assumption is that contraction is brought about by an increase, and relaxation by a decrease in ionized Ca^{++} available to contractile proteins.

If Cl is passively and rapidly redistributed[3] and the system is in steady state, the basis for the first assumption may be formally stated (430):

$$E_m = \frac{RT}{F} \ln \frac{P_{Na}[Na]_o + rP_K[K]_o}{P_{Na}[Na]_i + rP_K[K]_i}$$

where E_m is the resting potential, R the gas constant, T the absolute temperature, F Faraday's constant, the subscripts o and i denote whether the given ion is extra or intracellular, P_{Na} and P_K are the respective ion permeabilities, and r is the coupling ratio of the number of Na pumped out for each K pumped in.

The equilibrium potentials for a given ion will be given by the conventional Nernst equation, *e.g.*:

$$E_K = \frac{RT}{F} \ln \frac{K_o}{K_i}, \qquad E_{Na} = \frac{RT}{F} \ln \frac{Na_o}{Na_i}$$

It follows from the equation for E_m that a selective increase in membrane permeability to one ion will shift the membrane potential towards the equilibrium

[3] The assumption that Cl^- is passively distributed and rapidly permeant is adopted as a matter of convenience simplifying the mathematical treatment. There are as yet no comparative studies known to us (but *cf.* 606a, 636) of the Cl^- permeabilities of different vascular smooth muscles.

potential of that ion, which will carry the membrane current in a direction downhill to its electrochemical gradient. This implies that a selective change in ion permeability may depolarize or hyperpolarize the membrane, depending on whether the equilibrium potential of the given ion is more positive or more negative than the resting potential. A general increase in membrane permeability will result in a downhill flux of all permeant ionic species (for review, see 48, 156, 229). In terms of the known electrolyte composition of vascular smooth muscle, depolarization may be caused by an increase in Na permeability or by an electrogenic K pump, hyperpolarization by an increase in K permeability or an electrogenic Na pump. The effect of changing pump activity on the membrane potential will depend on the coupling ratio, which can be variable (553). Finally, if a given drug both induces a passive permeability change and activates or inhibits an electrogenic pump or changes the coupling ratio, the resultant potential changes may add or subtract and, if separated by a resolvable time interval, may be diphasic.

A. Sodium, potassium, and chloride

In spite of several attempts to relate the intracellular concentrations or the transmembrane gradients of monovalent ions directly to the contractile process (56, 194, 355), we find no evidence for a direct coupling of Na or K to contraction. With the benefit of hindsight it is apparent that earlier studies neither considered nor measured the changes in Ca concentration which may occur simultaneously with, or as a consequence of, changes in monovalent ion gradients. Also, in a number of experiments involving manipulation of ionic gradients, the effects of extracellular electrolytes on catecholamine storage and release were not considered and cannot be evaluated here. This objection is applicable when the effects of ions upon the response of vascular smooth muscle to 60 cycle sinusoidal current or low concentrations of K are determined. Alternating current stimulation of vascular strips results in a large indirect component of contraction, mediated by release of catecholamines and K releases catecholamines from adrenergic sites (Part II of this review). The increase in tone elicited by 9 to 15 mM external K, in the isolated pulmonary artery (56) and in the systemic circulation *in vivo* (233) may be due to direct, indirect, or combined effects. Peripheral vasoconstriction elicited in the hamster cheek pouch by 9 to 12 mM K is abolished by denervation or adrenergic blockade (585). The vasodilator action of K on the feline muscular bed is not influenced by *alpha* adrenergic blockade (338, 339).

K-free solutions produce contracture or delayed relaxation in the canine carotid artery and systemic resistance vessels (43, 233), in the bovine hepatic, coronary, and mesenteric arteries (414, 415, 509), and in the rabbit aorta (144). Brecht *et al.* (345a) found that when the external K concentration was 10 mM the tone of bovine facial arteries *in vitro* was the lowest, and it increased when the cation concentration was either increased or decreased. These effects of K were not influenced by tetrodotoxin. The contraction of rat portal vein elicited by removal of external K is associated with depolarization and discharge of

action potentials (26). The depolarization and contracture induced by withdrawal of K are probably direct effects, and not due to release of endogenous catecholamines. Depolarization and contracture also develop in avian slow striated muscles (which have no excitatory adrenergic innervation) when they are placed in a K-free solution (553). The depolarization of smooth and slow striated muscles by K-free solution could be explained, as in the frog semitendinosus (277), by a decrease in K conductance with a shift towards the Na equilibrium potential. Theoretically, this depolarization could also be caused by the removal of a K-coupled electrogenic Na pump's contribution to the resting membrane potential. Whether the contracture is due to persistent depolarization or to interference with a K-dependent Ca pump or Ca permeability is an intriguing question, with the present reviewers favoring the second alternative. Vascular smooth muscle depolarized with high potassium is relaxed by isoproterenol (311) while the K-free contracture does not appear to be relaxed by this agent (43). Omission of Ca from the bathing medium accentuates the K-withdrawal contraction of bovine coronary arteries, although it eliminates the response to increased K (415). These findings lead us to believe that the contractile effect of K-free solutions is not simply the result of depolarization.

Acute substitution of lactose, but not choline, for Na results in Na loss and vasodilation of the perfused rat tail artery (195). Sodium-withdrawal produces contracture of canine mesenteric (611) and bovine coronary and mesenteric arteries (416) and the rabbit aorta (121). In the latter Na withdrawal is also said to be associated with increased Ca influx (84; see also section VI C).

Studies with Na^{24} and K^{42} indicate (205, 236) a very high permeability of vascular smooth muscle to sodium. The kinetics of Na efflux suggests the presence of three predominant compartments. The first, with a half-time of exchange of 30 to 50 seconds is extracellular. The second Na compartment, with a half-time of exchange of 2.5 to 5 minutes (205, 236) is in all probability intracellular. In comparison, the half-time of efflux of intracellular K is 40 to 70 minutes (236). In view of the relatively high Na permeability (205, 236, 606), it may be anticipated that the resting membrane potential of vascular smooth muscle will not be a simple K equilibrium potential. The third compartment, with the slowest exchange time of 40 to 70 minutes is presumably bound at some undetermined site, but at any rate contains less than 2% of the total Na in the dog carotid artery (205) or the rat aorta (236).

Recent estimates (532) of intracellular cation concentrations are about 50 mM of Na and 98 mM K per liter intracellular water, the latter having been calculated on the basis of an inulin space of about 36% (205, 533). The above-cited value of intracellular Na is subject to certain assumptions regarding extracellular cation binding (205, 533). Without these assumptions the sum of the calculated intracellular Na and K ions (205, 532) would be in excess of that expected to be in osmotic equilibrium with the extracellular fluid (533). A technical consideration deserving reemphasis is that stripping off the adventitia or dissecting blood vessels in ice-cold solution can increase the Na content up to

50%, and decrease the K down to 30% of normal (237). Incubation in plasma accentuates, rather than prevents the "unavoidable" Na gain of vascular strips placed in salt solutions (234). The possibility that at least some of this unavoidable gain may be related to the degree or absence of stretch has not been explored.

In only one recent study (327) have intracellular Na and membrane Na permeability been reported to be very low, 7.3 mM/Kg cell H_2O and 0.18 picomoles/cm²/second, respectively. This may reflect a real difference in the electrolyte content of the sheep carotid artery or a spurious result of the assumptions in the calculations employed.

The higher electrochemical potential of extracellular Na suggests that an active process maintains the transmembrane Na gradient. There is now considerable evidence for the existence of an active Na pump in vascular smooth muscle (43, 129, 205, 236, 605). The extrusion of Na and uptake of K against their electrochemical gradients appear to be mediated by a system which has the properties of a Na, K-dependent ATPase (for review, see 549). The Na pump of vascular smooth muscle is inhibited by ouabain (205, 236) and by dinitrophenol (236, 605). The unusually high dependence of this system upon external K has been emphasized by Garrahan *et al.* (205), who found a 90% decrease in Na efflux of dog carotid arteries in K-free solutions. Prolonged incubation in Ca-free, EDTA-containing media or substitution of Li for Na also inhibits the transport of K and Na in rabbit aorta (129).

Whether chloride is partitioned in a manner compatible with a passive, Donnan distribution in canine carotid arteries has been the subject of two conflicting reports (606a, 636).

It can be anticipated from the foregoing that a generalized increase in membrane permeability will lead to net Na influx and to K efflux. Because of the possible effect of drugs on active electrolyte transport, measurements of short-term electrolyte transients need not parallel those obtained after prolonged (*e.g.*, 30 min) incubation of blood vessels with drugs.

The electrolyte shifts that occur during constriction of rat-tail arteries induced by norepinephrine, angiotensin, and vasopressin have been investigated with cation-sensitive electrodes by the Friedmans and their associates (191–194). The results are compatible with a transient increase in passive permeability, during which Na moves into and K out of the cell, following their respective downhill gradients. Maximal vasoconstriction precedes maximal Na uptake and the latter does not correlate well with the degree of vasoconstriction (191). This behavior of monovalent ion fluxes is also reflected in the limited temporal and quantitative correlation between the changes in membrane potential and tension development elicited by drugs (557). The norepinephrine-induced increase in the K permeability of the rat aorta is an *alpha* adrenergic effect which, unlike that caused by angiotensin, is blocked by phentolamine (505). Electrical stimulation of rat aorta also increases K efflux (235). The Na efflux of canine and feline carotid arteries appears to be increased by angiotensin (595), but the authors'

suggestion that this is due to stimulation of active transport, rather than due to mechanical "squeezing" (129) or an increase in passive permeability requires further investigation.

We have previously alluded to the possibility that drugs may stimulate the Na pump in vascular smooth muscle. This would tend to oppose the net effects of an increase in passive permeability. A dual action of catecholamines on Na flux may have been responsible for the observations that norepinephrine causes K loss consistently but Na gain only inconsistently (592) and that it prevents the Na gain by perfused canine femoral arteries (104). In sucrose-gap studies of the effects of norepinephrine on the membrane potential (557) we found, during recovery from contractions elicited by large (2×10^{-5}) doses of norepinephrine (558), hyperpolarization considerably below normal (section VI E) resting potentials (*e.g.*, to 90 to 100 mV). An electrogenic Na pump is the most probable mechanism causing this hyperpolarization well beyond the E_K calculated from the reported vascular K contents. Adrenergic amines are known to stimulate the Na pump of striated muscle (143). Investigation of the effects of *alpha* and *beta* adrenergic stimulation upon passive membrane permeability and active transport as a function of time would be of considerable interest.

It must be emphasized, finally, that the mechanisms regulating the electrolyte content of gradedly responsive vascular smooth muscle, such as the aorta, need not be quantitatively or even qualitatively identical with those of spike-activated smooth muscle.

B. Extracellular space and water content

Inulin is the commonly accepted marker for measurements of extracellular space, although in several types of smooth muscle the inulin space is significantly greater than the extracellular compartment estimated by electronmicroscopy or occupied by polyglucose or I^{131}-albumin (44, 96). Furthermore, although the mean inulin space of the aorta is about 35 %, it is quite variable (28 to 53 %) even within the same species (237, 265). There is also evidence of incomplete penetration of inulin into connective tissue, leading to an underestimation of extracellular space (606a).

The very high inulin spaces measured could be due to intracellular penetration dependent upon the functional state of smooth muscle. Thus, rather than causing an increase in the extracellular space, as had been suggested (265), epinephrine may increase intracellular penetration of inulin in the rabbit aorta. It is difficult to reconcile an increase in extracellular space during vasoconstriction with the H_2O loss that accompanies isotonic contraction of vascular smooth muscle (131, 505).

C. Calcium

The participation of calcium in cellular functions has been expertly reviewed in Bianchi's (61a) recent monograph. There is considerable evidence that, in striated and in smooth muscles, a rise in free intracellular Ca activates contraction, and a fall in cytoplasmic Ca activity leads to relaxation (60–61a, 128, 130,

150, 158, 228, 309, 445, 469, 520, 529, 613, 630). This activator Ca may arise from an increased influx of the extracellular ion or from translocation of Ca bound at the surface membrane or concentrated in an intracellular storage site (*e.g.*, sarcoplasmic reticulum). As already emphasized by Bianchi (60) in reference to skeletal muscle, Ca influx may be increased during a twitch without the net increase of intracellular Ca being quantitatively sufficient to account for activation. The Ca sinks may be extracellular or intracellular, but are generally thought to be regions of high (Ca) electrochemical potential, necessitating expenditure of energy for active transport of Ca uphill, during relaxation. Some part of this Ca-lowering process may also take place through binding to high-affinity sites.

An approximately 2- to 3-fold increase in Ca^{45} influx has been reported in two studies to occur during K- or epinephrine-induced contractions of rabbit aortas (83, 84). A puzzling feature of the first study (84) is the equality of fluxes into normal and glycerinated aortas, since glycerination is known to increase membrane permeability (585a). In the second study (83) both control and experimental strips appear to have been incubated in Ca-free Ringer's solution before determining flux rates. The control Ca influx, 0.25 mμmoles/g/sec, was approximately equal to that observed during a 50% increase in tension produced by K_2SO_4 (fig. 3 in 83). Since membrane Ca probably controls its own entry into cells, Ca-depleted aortas would be expected to have an increased, rather than normal, permeability to subsequently added Ca. Binding of Ca to extracellular sites that become unsaturated in Ca-free solutions would also be a function of added Ca concentration and indistinguishable from Ca influx. The latter consideration is also applicable to the effects of low Na solutions on measured Ca influx, since it has been shown by Catchpole *et al.* (106) that Na and Ca compete for anionic binding sites in the ground substance. Since the experiments cited (83, 84) were conducted at pH 6.9, they were probably not complicated by microprecipitation of Ca phosphate. That the latter may occur has been suggested by Goodford (220), who found a significant increase in Ca content of taenia coli, incubated in phosphate buffered media above pH 7. This finding should be considered in future studies of Ca fluxes in smooth muscle.

There is evidence that drugs can affect excitation-contraction coupling by a common mechanism that is not entirely dependent on depolarization of the membrane. Several smooth and striated muscles bathed in Ca-free media contract in response to drugs for some time after the loss of the contractile response to high K or electrical depolarization. Examples are the effects of norepinephrine on rat tail artery (273) and of acetylcholine on rat uterus (158), the taenia coli (150), the toad stomach (566), and the striated muscle of the frog extensor longus digitis (189). A related property of a number of smooth muscles (in normal Krebs-Ringer solution) is their greater maximal contractile response to drugs than to complete depolarization with potassium. Examples are the maximal responses of vascular smooth muscle to norepinephrine (276, 557, 611, 612), and the responses of uterine (158), intestinal (166), and tracheal (552) smooth muscle to acetylcholine. The maximal responses of vascular smooth

muscle to different drugs also vary within the same vascular segment and the difference persists in the depolarized state (553, 554, 557). The characteristics listed could be due to the ability of drugs to translocate Ca into the cytoplasm from a compartment not accessible to depolarization. An alternative possibility is that drugs produce a longer and more persistent increase in the permeability of the plasma membrane to Ca than does K: in this event Ca bound to the basement membrane and in equilibrium with the contaminant Ca present in "Ca-free" solutions (of the order of 10^{-5} M without chelating agents) could activate contraction.

Several authors have suggested the existence of two separate Ca sites to account for the different responses of smooth muscle to drugs and to K (128, 130, 273, 290, 308). Van Breemen and Daniel (597) have proposed that the superficial and deep sites are in parallel. This hypothesis was based on the assumption that Ca removed by K from a superficial site that is in series with the deep site would be preferentially replenished from the latter, rather than from the extracellular fluid. Since efflux in these experiments was determined in a Ringer's solution containing 1.2 mM nonradioactive Ca, there seems to be no *a priori* reason to expect that a superficial site could not be repleted from this source. The decline of drug-induced contractions in Ca-free media can be accelerated by chelating agents (158, 273). This finding does not favor the hypothesis that the drug-induced contractions are preferentially utilizing intracelluar Ca.

Several findings, rather than supporting the multiple site hypotheses, are more compatible with the assumption that drugs have a more pronounced effect on Ca permeability than does depolarization. When graded amounts of Ca are added to a Ca-free medium, the contractile response of the rat-tail artery to high K increases gradually over a wide (2.5 mM) range of external Ca concentration, while the response to norepinephrine rises steeply reaching near maximum at 0.75 mM external Ca (273, 275). Similarly, when the toad stomach muscle is depleted of Ca, the contractile response to alternating current stimulation is lost at higher residual tissue Ca levels (0.31 mM/Kg net weight) than the response to acetylcholine (0.14 mM) (566). However, the full contractile response to acetylcholine is also restored at much lower concentrations of Ca than the response to electrical stimulation.

A number of the experimental findings could be accounted for by two permeability barriers to Ca in series. The external barrier, containing high-affinity sites for Ca, could concentrate the cation from the external medium and, if fully utilized, support maximal contractions in the presence of very small amounts (0.2 mM) of total tissue Ca (566). The basement membrane or some less specialized condensation of ground substance probably has the required Ca-binding properties (106). It has been recently reported that 90% of the Ca content of cultured HeLa cells is in the extracellular coat (73). Without implying that the properties of these cells are necessarily similar to those of smooth muscle, one notes with interest that in this system the Ca uptake by the extracellular coat, but not the Ca entry into the "naked cell," is a function of the Ca concentration

of the external medium. Parathormone on the other hand affects Ca uptake by the trypsinized "naked cell" and not by the extracellular coat. There is, to our knowledge, only a brief report suggesting that enzymes may decrease or abolish the Ca-binding activity of vascular strips *in vitro* (173).

The inner barrier to Ca in the above model would be the plasma membrane, whose permeability to Ca and other solutes would be controlled by the membrane potential, by labilizing or stabilizing drugs, and by Ca itself. As long as the stabilizing action of Ca on membrane permeability persists, influx from the external site (Ca bound to the basement membrane or free in the extracellular fluid) would be subject to "autoinhibition." This could account for the relatively high Ca requirement for K-induced contraction. In contrast, drugs that eliminate the stabilizing effect of Ca either by removing it from the plasma membrane or through some other mechanism would permit maximal influx from the external site into the myoplasm. This model, which is essentially analogous to the one proposed by Hurwitz *et al.* (294, 295) for the mechanism of action of acetylcholine on intestinal smooth muscle, is more compatible with the experimental findings than some of the multiple site theories.

A multiple barrier system to Ca has been proposed by Goodford (218) to account for the relatively low energy of activation of Ca efflux (see below) from taenia coli (219). According to this model, there is a region of high energy into which Ca must be actively transported first and whence it escapes downhill by a passive, but rate-limiting process. A double permeability barrier would account for the failure of 1 to 2 mM external Ca concentrations to interfere with the relaxant effect of isoproterenol in Schild's experiments (529), even if relaxation was due to externally-directed Ca pump action. If the free energy of Ca within the barrier exceeds the free energy in normal Ringer's solution, then the external Ca would have to be increased above 1 to 2 mM before it interfered with an externally directed Ca pump. According to another study, higher concentrations (8.3 mM) of Ca do abolish the relaxant effects of epinephrine on tracheal smooth muscle (421). Schild's (529) experiments, however, were conducted on depolarized myometrium, and the two studies are not entirely comparable.

Active transport of Ca into the extracellular phase (527) suggests itself as a possible mechanism of relaxation in smooth muscle. Attempts to demonstrate active extracellular transport in taenia coli (218, 219) or uterus (598) have not been successful. In only one of the studies reviewed could we find evidence of a high degree of temperature-dependence of Ca efflux (fig. 2 in 439) suggestive of an active process. It is possible that, under the usual experimental conditions, the fraction of resting efflux due to active transport of Ca is too small to be detectable. If vasodilators (smooth muscle relaxants) stimulate the active extrusion of Ca, then the Q_{10} of this (still hypothetical) stimulated transport should be higher than values obtained during resting efflux.

It is difficult to estimate the contribution of the sarcoplasmic reticulum to Ca release and uptake in smooth muscle. Because of the small amount of smooth endoplasmic reticulum and the short diffusion distance in smooth muscle, Peachey and Porter (470) suggested that influx of extracellular Ca activates

contraction. The scarcity of smooth endoplasmic reticulum in aortic smooth muscle has been noted (for references, see section I), but no comparative studies have been made of the proportional volume of smooth endoplasmic reticulum in different types of smooth muscle. We have suggested elsewhere (557) that the relative contribution of, respectively, the Ca stored in the sarcoplasmic reticulum and the extracellular Ca may vary in different types (*e.g.*, phasic or tonic) of smooth muscle (see also section VI G).

The microvesicular fractions obtained from vascular smooth muscle (482) may be smooth or denuded rough endoplasmic reticulum or even micropinocytic vesicles detached from the plasma membrane. When Ca^{45} is injected *in vivo*, 26 to 48 % of the vascular radioactivity is found in the fraction with the sedimentation characteristics (123,000 $\times$ *g* fraction) of microsomal vesicles (458). Microsomal fractions with the properties of particulate relaxing factor have been isolated from intestinal smooth muscle (588) and there are preliminary reports of its isolation from uterine (586) and vascular smooth muscle (540). However, if a significant proportion of the particulate relaxing factor consists of detached micropinocytic vesicles, then this finding would favor the existence of externally directed active transport, rather than intracellular translocation.

There is some evidence that not all blood vessels are equally sensitive to Ca depletion, but the results of different laboratories (95, 443) are somewhat difficult to compare. Brecht *et al.* (80) found that when spontaneous activity of bovine artery and vein strips was abolished in Ca-free media, addition of lower concentrations of Ca (0.1 to 0.2 mM) was sufficient for tension development in arteries than in veins (0.3 to 0.5 mM). The response of the rabbit aorta to norepinephrine persists, though diminished, for an hour in solutions containing no added Ca (443, 558), while the majority of mesenteric veins fail to respond after 6 min of Ca withdrawal (558). It has been suggested that loss of responsiveness during Ca depletion may vary with the thickness of the preparations (501). The Ca requirement (ED50) of depolarized rabbit aorta is 3 to 4 times lower than that of the portal-anterior mesenteric vein even when Ca withdrawal of the aorta is prolonged to eliminate differences due to diffusion (553, 558). After incubation in Ca-free depolarizing solution, in particular with Dibenamine (1.5×10^{-6} w/v) added, the contractile response of mesenteric veins to cumulatively added Ca is depressed (553). Larger K-contractures can be elicited again, after repolarization of the strips in Ca-free solutions containing Na (553). This behavior and the large phasic component of the depolarization contractures of the mesenteric vein resemble the uncoupling of depolarized fast striated muscles (60, 122a, 189). Under the above described experimental conditions the Ca contracture of depolarized aortic smooth muscle persists; this suggests, but only indirectly, a higher state of maintained Ca permeability. It remains to be confirmed whether the increased permeability of the depolarized rabbit aorta is unidirectional (influx only), as had been suggested (83). The lesser sensitivity of the rabbit aorta to depression of K contracture by cinnarizine (216a) correlates well with the usual absence of action potentials in this type of smooth muscle. Whether the greater sensitivity to cinnarizine of the smaller

vessels studied by Godfraind *et al.* (216a) is related to spike-generating, phasic properties of their smooth muscle fibers, as we would suggest, will have to be determined by electrophysiologic studies. This assumption is at least supported by the sensitivity of the rabbit mesenteric vein to the depressant effects of Dibenamine (553).

Isojima and Bozler (302) found that the response of turtle aorta to acetylcholine, which had been abolished in Ca-free NaCl solution, was restored in Ca-free KCl solution. The mechanism of this interesting observation remains to be determined.

D. Magnesium

The Mg requirements of isolated contractile proteins and glycerinated vessels have been mentioned earlier (section IV A). Whether the intracellular activity of Mg can vary sufficiently in living fibers to affect contractile activity is uncertain. Although about 50% of the total Mg can be removed from canine iliac arteries incubated in a Mg-free medium, the strips so depleted still respond normally to epinephrine, angiotensin, and serotonin (560). Assuming an extracellular space of 35%, and all the remaining Mg intracellular, these preparations still contain 4.0 mM Mg/Kg cell water, while ATPase activity of vascular actomyosin is maximally activated by 3.0 mM Mg (531).

A large proportion of exchangeable Mg has also been demonstrated in the canine aorta *in vivo*, with approximately 33% of Mg exchanging in 4 hr in normal (609), and about 60% in hypermagnesemic animals (608). The reported values of normal vascular Mg content show considerable variation (94, 182, 560, 608) and may include an extracellularly bound fraction (106, 608).

The vasodilator effects of Mg in intact animals (232, 590) are outside the scope of this review. The specific interaction between Mg and neurohypophysial peptides (560) will be discussed at a later date (Part II of this review).

E. Resting membrane potential, automaticity, and conduction

The technical limitations of the sucrose gap and intracellular microelectrode methods employed in recent studies of smooth muscle have been discussed in detail previously (96, 557). Rothstein's review of membrane phenomena (506) provides an excellent survey of the general biophysical processes underlying the specialized case of excitation and inhibition in vascular smooth muscle.

The resting membrane potentials measured in vascular smooth muscle are summarized in table 1. In common with other smooth muscles (96), the resting potentials in vascular smooth muscle are lower than in fast striated muscle. Low, unstable potentials are usually associated with spontaneous spike activity.

The K dependence of the vascular smooth muscle's resting potential is now well established: high concentrations of K invariably depolarize the membrane (26, 124, 323, 557, 584). The possibility that some effects of 5 to 20 mM K are indirect (neurally mediated), and the depolarizing action of complete K withdrawal have been discussed (section VI A).

Withdrawal of Ca depolarizes the sheep carotid artery (326), rabbit aorta

TABLE I

Electrophysiology of vascular smooth muscle

Species	Vessel	Resting* membrane potential	Action potentials	Method	Reference
		mV			
Frog	Tongue 20–100 μ Arteries and veins	41	Two types; evoked, not spontaneous	Intracellular	197
Frog	Abdominal cutaneous 50–100 μ Arteries and veins	25	Spontaneous	Intracellular	198
Frog	Tongue Arteriole 20–80 μ	34 (18–59)	Evoked, not spontaneous	Intracellular	544
Frog	Tongue Blood vessels 50–150 μ	65	Evoked in 10%	Intracellular	576
Frog	Abdominal skin 50–150 μ	44		Intracellular	576
Turtle	Abdominal aorta, vena cava	40	Spontaneous	Intracellular	503
Rat	Portal vein	39 (30–65)	Spontaneous and slow waves	Intracellular	199
Rat	Mesenteric arterioles	39 (30–50)	Spontaneous and slow waves	Intracellular	576
Guinea pig	Mesenteric arterioles 100–450 μ	39 (26–59)	Spontaneous and slow waves	Intracellular	568
Guinea pig	Anterior mesenteric vein	51 (41–62)†	Spontaneous and slow waves	Intracellular	440
Sheep	Carotid artery	61 (47–77)	? Evoked with drugs	Sucrose gap	323
Pig	Carotid artery	50		Sucrose gap	97
Pig	Renal vein	53		Sucrose gap	97
Rabbit	Branch of posterior mesenteric artery	54		Intracellular	569
Rabbit	Ear artery	62		Intracellular	569
Rabbit	Portal anterior mesenteric vein	40–50	Spontaneous and slow waves	Sucrose gap	282
Rabbit	Portal anterior mesenteric vein	46	Spontaneous and slow waves	Sucrose gap	557
Rabbit	Portal anterior mesenteric vein	33	Spontaneous and slow waves	Intracellular	123
Rabbit	Main pulmonary artery	52		Intracellular	584
Rabbit	Main pulmonary artery	52		Sucrose gap	557

* Mean; values in parentheses are the range.

† Resting membrane potentials below 30 mV excluded.

(541), and rat portal vein (26), although the effects on spike activity differ (see below).

Spontaneous rhythmic contractions, indicative of automaticity, were first described in 1852 in the pulsatile veins of the bat wing (Wharton Jones, cited

by 23). Some 20 years ago, Bozler (75) pointed out that these pulsatile, non-innervated veins should be classified as single-unit smooth muscle. Spontaneous rhythmic activity of this type has since been described in the portal veins of chickens (23), rats, dogs, rabbits, and other mammals (26, 71, 123, 124, 199, 282, 283, 440, 557), human umbilical vessels (136, 559, 564), the longitudinal mesenteric arterial muscle (69, 70) and small mesenteric arteries (558) of the fowl, some small canine cutaneous arteries (310), and the turtle aorta and inferior vena cava (503). In all the preparations that have also been studied with electrophysiologic methods, rhythmic mechanical activity was found to be associated with action potentials, usually arising from slow waves. The rate of spontaneous contactions and action potentials increases in response to stretch (23, 71, 123, 310).

Myogenic propagation of the action potential is implied by the definition of single-unit muscle (75). The propagated activity of nerve-free vascular smooth muscle (*e.g.*, bat veins, umbilical vessels) is self-evident. The automaticity and conduction in the longitudinal muscle of rat and rabbit portal-anterior mesenteric vein are also myogenic, and are not eliminated by tetrodotoxin, adrenergic blocking agents, or nerve-blocking concentrations of local anesthetic (282, 312). Spontaneous vasomotion of the microcirculation (643) indicative of conducted activity, may represent either myogenic single unit or neurally coordinated multiunit behavior. In innervated vascular beds some of this electromechanical activity is influenced by, though not totally dependent on, excitation of nerves (15, 31, 177, 544, 576). Altura (15) recently produced considerable evidence of adrenergic and cholinergic modulation of microcirculatory vasomotion. In denervated vascular beds vasomotion persists and is undoubtedly myogenic (626). The automaticity and the excitability of vascular smooth muscle may fluctuate with such variables as the degree of stretch, depth of anesthesia, and temperature (177, 282, 543, 557, 568, 576).

The nexus, the site of close membrane apposition which is considered to be the low-resistance path for cell-to-cell propagation (42; and section I D) has been demonstrated in rabbit (282) and canine (140) mesenteric veins, both of which show well-developed conducted activity (557). Areas of close apposition are also present in the rat portal vein (199) and the human umbilical artery (570) and occur with increasing frequency in the terminal vascular bed (494). Nexuses are absent in the aorta (section I D). The available evidence indicates a good positive correlation between the presence of nexuses and conducted action potentials.

F. Electrical responses to drugs: action potentials and graded depolarization

In certain types of vascular smooth muscle action potentials arise spontaneously or can be evoked by drugs or direct electrical stimulus (table 1). The shape of the action potentials varies from spikes of 40 to 50 msec duration in frog and rat arterioles (197, 576) to the plateau-type action potentials lasting 15 sec in the turtle aorta (503). The action potentials frequently arise from slow waves, occasionally overshoot, or exhibit positive afterpotentials (26, 69, 123,

124, 197–199, 402, 440, 503, 544, 557, 568, 576). A more thorough analysis of action potentials in frog and rat blood vessels can be found in Steedman's (576) excellent study.

It is apparent from the survey of even the limited number of published studies that the morphology of vascular action potentials of different species, vascular beds, and functional states covers perhaps the entire range encountered in all other types of smooth muscle (for review, see 96). Different electrophysiologic properties of smooth muscle cells in close proximity of each other are indicated by the considerably different action potentials that can be evoked from precapillary sphincters (50 msec duration) and from the adjacent arteriolar (200 msec) smooth muscle (197). On direct external electrical stimulation, in only 10% of the cases could action potentials be elicited in blood vessels of the frog tongue (576).

The usual electrical response of spike-generating vascular smooth muscle to vasoconstrictors is depolarization, initiation of action potentials, or an increase in spike-frequency. These electrical effects can be elicited by potassium, *alpha* adrenergic agents (124, 199, 282, 311, 440, 503, 544, 557, 568, 576), histamine (124, 557), 5-hydroxytryptamine (557), and vasopressin (576). *Beta* adrenergic agents (69, 124, 282, 311) and the phosphodiesterase inhibitors theophylline (124, 311) and caffeine (557) produce inhibition of spike electrogenesis, sometimes preceded by an initial stimulatory period. Acetylcholine can produce an initial hyperpolarization associated with action potentials and contraction, followed by depolarization (197, 199, 440), while in other mammalian vascular beds it abolishes spike activity (576). Cholinergic excitation with increased frequency of action potentials is seen in the longitudinal mesenteric arterial muscle of the chicken (69). In spite of the excitatory and inhibitory electrical effects of, respectively, vasoconstrictors and vasodilators, in the majority of cases investigated their mechanical effects are not solely dependent on electromechanical coupling (section VI H).

The *beta* adrenergic inhibition of action potentials has been reported to be associated with depolarization (311) and also with hyperpolarization (282). Hyperpolarization may be ascribed to an increase in K permeability, activation of an electrogenic Na pump, or both effects. Depolarization may be due to an increase in Na permeability or stimulation of an electrogenic K pump. In the slow striated muscle (latissimus dorsi) of the pigeon, isoproterenol usually has a depolarizing action when the K concentration of the Krebs' solution is 5.9 mM and a hyperpolarizing action when it is 1 mM (553). A tentative explanation of the latter finding is that the K/Na coupling ratio, and therefore the sign of the pump electrogenesis, may vary as a function of external K concentration. Inasmuch as isoproterenol has a *beta* adrenergic relaxant effect on avian slow muscles and on vascular smooth muscle, these observations may have some applicability to the electrogenic effects of catecholamines on smooth muscle. In fact, the authors (311) who reported a depolarizing action of isoproterenol on vascular smooth muscle used a solution containing more K (5.94 *versus* 5.0 mM) than employed by Holman *et al.* (282). Whether this small difference in K

concentration could be responsible for the differing results in uncertain, particularly since in one case the rat and in the other the rabbit portal vein was investigated.

Sucrose-gap records of sheep carotid arteries stimulated with K, norepinephrine, or histamine have been interpreted as showing spike or plateau-type action potentials (323–325). In our opinion the published records could represent minimal spike activity superimposed on a predominantly graded (see below) electrical response. Clearly distinguishable spike activity is elicited in the sheep carotid artery by a combination of acetylcholine or nicotine and endogenously released norepinephrine (324). Characteristically, these electrical effects are accompanied by only minimal contraction; and this suggests that spike electrogenesis is not the predominant link in excitation-contraction coupling. Repetitive action potentials are also produced in low Ca media (326). We have emphasized elsewhere in reference to vascular smooth muscle (557) the general phenomenon (506) that stable membranes may be labilized to produce action potentials. The question relevant to excitation-contraction coupling is not whether spikes can be artificially produced, or demonstrated in a small number of phasic fibers within a tonic population, but whether they represent a normal step in the activation process of the entire cell population.

Graded depolarization, rather than action potentials, appears to be the normal electrical response of certain types of mammalian vascular smooth muscle (553, 554, 557, 583). The arguments in favor of this view have been discussed in detail earlier by the present authors (557) and will not be recapitulated here. Norepinephrine, 5-hydroxytryptamine, and histamine produce graded depolarization in large, multiunit blood vessels. On the rabbit main pulmonary artery a depolarizing action of norepinephrine, which could not be observed in earlier microelectrode studies (584) of intimal smooth muscle, was conclusively demonstrated by the sucrose-gap method (557).

Graded excitatory junction potentials accompanied by contraction, but without action potentials, have been recorded intracellularly in isolated rabbit arteries (569). The authors' argument that failure to elicit action potentials is an artifact due to recording *in vitro* is not convincing, since action potentials can be recorded *in vitro* from other types of vascular smooth muscle. The action potentials of small (100 to 450 μ diameter) guinea pig arteries recorded *in vivo*, referred to by Speden (568, 569) need not, and probably are not, representative of the electrophysiological properties of the larger arteries of the rabbit.

The classification (553, 557) of vascular smooth muscle into spike-generating (phasic) and gradedly responsive (tonic) is not advanced solely on the basis of electrophysiologic studies. The K contractures and apparent Ca permeabilities of the spike-generating mesenteric vein and the gradedly responsive aortic and pulmonary arterial smooth muscle are also clearly different (section VI C). Short (10 msec) external electrical pulses produce contractions in spike-generating (551) but not in gradedly-responsive vascular smooth muscle (558). The different degrees of phasic and tonic behavior appear not to be limited to blood vessels but may be a more general property of mammalian smooth muscles. K con-

tractures of rabbit tracheal smooth muscle are tonic while those of small (300 to 500 μ) bronchioles are phasic (552).

G. Electromechanical coupling

Given that spontaneous or evoked electrical activity of the membrane triggers contraction by increasing the sarcoplasmic concentration of free Ca, there remain to be established the source of the Ca and the mechanism of its release. Intimately related to the problem of electromechanical coupling is the identity of the ions carrying the charge represented by the action potential.

The spontaneous action potentials of portal-mesenteric veins are undoubtedly triggering the associated contractions. In view of the similarity of this preparation to the taenia coli, one must consider the suggestion (220) that the action potentials are Ca spikes, which also provide the activator Ca for contraction. The major alternative to the former hypothesis would be that spikes cause the translocation of Ca from an intracellular storage site (557), in analogy with the well known mechanism in fast striated muscle (520). Tetrodotoxin does not abolish the action potentials of rabbit mesenteric veins (282) but, as emphasized by Kao (317), resistance to tetrodotoxin is not sufficient evidence against Na being the charge carrier. Ca-free solutions, after an initial period of enhanced spike activity, depolarize and abolish the action potentials of rat portal veins. Contraction amplitude decreases before the spike amplitude (26), and norepinephrine can initiate spike activity without contractions in Ca-free solutions (311). These findings seem difficult to reconcile with the joint proposition that Ca current is responsible for spike activity and that the amount of Ca charge is sufficient to activate contraction. This hypothesis was advanced on the basis of experiments on taenia coli (92, 220, 451). Axelsson (25), however, found that spike activity was enhanced, although the taenia was relaxed, when Li was substituted for Na or in Ca-free solutions. If the action potentials carry sufficient Ca for contractile activation, then secondary postulates have to be invoked to explain the paradox of electromechanical dissociation. Of course, the charge-carrier need not be unique but may be one ion or another, depending on experimental conditions (344, 506). Therefore, Axelsson's findings do not destroy but, in our opinion, greatly weaken the case that the Ca charge carried by the spike is sufficient to directly activate single unit mammalian smooth muscle. If, on the other hand, action potentials discharge Ca from an intracellular storage site in spike-generating vascular smooth muscle, one may anticipate ultrastructural evidence of greater amounts of sarcoplasmic vesicles than found in gradedly responsive vascular smooth muscles. The spike-generating smooth muscle of the canine portal vein (557, 558) does contain a relatively voluminous sarcoplasmic tubular system (140).

The repetitive action potentials evoked in sheep carotid arteries by removal of Ca from the medium are accompanied by Na influx (326, 327). These Na spikes generally do not trigger individual contractions. They are interesting, experimentally induced oscillations, rather than a normal link in excitation-contraction coupling.

The coupling mechanism that links the graded depolarization of smooth muscle in large, multiunit vessels to contraction is also uncertain. The possibility that depolarization in this type of smooth muscle is associated with a maintained increase in Ca permeability will be considered in the next section (see also section VI C).

H. Pharmacomechanical coupling, maintained tension, and relaxation

The discovery that depolarized smooth muscle can be contracted or relaxed by drugs (166, 529) has been amply extended to vascular smooth muscle (273, 289, 311, 325, 402, 557, 560, 611, 612). It has also been shown that action potentials in spike-generating vascular smooth muscle can be abolished without depolarization, while the contractile effect of drugs persists (557). In the same study, it was also established that the inequality of the maximal contractile response of polarized vascular smooth muscle to different drugs (554) persists in the depolarized state. Antagonists and potentiators exert similar effects in polarized and in depolarized vessels (560, 611) and there is only limited correlation between the electrical and the mechanical effects evoked by drugs (124, 557). All of these findings support the importance of pharmacomechanical coupling: the role of nonelectrical processes in mediating drug-induced contractions. The possibility that the process responsible for the drug-induced contractions of depolarized muscle is an increased state of permeability to Ca has been considered by several authors (150, 166, 612). There have also been several advocates of the idea that certain membrane-active agents act by displacing Ca from the cell membrane (171, 343, 538, 557) and that tonic contractions are associated with a maintained increase of Ca influx (83, 539). The working hypothesis outlined below has numerous antecedents within and beyond the material surveyed in this review. In fitting the pieces together we have tried to follow A. V. Hill's (271) admonition that publication of a hypothesis is permissible only if it can be tested experimentally.

A survey of smooth and striated muscles indicates that either one may be phasic or tonic. Phasic muscles respond to depolarizing agents with a twitch and transient Ca influx (60), they are normally activated *via* action potentials and may be either single- or multiunit. In tonic muscles depolarization produces a sustained state of contraction and increased permeability to Ca (section VI C). The presence of both components, phasic and tonic, in a given muscle may be due to a heterogeneous (phasic and tonic) cell population (24, 28, 469) or to an intermediate type of muscle fiber. Phasic components of contraction are absent in depolarized muscle stimulated with drugs, the slow response of these preparations having been noted in the original work of Schild and his co-workers (166). The tonic component, when present, can be selectively abolished in the uterus by lowering the temperature (166) and in vascular smooth muscle by Ca-free solutions (fig. 3 in 612) by desipramine (289), or by cinnarizine (216a). Prominent phasic components of contraction are seen in small vessels (67) and in mesenteric veins (553), while tonic behavior is characteristic of the rabbit main pulmonary artery and aorta. Activation with action potentials appears to occur in phasic

vascular smooth muscle, and graded depolarization (summated junction potentials) in tonic vascular smooth muscle (557).[4] Spike-activated muscles may be single-unit or multiunit, depending upon the extent of cell-to-cell conduction, rather than the cellular mechanisms of excitation and contraction. Gradedly responsive muscles are, by necessity, multiunit.

It is tentatively proposed that, as in many other systems (for review, see 506), membrane-bound Ca regulates the permeability of the membrane to ionized Ca itself as well as to other solutes. Depolarization removes some membrane-bound Ca: the resultant permeability increase is transient in phasic and sustained in tonic vascular smooth muscles. Some drugs may release membrane-bound Ca and, as suggested by Feinstein (171), exert an even greater stabilizing action than Ca itself. Other agents, like acetylcholine (294, 295), norepinephrine, and other active amines or peptides, may eliminate the stabilizing effect of Ca with or without removing it from bound sites. Activator Ca under these conditions may arise in varying quantities from the plasma membrane itself, from binding sites within the ground substance or from the extracellular fluid. This mechanism of permeability change would be compatible with the steep Ca "dose-response curves" in the presence of highly potent agonists (section VI C). The unequal efficiency of pharmacomechanical coupling obtained with different drugs (557) could thus be accounted for by an unequal ability to overcome the stabilizing action of Ca and thereby produce a sustained increase in membrane permeability.

Nonelectrical coupling of inhibitory stimuli to relaxation may be caused either by a decrease in permeability to Ca (passive or the active exclusion of Shanes, 538), an enhanced operation of the Ca pump, or a combination of both factors. Electrical inhibition may be due to hyperpolarization, or inhibition of spike electrogenesis, or both (282, 311, 325, 557).

Tone, the maintained contraction of vascular smooth muscle, may be due to a true tetanus caused by random discharge of action potentials of phasic fibers (26, 177, 552) or to graded depolarization of tonic smooth muscle (553, 557). Tone may also be caused by an increase in the permeability of the membrane to Ca or, theoretically, by an inhibition of Ca pumping, either of which may occur relatively independently of changes in the membrane potential. Either the latter mechanism or sustained depolarization may be responsible for the "catch-like" state of rabbit main pulmonary artery which follows strong electrical stimulation (556). It is important to recognize that the mechanical records of a tetanus and a tonic contracture are often indistinguishable. The basic cellular mechanisms of excitation-contraction coupling, in particular the sources and sinks of activator

[4] Tonic, gradedly responsive fibers and phasic, spike-generating fibers are extreme and simplified examples of what is probably a continuous spectrum. Sustained K-contractures, for example, in avian slow muscles (558) are not incompatible with the ability of the cell membrane to generate action potentials (553). A possible correlation has been pointed out between sustained contractures maintained by Ca-influx and the contribution of Ca to the action potential in some muscles (158a).

calcium, may be quite different in the two types of maintained tension. All the excitatory influences are coupled to a single actomyosin system (section IV A). There is overwhelming evidence against the operation of a "catch" mechanism of specialized protein crystallization even in those invertebrate muscles which, unlike mammalian muscles, contain paramyosin (for review, see 162, 444). There is a measurable energy cost of maintaining contraction in vascular (126) and in invertebrate "catch" muscle (32), although it is less than that required for developing tension. This result is encouragingly consistent with thermodynamic considerations of the relative amounts of work involved, and fully confirmatory of Bozler's (75–77) views on the nature of tone in smooth muscle.

It is evident that current views, including our own, regarding the nature of excitation-contraction and inhibition-relaxation coupling owe a large debt to inference and speculation. Experimental evidence based on concomitant electrophysiological studies linking, respectively, graded depolarization with tonic and action potentials with phasic mechanical properties is limited to a few blood vessels investigated in our laboratory. Correlation of the time course of K contractures and Ca fluxes with the type of electrical activity, in a variety of smooth muscles, will be required to establish the general validity of some of the concepts outlined here. Reassessment of the electrophysiology of nonvascular smooth muscle may also reveal the existence of forms that, unlike the taenia coli, are activated by graded depolarization. Significant advances in this field can come from rigorous exploration of the fundamental biophysical processes that regulate the excitability of the membrane and convey phasic and tonic properties to smooth and striated muscles.

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