THE PHARMACOLOGY OF VASCULAR SMOOTH MUSCLE[1]

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TABLE OF CONTENTS

Introduction 184
I. Some Anatomical and Histological Considerations 185
a. Localization of vascular smooth muscle responding to drugs 185
b. Orientation of muscle in blood vessels 186
c. Efferent innervation 187
II. Some Physiological Considerations 188
a. Electrophysiology of vascular smooth muscle 188
b. Tone and phasic variations in tone 189
c. Responses to mechanical stimulation and pressure 192
d. Response to electrical stimulation 193
e. Response to light 194
III. Some Biochemical Considerations 195
IV. Responses of Vascular Smooth Muscle to Various Drugs and the Concept of Specific Receptors 197
a. Preliminary comments on the development of the receptor hypothesis 197
b. Epinephrine 200
c. Norepinephrine 203
d. Isopropylarterenol 204
e. Other sympathomimetic amines 205
f. 5-Hydroxytryptamine 207
g. Pitressin and hypertensin 207
h. Histamine 208
i. Acetylcholine 209
j. Adenylic acid derivatives 209
k. Nitrites, organic nitrates and azide 210
V. Effects of Specific Antagonists or Blocking Agents on Vascular Smooth Muscle. 211
a. Receptor theory for "reversible competitive antagonism" and its application 211
b. Receptor theory for non-competitive antagonisms and "irreversible competitive antagonism" 214
c. Kinetics of the development of and recovery from reversible competitive antagonism 218
d. Kinetics of the development of and recovery from irreversible competitive antagonism 223
e. On the relative specificity of specific antagonists 225
f. Some comments on the use of antagonists in studies on vascular pharmacology and physiology 226
VI. Local Potentiation of Drug Action on Vascular Smooth Muscle 229

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a. Modes of local potentiation 229
b. A possible source of error in studies on the potentiation of responses to epinephrine and norepinephrine 233
c. The "monoamine oxidase hypothesis" of potentiation of responses to epinephrine and norepinephrine by various drugs 234
d. Potentiation by cocaine 236
e. Potentiation by ephedrine and related sympathomimetic amines 238
f. Potentiation by other drugs 240
g. Potentiation by substances of physiological origin 241
h. Potentiation of responses to drugs following denervation 242
VII. Local Indirect Action of Certain Drugs on Vascular Smooth Muscle 244
a. Possible modes of local indirect action 244
b. Local indirect action of acetylcholine, nicotine, and related substances 246
c. Local indirect action of tyramine, ephedrine, and related sympathomimetic amines 246
d. Local indirect action of 5-hydroxytryptamine and tryptamine 248
e. The relation between the local indirect action of certain drugs and tachyphylactic phenomena 249
Concluding Remarks 253

"*Quantitative pharmacology is such an undeveloped subject that it is hopeless to expect formal proof for any hypothesis and equally hopeless to expect any hypothesis to explain all the facts observed.*"—A. J. Clark, 1937.

INTRODUCTION

The present review will be primarily concerned with the local actions of drugs on vascular smooth muscle. Such actions are those which are spatially limited to the region of tissue containing the smooth muscle cells affected by the actions. Local actions may be of two types. The first type, which will be called "direct action", results from a reaction of a drug with certain components of the smooth muscle cells on which the functional activity of the cells depends. These are the components which are commonly referred to as the "receptors" for the drug in question. The second type, which will be called "local indirect action", results from a reaction of a drug with some other components of the cells or of adjacent tissues, leading to the release or accumulation of a second substance which exerts a direct action on the cells. In the case of many drugs it is possible to deduce from experimental evidence whether action is of a direct or indirect type or a combination of both types; while in the case of some drugs the type of action exerted is not yet clear.

Beyond the scope of this review are "remotely initiated indirect actions" of drugs on vascular smooth muscle—that is, actions initiated either by the direct or indirect stimulation or inhibition of structures remote from the region of the smooth muscle affected. Among such structures, which are of fundamental importance in the nervous and humoral control of vascular tone, are sympathetic ganglia, the adrenal medulla, chemoreceptors, baroreceptors, mechanoreceptors, and certain parts of the central nervous system. In any complete pharmacological investigation of the effects of drugs on vascular smooth muscle in the body,

consideration must necessarily be given to remotely initiated indirect actions as well as to local actions. However, because remotely initiated indirect actions frequently mask or modify the local actions of drugs on vascular smooth muscle, the present review, with its emphasis on local actions, will be limited largely to a discussion of results obtained with experimental preparations in which such indirect actions are either excluded or unlikely to influence the results.

Together the topics composing this review constitute part of the general pharmacology of mammalian vascular smooth muscle. Since many aspects of the general pharmacology of this type of smooth muscle are also aspects of the general pharmacology of all mammalian smooth muscle, consideration will often be given here to experimental results obtained with smooth muscle other than that of blood vessels. As will become apparent, considerations of such results frequently lead to a better understanding of results of experiments on vascular smooth muscle itself.

In any treatment of the general pharmacology of vascular smooth muscle consideration should be given to certain aspects of the anatomical arrangement, histology, physiology, and biochemistry of this type of muscle. The first three sections which follow are therefore devoted to these subjects.

I. SOME ANATOMICAL AND HISTOLOGICAL CONSIDERATIONS

a. Localization of vascular smooth muscle responding to drugs. Most recent studies on the local actions of drugs on vascular smooth muscle have been made by determining the effects of drugs on the pressure-flow relationship of the circulation through a particular organ or tissue. Both natural and artificial circulation and both direct and indirect methods of measuring flow have been used. (See articles on methods of measuring blood flow in *Methods in Medical Research*, vol. I, 1948 (291)). Changes in resistance to flow are determined from changes in the pressure-flow relationship. Changes in resistance to flow produced by drugs are frequently assumed to be due to changes in caliber of the small arterioles brought about by changes in the tone of the smooth muscle of these vessels, since the greatest resistance to flow in most vascular beds is usually in these vessels. However, it is often not possible to determine from changes in resistance alone whether the smooth muscle affected is that of the arterioles, or of the capillaries,[2] or of arterio-venous anastomoses (when present), or of veins, or of two or more of these structures. In the case of some organs various indirect methods have been used in attempts to determine what vascular elements are responding when a drug causes a change in resistance to flow. For details of several interesting and diverse methods used with different organs and tissues

[2] The term smooth muscle of capillaries will be used in this review in a broad sense to designate any contractile elements which can cause constriction or dilation of capillaries independently of the constriction or dilation of the small arterioles and venules which the capillaries connect. One contractile element of this type is the precapillary sphincter (smooth muscle) found by Chambers and Zweifach (71, 72) in mesentery and omentum. Another type in certain mammalian vascular beds may be the endothelial cell of the capillary wall itself; however, this is a controversial matter. For some comments concerning the controversy over the contractile elements of capillaries, see Lutz *et al.* (280) and Boyd (34).

the reader is referred to the paper of Daly *et al.* (102) on the lungs (epinephrine and histamine on arterioles and veins); the paper of Hilton and Holton (223) on the rabbit ear (adenosinetriphosphate and other vasodilators on both the arterioles and capillaries); the papers of Hürlimann and Bucher (235, 236) on the rabbit ear (epinephrine and histamine on arterio-venous anastomoses); and the book of H. W. Smith (358) on the kidney (chapter 14 on the effects of various drugs and chapter 18 on renal hemodynamics).

In addition to indirect methods of determining what vascular smooth muscles react to drugs in an intact vascular bed, direct microscopic observations have been used with certain preparations. For example, with transparent chambers inserted in rabbit ears (84, 119, 339a) several studies have been made on the effects of drugs on the various anatomical components of the vascular bed (1, 270, 391). Also, the mesenteric and omental preparations of Zweifach (402) have been used for pharmacological studies (210, 211, 402). One advantage of the Zweifach type of preparation is that it permits topical application of drugs to the region under observation. With thicker tissues the peripheral vascular bed can sometimes be satisfactorily illuminated for microscopic observation with a fused quartz illuminator (225, 248). Using this type of illuminator Seneviratne (348) studied the response of various vascular elements of the liver of mice and rats (arterioles, sinusoids and hepatic and portal venules) to histamine, acetylcholine, and epinephrine. Microscopic observation and measurement of diameter changes of small arteries *in situ* on local application or injection of drugs have also been used for quantitating the response of such vessels to drugs under various experimental conditions (9, 43–45, 198).

b. Orientation of muscle in blood vessels. Blood vessels generally have smooth muscle fibers oriented circularly or in a close spiral. (See Benninghoff (24) for an extensive review of the microscopic anatomy of blood vessels, and Strong (364) for impressive evidence for a close spiral arrangement of muscle in the media of distributing arteries.) However, as noted by Benninghoff and others, many vessels contain longitudinally as well as circularly or spirally oriented smooth muscle. This is particularly true in many veins, where the longitudinal muscle is often the predominant type. In most arteries there is little if any longitudinal muscle, although there are a number of exceptions. For example, the main human coronary arteries have about as much longitudinal as circular muscle, while the main human pulmonary artery has almost exclusively longitudinal muscle (24).

The orientation of the smooth muscle fibers in large vessels should be taken into consideration whenever isolated viable preparations (closed or open rings, spiral strips, or tubular segments) of such vessels are used for pharmacological studies. (See 292 for the first report on isolated rings; and 51, 299, 338, 339, 354 for reviews of earlier literature and for useful modifications of such *in vitro* preparations.) Several workers have demonstrated that segments of arteries of the type which contain no significant longitudinal muscle lengthen when drugs are applied which cause contraction of the circular muscle (215, 345, 359). This lengthening, which appears as a "relaxation" in kymograph tracings, is thought to be due to an increase in thickness of the circular muscle fibers on contraction.

In view of this it would not be surprising to obtain an apparent relaxation with stimulating drugs and an apparent contraction with inhibitory drugs on rings or spiral strips cut from vessels containing almost exclusively longitudinal smooth muscle. Such a situation might possibly account for the early observation of Macht (282) that rings of pulmonary artery contracted with $NaNO_2$. However, it apparently does not account for the relaxation produced by epinephrine on rings of coronary arteries from many species. Barbour (18, 19) excluded this possibility by showing that longitudinal strips from beef coronary arteries did not contract significantly with concentrations of epinephrine which produced marked relaxation of rings of the same artery.

In the case of spirally cut strips from arteries which have practically no longitudinal muscle, the direction of cutting the spiral may be of some importance. Häusler (215) reported that strips cut in left handed spirals from ox mesenteric arteries gave markedly greater contractions than those cut in right handed spirals. This he assumed to be due to a left handed spiral or helical arrangement of the muscle fibers in these arteries. (See also 216, 322 for more recent work on the relationship between responses of strips to drugs and the angles of cutting of strips from several arteries.) With rabbit thoracic aorta used in our laboratory, we have found no significant difference in response of right and left handed spiral strips. This is in accord with the results of histological examinations which show an essentially circular orientation of the muscle in this vessel. The histological examinations also show that there is practically no longitudinally oriented smooth muscle in rabbit aorta, and that the circularly oriented muscle constitutes almost half of the contents of the vessel wall—a surprisingly high fraction for an aorta (Elchlepp and Furchgott, unpublished observations).

c. Efferent innervation. The nature of the terminal efferent innervation of blood vessels has been a matter of much debate among neurohistologists. According to Boeke (31, 32) there is a terminal nerve net or reticulum of fine anastomosing fibers surrounding the smooth muscle cells and even sending processes into the protoplasm of these cells. Nonidez (315, 316) and others have considered this terminal net to be a non-nervous structure and an artifact of the staining techniques used and have presented evidence for a much less dense terminal innervation with discrete endings on some smooth muscle cells. With the notable exception of Jabonero (239), most recent investigators (220, 294, 382) have not supported the concept of a terminal net, and even Jabonero does not find intracellular endings. Hillarp, using methylene blue staining, has described a fairly dense plexus of non-myelinated, beaded fibers surrounding vascular smooth muscle cells. These fibers may be in close proximity to one another but they do not generally anastomose, so that a true reticulum is not formed.

From the standpoint of the neuro-humoral theory of transmission, it does not matter whether the terminal portions of the efferent nerves affecting the smooth muscle cells are rather widely separated discrete fibers or are arranged in a dense plexus or reticulum, as long as they liberate their transmitter in close proximity to smooth muscle cells. However, in view of the present emphasis on transmitters probably acting by altering the membrane permeability of effector

cells which they reach from the outside, it seems unlikely that there should be intracellular efferent nerve endings. Yet it must be admitted that a concept consonant with that of intracellular nerve endings has been used occasionally by pharmacologists in attempting to explain why certain antagonists which completely block the action of mimetic drugs on certain effectors do not effectively block the action of nerve stimulation. For example, Dale and Gaddum (96), in trying to explain why atropine blocks vasodilation in the submaxillary glands due to injected acetylcholine or pilocarpine but not that due to chorda tympani stimulation, proposed that atropine creates a barrier which prevents the parasympathomimetics from gaining access to the receptive mechanism within the barrier; but that this barrier is ineffective against acetylcholine liberated by nerve stimulation, because the liberation is from nerve terminals within the barrier. (But see section V, f, of this review for other ideas on this matter.)

Another aspect of the problem of innervation which is important from a pharmacological standpoint is whether the vascular smooth muscle under study has to have (or have had, if it is denervated) adrenergic innervation in order to react to sympathomimetics and cholinergic innervation in order to react to parasympathomimetics. The finding of von Euler (120) that the perfused, nerve-free vascular bed of the human placenta reacts to both epinephrine and acetylcholine indicates that autonomic innervation is not a requisite for response to these drugs. However, the relatively high concentrations of epinephrine and acetylcholine required to produce effects in von Euler's experiments also suggest that autonomic innervation may be needed to insure high sensitivity of vascular smooth muscle to mimetic drugs. In line with this idea, one might attribute the high sensitivity to acetylcholine of blood vessels of limb muscles (which have sympathetic but no parasympathetic innervation) to innervation by cholinergic fibers within the sympathetic nerve trunks (48, 49, 152, 154, 158). On the other hand, Folkow *et al.* (160) and Celander and Folkow (68) were not able to demonstrate cholinergic innervation of skin vessels, which are also highly sensitive to acetylcholine; and Clark and Clark (83) reported that newly formed muscular arteries in rabbit ears (transparent chamber technique) respond actively to epinephrine before they become innervated. Thus, for the present any attempt at relating high sensitivity to mimetic drugs to appropriate autonomic innervation seems unwarranted.

II. SOME PHYSIOLOGICAL CONSIDERATIONS

a. Electrophysiology of vascular smooth muscle. The electrophysiology of vascular smooth muscle has been the subject of only a small number of investigations. With certain other types of smooth muscle it has been demonstrated that tonic contraction (or contracture) induced by stimulating drugs is associated with a sustained decrease in the positive membrane potential, and that relaxation induced by inhibitory drugs is associated with an increase in the membrane potential. (For examples of older work with extracellular electrodes, see 17, 337; and for newer work with intracellular electrodes see 47, 50, 397.) It is generally assumed that the changes in membrane potential lead to the changes in the me-

chanical state of the smooth muscle and not *vice versa*. Determinations of membrane potentials of vascular smooth muscle at various levels of tone due to applications of drugs have not yet been made, but in view of the results with other smooth muscles it seems likely that sustained changes in tone would be associated with sustained changes in membrane potential.

Although no determinations of changes in membrane potential of vascular smooth muscle with sustained changes in tone have been made, several investigators have reported on "action potentials" obtained with external electrodes from perfused segments of surviving arteries and veins. Wybauw (399) and Luisada (277, 278, 279) obtained small, transient variations in potential (peak differences of the order of 1 millivolt) between two pick-up electrodes attached to the vessel wall whenever the perfusion pressure was suddenly raised. Epinephrine and other drugs modified the rate of change and amplitude of the potential differences. Peterson (321) recorded "action potentials" up to 3 millivolts occurring at the beginning of each spontaneous contraction of isolated segments of ox carotid artery. All three of the investigators cited presented evidence against the possibility that the potential changes observed were artifacts resulting from mechanical changes. The work of Peterson (321) in particular indicates that rhythmic spontaneous contractions of vascular smooth muscle (see following section) may be due to rhythmic conducted action potentials. Unfortunately, none of the investigators attempted to determine speed of conduction of the apparent action potentials. It would be of interest to know whether this is of the same order as the slow speed of conduction (.5 to 3.0 mm. per sec.) of contraction waves along isolated arterial segments, reported by Monnier (299) and Bürgi (51). If conducted action potentials can occur in blood vessels, what is the nature of the conducting pathways between the small smooth muscle cells? Even in the case of much more extensively studied smooth muscle (from intestine, ureter, uterus, etc.) there is still a controversy over whether these pathways consist of a nerve plexus or of protoplasmic bridges between adjoining cells. Vaughn Williams (374) has recently reviewed this subject in relation to intestinal smooth muscle.

b. Tone and phasic variations in tone. Vascular smooth muscle, like most other smooth muscle, can exhibit inherent or spontaneous tone (an active sustained contraction not demonstrably dependent on stimuli from outside the muscle). Inherent tone in varying degree may occur in the smooth muscle of the vascular bed of perfused organs or tissues or in the muscle of isolated strips or segments of larger arteries and veins. Variations in tone from the inherent level may be brought about by stimulation of nerves to the muscle, direct electrical stimulation, changes in physical environment and addition of chemical agents.

In perfusion experiments the composition of the perfusion fluid may have a marked influence on vascular tone (98, 303). It has long been known that perfusion with defibrinated blood or serum leads to much greater resistance to flow than does perfusion with heparinized whole blood or plasma. This increased resistance is now thought to be due to the presence of 5-hydroxytryptamine (serotonin) liberated from platelets during the clotting process (317, 323). Even

heparinized blood probably contains very small amounts of 5-hydroxytryptamine, norepinephrine (122, 123) and other vasoexcitor substances sufficient to produce some increase in tone of vascular smooth muscle in perfused organs. According to Brauer (38) heparinized blood contains an hepatic vasoconstrictor which may be differentiated from epinephrine, norepinephrine, pitressin, and 5-hydroxytryptamine. On the other hand, a vasodilator may be released into blood used in perfusion as a result of hemolysis of red cells (73, 150). Chambliss *et al.* (73) have presented evidence that rapid injections of isotonic saline into blood may traumatize red cells and cause them to liberate a vasodilator. Folkow (150) has reported that resistance to flow in the perfused cat limb falls if the blood has to pass through a simple mechanical pump on the in-flow side. He attributes this fall to a vasodilator released as a result of trauma to the blood cells (probably red cells) during passage through the pump; and speculates that various types of flow meters used on the in-flow side of a perfused organ or limb may also lead to such a release. Both, Folkow (150) and Chambliss *et al.* (73) feel that the vasodilator substance released from red cells may be ATP.

In experiments on the effects of drugs on vascular smooth muscle the initial tone of the muscle is sometimes of great importance. This is strikingly illustrated in the studies by Dale and coworkers on the responses of isolated perfused limbs to histamine. In the first study, histamine was found by plethysmography to produce vasoconstriction rather than vasodilation (97). However, later studies (56, 98) showed that the earlier failure to obtain vasodilation was due to the very low initial tone of the vascular smooth muscles which histamine relaxes—namely, the muscles controlling the caliber of terminal arterioles and capillaries. Vasodilation with histamine could be obtained if the initial tone of these muscles was increased by the inclusion of small amounts of epinephrine or vasopressin in the blood used for perfusion. The vasoconstriction of the early studies was attributed to the contracting effect of histamine on larger arterioles. Interestingly enough, acetylcholine and nitrites can produce vasodilation in the same perfused limbs in which histamine causes vasoconstriction. This apparently is due to the ability of acetylcholine and nitrites to reduce the initial tone of the larger arterioles.

The importance of initial tone of vascular smooth muscle has also been stressed by more recent workers. For example, Hilton and Holton (223) found that ATP and extracts of spinal root nerves caused an increase in blood flow through rabbit ears only when the initial tone in the "larger vessels" was low. According to them the vasodilation of the "capillary bed" brought about by these agents cannot appreciably alter resistance to flow if there is high initial tone in the larger vessels, since such tone will continue to determine the peripheral resistance. Thus it can be seen that in the case of testing specific vasodilators in perfusion experiments, one must frequently be aware not only of the general vascular tone, but also of the tone in different anatomical regions of the vascular beds being used. It might also be noted that Ahlquist (2) feels that the ability of certain drugs to reverse the vasodepressor action of epinephrine in animals treated with adrenergic blocking agents is due to the ability of these "anti-adrenolytic" drugs to produce

marked vasodilation (decrease of peripheral vascular tone), so that further dilation by epinephrine is not possible.

Just as in the case of perfused tissues or organs, initial tone of vascular smooth muscle has to be considered in testing the effects of drugs on segments or strips of large vessels *in vitro*. Many such preparations develop an inherent tone of moderate degree during the course of an experiment (338, 339), and may then be used directly for studies on relaxing drugs. However, certain preparations of this type, especially spiral strips of rabbit aorta, usually have very little and sometimes no demonstrable inherent tone (170). In order to study relaxation with drugs on such preparations, it is first necessary to place them in a state of initial tone by the prior addition of a stimulating drug. Although the development of inherent tone in isolated arteries or veins may be useful on occasion, it is a definite handicap in any quantitative studies of the relation between contractile response and concentration of added stimulating drug, since one can never be certain of the influence of the initial tone on the response to the added drug.

The mechanism for the development of inherent or spontaneous tone is not understood. In the occasional experiments in which a moderate level of inherent tone develops in spiral strips of rabbit aorta, it can be readily abolished with nitrites but is not lowered at all by blocking agents such as dibenamine, atropine, and antihistaminics (170). Thus it would appear unlikely that the inherent tone is due to a gradual release within the arterial strips of such substances as epinephrine, norepinephrine, 5-hydroxytryptamine, acetylcholine or histamine. Rabbit aortic strips exhibiting moderate inherent tone also show no significant increase in sensitivity to the stimulating drugs mentioned above that of strips with negligible tone; but with one such strip sensitivity to the stimulating effect of added potassium ions was found to be increased about ten-fold over the sensitivity found in the more usual strip with negligible inherent tone (Furchgott, unpublished results).

Frequently associated with the development of inherent tone in isolated segments or strips of arteries and veins is the development of spontaneous phasic variations in tone. Factors influencing the frequency and intensity of the rhythmic contractions resulting from these phasic variations have been studied by a large number of workers (*e.g.*, 51, 52, 109, 161, 283, 299, 300, 301, 320, 321, 338, 339, 377). Addition of serum or certain stimulating drugs such as epinephrine may often initiate rhythmic contractions, or if such contractions are already present, may increase their frequency. The mechanism by which rhythmic contractions are produced in isolated vessels is unknown, although Peterson's results (320, 321) (see preceding sub-section) raise the possibility that they may be initiated by rhythmic action potentials, just as rhythmic contractions are initiated in certain visceral smooth muscles (35, 36).

Phasic variations in tone are a well-recognized phenomenon in small peripheral blood vessels *in situ* as well as in isolated preparations of larger vessels. Direct microscopic observations of these variations (vasomotion) have been

made by numerous investigators (*e.g.*, 44, 72, 83, 314, 391, 392, 393, 403, 404). Vasomotion in some peripheral vascular beds appears to be dependent on intact efferent innervation (observations by Chambers and Zweifach (72) on rat mesoappendix, and by Clark and Clark (83) and by Wilson (391–393) on rabbit ear). However, in other cases vasomotion appears to be independent of innervation (observations by Nicoll and Webb (314) on bat wings, and by Brun (44) on small arteries of rat abdominal muscles). In recent studies Zweifach (403) has found that although sympathetic denervation abruptly inhibits vasomotion of metarterioles and precapillary sphincters in the rat mesoappendix, vasomotion of these vascular elements returns within four days. As in the case of phasic variations in tone of large vessels *in vitro*, vasomotion of small vessels *in situ* may be modified by the application of drugs. In general, drugs which increase the average level of tone of the vascular smooth muscle of small vessels also enhance vasomotion, while drugs which decrease average tone depress vasomotion. However, there appear to be exceptions, for Wilson (391) found pitressin to inhibit vasomotion of small arteries of the rabbit ear, Brun (44) found ephedrine to inhibit vasomotion of small arteries of rat abdominal muscle, and Zweifach *et al.* (404) obtained essentially no change of vasomotion of metarterioles and precapillary sphincters of the rat mesoappendix with certain "vasodilators" including acetylcholine and adenylic acid. Vasomotion cannot generally be detected in perfusion experiments. However, in such experiments small phasic fluctuations in resistance to flow have occasionally been noted (see, for example, 149, 372) and attributed to synchronized phasic variations in vascular tone.

c. Responses to mechanical stimulation and pressure. That vascular smooth muscle of small vessels *in situ* will contract in response to mechanical stimulation has been known for a long time. The "white reaction" of the skin to the proper type of stroking was attributed by Lewis (272) to a contractile response of small skin vessels. In arteries of the rabbit ear observed through the microscope Grant (198) obtained constriction following application of moderate pressure. Zweifach (401, 403) using micromanipulative procedures produced contraction of individual smooth muscle cells of arterioles and precapillary sphincters in the mesentery of rats and cats by prodding these cells with a micro-needle. Dilation of small blood vessels *in situ* on mechanical stimulation of the area of tissue containing them is an even better known phenomenon (198, 272). The dilation is generally attributed to the action of a substance (H-substance of Lewis) or substances released from the adjoining tissue cells as a result of the mechanical stimulation.

As long ago as 1902 Bayliss (23) presented evidence that the smooth muscle of larger arteries also reacts to mechanical stimulation. He observed a contraction of such vessels in response to an increase in internal pressure and a relaxation in response to a decrease. That even isolated segments or strips of arteries frequently respond to a sudden increase in internal pressure or tension with contraction has been reported by several workers (51, 108, 299, 377). The contraction is generally thought to be myogenic in origin. It should be noted that it is most often observed in isolated preparations possessing good inherent tone.

We have never been able to induce contractile responses to sudden increases in tension with spiral strips of rabbit aorta, whether such strips were initially in a state of negligible inherent tone or in a state of moderate tone due to the presence of a stimulating drug.

In the last several years interest in the responses of blood vessels to changes in internal pressure has been revived, since such responses may afford an explanation of two vascular phenomena. The first is that of an increased resistance to flow with increased arterial pressure in certain vascular beds. This is best known in the case of "autoregulation" of flow in the kidney (293, 347), but Folkow (149) has also reported a less striking but similar situation in the denervated vascular bed of limb muscles. Folkow attributes this to an increase in contractile activity of vascular smooth muscle with increase in stretching tension due to increase in intermal pressure. The second phenomenon which Folkow (146) has attempted to explain on the basis of such a response is the transient reactive hyperemia in a limb following a brief period of arterial occlusion. He reasons that during occlusion the smooth muscles of the vascular bed are no longer subjected to the stimulus of stretch and consequently lose tone; and that the regeneration of tone in these slowly reacting muscles lags behind the sudden build-up of pressure on removal of occlusion, so that a state of relative vasodilation temporarily occurs. Results supporting this interpretation of post-occlusion reactive hyperemia have also been obtained by Hilton (221) in the case of cat limb muscles, and by Patterson and Shepherd (319) and Greenfield and Patterson (205) in the case of human limb muscles.

At very low internal pressures in small vessels a new factor has to be considered (63, 64, 190, 307). This is the so-called "critical closing pressure"—that internal pressure at which a vessel closes completely. Burton concluded that because of the physical properties of blood vessels, they can no longer remain open when the internal pressure falls to the "critical" level at which the equation of Laplace for equilibrium of forces in a cylindrical vessel (*i.e.*, $T = P \times R$, where P is excess hydrostatic pressure inside vessel over outside in dynes/cm.^{2}, T is tension in dynes/cm. length of wall, and R is radius of cylinder in cm.) can no longer be satisfied. According to Burton's calculations the critical closing pressure would increase with increasing tension in the wall (vasomotor tone) and with decreasing size of the vessel. In perfusion experiments in which flow is obtained as a function of pressure, the intercept of the flow-pressure curve on the pressure axis at zero flow is taken as the critical closing pressure of the small vessels controlling resistance to flow. In the rabbit ear the critical closing pressure is around 10 mm. Hg, but by perfusing vasoconstrictor drugs such as epinephrine or naphazoline, or by stimulating the cervical sympathetic nerve it may be raised to several times this level. A point which is of considerable importance in pharmacological studies is that the same dose of a vasoconstrictor drug (or same degree of sympathetic stimulation) will cause a very much greater increase in resistance to flow at low perfusion pressures (*e.g.*, two to three times the critical closing pressure) than at high perfusion pressures.

d. Response to electrical stimulation. Fulton and Lutz (165) observed that

direct stimulation with microelectrodes caused constriction of arterioles of the frog retrolingual membrane in which the nerves were blocked with cocaine. Although it is probable that vascular smooth muscle of arterioles of mammals would also contract on direct electrical stimulation, actual studies on the reactions of mammalian vascular smooth muscle to such stimulation seem to have been limited to larger vessels *in situ* or preparations of arteries *in vitro*. Meyer (292) in the first paper on the behavior of arterial rings reported that they contracted in response to single strong inductorium shocks. However, like many other smooth muscle preparations, arterial rings or strips generally respond much more vigorously to short periods (five to ten seconds) of stimulation with a faradic or alternating current (167, 254) than they do to single shocks.

Furchgott found that end-to-end stimulation of spiral strips of rabbit aorta with an alternating current produced a relatively rapid contraction while the current was on, followed by a continued slow contraction of about a half minute duration and finally a very prolonged relaxation when the current was turned off. Addition of Dibenamine to such strips did not alter the on-current contraction, but it completely blocked the off-current contraction and greatly accelerated the relaxation process. The continued slow contraction and prolonged relaxation after cessation of current was attributed to the release of an "epinephrine-like" substance within the tissue (possibly from sympathetic nerve endings) during electrical stimulation, since Dibenamine would be expected to block the contracting effect of such a substance. That arterial walls do contain measurable quantities of "epinephrine-like" material (as well as histamine and acetylcholine) has been shown by Schmiterlöw (344).

e. Response to light. Furchgott *et al.* (176) have recently reported that spiral strips of rabbit aorta, maintained in a state of tone (either inherent or drug-induced) relax partially during exposure to strong illumination. The relaxation is reversible. The light may be sunlight, strong skylight, or light from a tungsten lamp. Spiral strips from dog carotid and femoral arteries behave similarly. Relaxation on illumination is greatest (up to 50 per cent loss of tone in some strips) when the maintained tone is about half-way between zero and maximal tone. The action spectrum for this photodynamic effect in the case of rabbit aorta was obtained with the use of a monochromator. The maximal relaxing effect occurred at 365–370 mμ, regardless of the drug used to maintain intermediate tone (usually epinephrine or histamine). The relaxing effect fell off on either side of the peak and disappeared below 340 mμ and above 450 mμ. Apparently, some substance in the vascular smooth muscle with an absorption peak near 365 mμ is activated by light and initiates certain chemical reactions which lead to relaxation.

In the presence of $NaNO_2$ (1 to 5 $\times 10^{-4}$), with tone maintained with appropriate concentrations of stimulating drugs, the relaxing effect of visible radiation is markedly potentiated. This potentiation is associated with the appearance of a new action spectrum with a major peak at 410 mμ and two minor peaks at 500 and 540 mμ. Thus it would appear that $NaNO_2$ causes the production of a new photodynamic substance in the muscle with absorption peaks in the visible range of the spectrum. In contrast to $NaNO_2$, glyceryl trinitrate and mannitol

hexanitrate (10^{-5} to 10^{-4}) largely or completely abolish the photodynamic relaxation, even when added after $NaNO_2$. Sensitivity to light is also significantly reduced after additions of NaCN and NaN_3; yet it is not reduced by anoxia (replacement of O_2 by N_2 in the muscle chamber).

This reaction of isolated arteries to light differs in three major respects from previously reported reactions to light of several other smooth muscle preparations. (See 30 for references.) First, in the case of arteries no photodynamic substances, such as fluorescent dyes or porphyrins, need be added as in the case of other preparations. Secondly, oxygen is not required as with the other preparations. And finally, the response with arteries is relaxation, whereas the response reported with other smooth muscle preparations is contraction.

As yet no studies have been made on the effect of light on small peripheral vessels. However, if it is also found that light can cause relaxation of the smooth muscle of such vessels, then some re-evaluation of the results of microscopic observations on such vessels under intense illumination may be required.

III. SOME BIOCHEMICAL CONSIDERATIONS

The number of studies on the biochemistry of smooth muscle in general is very small as compared with the number of such studies on skeletal muscle and cardiac muscle. In particular, the biochemistry of vascular smooth muscle has been a sadly neglected field.

There have been a few studies of the oxygen consumption of isolated arteries by the Warburg method. Briggs *et al.* (39) and Krantz *et al.* (257) reported dry weight Q_{O_2} values of about 1.0 for rat thoracic aorta in Krebs-phosphate medium. We have also obtained a Q_{O_2} of almost 1.0 for rabbit thoracic aorta. If it is assumed that the oxygen consumption is primarily that of the smooth muscle, and that this constitutes about fifty per cent of the vessel wall (see Section I, b), then the corrected dry weight Q_{O_2} of the aortic smooth muscle would be about 2.0 (or wet weight Q_{O_2} about 0.4). This Q_{O_2} is considerably lower than values reported for cardiac and skeletal muscle from small animals, but it is of the same order of magnitude found for smooth muscle isolated from the rabbit small intestine (166). No studies have yet been made on the oxygen consumption of actively contracting vascular muscle. However, it seems likely that increased contractile activity or tone of vascular smooth muscle produced by stimulating drugs would lead to an increased oxygen consumption, just as Bülbring (46) found in the case of the smooth muscle of the taenia coli of guinea pigs.

It has long been known that the maintenance of good inherent or spontaneous tone by vascular smooth muscle is dependent on an adequate supply of oxygen. (See Dale and Richards (98) for the case of perfused vascular beds, and Rothlin (338) for that of isolated strips of large vessels.) We have found that spiral strips of rabbit aorta suspended in Krebs solution containing glucose still respond to stimulating drugs such as epinephrine and histamine under anaerobic conditions; however, the sensitivity to such drugs is much lower than under aerobic conditions and maximal contraction heights obtainable with them are reduced by twenty-five to fifty per cent (Furchgott (167) and unpublished observations).

Apparently the rate of energy production from anaerobic glycolysis is considerably less than that from aerobic oxidations. Südhof (365) has found that the rate of lactic acid production by rings of beef arteries is about two to three times as great under anaerobic as under aerobic conditions.

The pathways for oxidation of energy-yielding substrates in vascular smooth muscle have been studied only indirectly. Briggs *et al.* (39) determined the effects of various added substrates and metabolic inhibitors on oxygen consumption of rat thoracic aorta. Their results provide presumptive evidence for the oxidation of carbohydrate in this tissue by way of the Meyerhof glycolytic pathway and the Krebs tricarboxylic acid cycle. Another indirect method of studying metabolic pathways, rather similar to that used in studying metabolic pathways in intestinal smooth muscle (166, 171, 173, 174), has recently been applied to strips of rabbit aorta (Furchgott, unpublished). Such strips are initially suspended in oxygenated Krebs-bicarbonate solution containing no glucose or other substrate. At intervals the contractile response to a standard dose of epinephrine or a standard electrical stimulus is tested, and when the response has decreased markedly, it is assumed that the endogenous energy-yielding substrates of the muscle have been largely depleted. The ability of various added substances to restore response is then tested. Among the substances found to be effective are glucose, pyruvate, acetate, butyrate, and succinate. With succinate the pH of the medium must be low enough to ensure good penetration (174). From these results with added substrates, along with other results on the inhibiting action of such metabolic blocking agents as iodoacetic acid, glyceraldehyde and fluoroacetate, it appears very likely that there is not only a glycolytic pathway but also a fatty acid oxidation system in vascular smooth muscle—both feeding "active acetate" into the Krebs cycle system.

As with striated muscle, the immediate source of energy for contraction in smooth muscle is probably that of the "high energy phosphate" bonds of ATP, ADP, and phosphocreatine. Recent determinations of these compounds in rabbit thoracic aortas, freed of extraneous fat and connective tissue and incubated in oxygenated Krebs-glucose solution at 37° for 45 minutes, give average values of about 0.2 micromoles per gram (wet weight) for phosphocreatine and 0.7 micromoles per gram for labile phosphate of ATP and ADP. (Furchgott and de Gubareff, unpublished data). If these compounds are assumed to be largely in the smooth muscle, then the corrected values per gram of smooth muscle would be about twice those given. These corrected values are only about one-third as high as average values obtained for phosphocreatine and labile phosphate of ATP and ADP in isolated strips of smooth muscle from rabbit stomach treated in the same manner as the aortas, but are of the same magnitude as the highest values reported for uterine smooth muscle of rats and rabbits (380). They are very much lower than the values reported for skeletal muscle or for cardiac muscle (175, 296).

The contractile proteins of vascular smooth muscle have not been investigated. From smooth muscle of the uterus, Czapó (92) obtained less actin and myosin than from skeletal muscle, and also found the actin to myosin ratio to be lower

than that of skeletal muscle. On addition of ATP, actomyosin threads from uterine muscle contracted more slowly and to a smaller extent than those from skeletal muscle.

Few studies have appeared on the activities of enzymes in blood vessels. Determinations of monoamine oxidase activity and cholinesterase activity in different vessels have been made by Thompson and Tickner (369, 370). With rabbit vessels they found monoamine oxidase activity to be highest in the aorta and higher in carotid, pulmonary and renal arteries than in brachial, femoral and ear arteries, and great veins. The cholinesterase activity found in arteries of different species appeared to be chiefly due to pseudo-cholinesterase. Adenosine triphosphatase activity in various vessels of dog, rabbit, and rat has been measured by Carr *et al.* (67). The dog vessels in descending order of activity were aorta, carotid, coronary, renal, and femoral arteries, and veins. Although by no means certain, it seems likely that the enzyme activities found by these workers in blood vessels (especially the muscular arteries) belong to the smooth muscle of the vessels.

IV. RESPONSES OF VASCULAR SMOOTH MUSCLE TO VARIOUS DRUGS AND THE CONCEPT OF SPECIFIC RECEPTORS

a. Preliminary comments on the development of the receptor hypothesis. A complete survey of the responses of vascular smooth muscle to the multitude of drugs which act upon it is beyond the scope and purpose of this review. However, in this section, in order to introduce the important concept of specific receptors for specific drugs, the responses of vascular smooth muscle to some of the drugs most commonly used in pharmacological studies will be discussed briefly. More space will be devoted to the effects of certain sympathomimetics than to other drugs, because a consideration of their effects best illustrates the usefulness of the receptor theory.

The concept of two types of receptors with which epinephrine can react in vascular as well as other kinds of smooth muscle was first proposed by Dale in 1906 (93). According to him, epinephrine produced a contraction of smooth muscle in which the "motor" type of receptor ("receptive substance" of Langley (267)) was dominant and a relaxation of smooth muscle in which the "inhibitory" type of receptor was dominant. Ergot alkaloids, by selectively blocking the action of epinephrine on "motor" receptors, prevented the contracting effect of epinephrine; and in many vascular smooth muscles, even reversed a contracting to a relaxing effect because of the now unmasked action of epinephrine on unblocked "inhibitory" receptors. In 1910 Barger and Dale (21a) extended this concept of receptors in interpreting the quantitative and qualitative differences in effects of various "sympathomimetic" amines on blood pressure and on activity of certain smooth muscles. They discussed in a general manner not only the importance of the affinities of a particular drug for the two types of receptors in determining its quantitative and qualitative effect but also the possibility that quantitative differences between the different drugs might in part be due to differences in physical and chemical properties which permitted differences in

concentration ratios (partition coefficients) between the extra-cellular fluid and that part of the cell (probably the membrane) containing receptors. Following this early work, the concept of specific receptors with which specific drugs acted in producing their effects on cells came into general use.

In recent years some attempts have been made to classify adrenergic receptors mediating specific responses in different effector organs on the basis of the order of sensitivity of the responses to a series of sympathomimetic amines (1a, 265). The classifications so arrived at are interesting, but are not properly within the scope of this review. It will suffice to say that the adrenergic "motor" and "inhibitory" receptors of vascular smooth muscle are respectively classified as α and β by Ahlquist (1a) and as A_e and A_r receptors by Lands (265).

A problem of more immediate importance in relation to the two types of adrenergic receptors in vascular smooth muscle is whether the individual muscle cell must possess exclusively one or the other type, or whether it may possess both types. In experiments on spiral strips of rabbit aorta it has been observed that isopropylarterenol causes relaxation of tone produced by the prior addition of epinephrine or norepinephrine (168). Since the strips used had negligible inherent tone, isopropylarterenol must have relaxed the very same muscle cells which epinephrine or norepinephrine contracted. Since other experiments had indicated that all three of these drugs act on the same set of inhibitory receptors as well as the same set of motor receptors in rabbit aorta, it was concluded that individual muscle cells may possess both types of adrenergic receptors.

A. J. Clark and his co-workers were the first to use the concept of drug-receptor interaction in interpreting the quantitative relation between concentration of an applied drug and its action on a tissue (usually isolated preparations of smooth muscle or heart). (For details see Clark's extensive review (79).) In order to derive an equation relating these variables, Clark made the simplifying assumptions that the reaction between a specific active drug and its specific receptors is reversible and obeys the law of mass action; that the receptors are all uniform in their affinity for the drug; and that the magnitude of the response elicited by the drug is directly proportional to the fraction of the total number of receptors combined with the drug. He also assumed that when different active drugs (agonists) with different affinities for the same specific receptors combined with the same fraction of receptors, equal responses would be obtained; whereas, when antagonists combined with receptors in the absence of agonists, no response would be detectable. In effect, this assumption demands an all-or-none action on receptors by drugs combined with them.

Clark's well-known equation (with different symbols and re-arranged for convenience in this discussion) relating action to drug concentration is

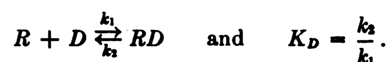
$$A = \frac{A_M(D)}{K_D + (D)}, \qquad \text{(Equation 1)}$$

where A is the measured action, A_M is the maximal action possible (when all receptors are occupied), (D) is the concentration of free drug (assumed to be equal to the concentration of added drug, because concentration of drug greatly

exceeds concentration of receptors), and K_D is the dissociation constant of the drug-receptor complex.[3] This equation, in which A is a hyperbolic function of (D) is analogous to the equation relating velocity of an enzyme reaction to concentration of substrate. If A is plotted against log (D), the familiar symmetrical sigmoid curve is obtained; or if $1/A$ is plotted against $1/(D)$ a straight line is obtained with slope equal to K_D/A_M and intercept equal to $1/A_M$. Clark appreciated the limitations of the above equation as applied to such a complex phenomenon as the response of a tissue to a drug. However, he found it useful in interpreting the mode of action of drugs, for the data obtained in many concentration-action experiments with different isolated muscle preparations and different active drugs could frequently be fitted reasonably well by a curve obtained with this equation. (See 79 for references, and also 325.)

In the particular case of a vascular smooth muscle preparation, namely, an isolated strip from beef carotid artery arranged for either isotonic or isometric recording, Wilkie (389) found contractile responses over a wide range of epinephrine concentration to fall closely along a theoretical curve given by Clark's equation. We have more recently tested the applicability of Clark's equation in the case of strips of rabbit aorta, which have the advantage of possessing practically no inherent tone (170). Experimental curves (plotted as isotonic contraction height against log of concentration) obtained in first epinephrine "concentration series" on individual strips usually deviated considerably at higher levels of contraction from a theoretical curve, but experimental curves obtained in second "concentration series" on the same strips often closely approximated a theoretical curve. In unpublished experiments on aortic strips a number of other drugs, including norepinephrine, phenylephrine, acetylcholine, and 5-hydroxytryptamine, have often given concentration-action curves quite similar to those reported for epinephrine. Histamine, on the other hand, usually gives curves much steeper than curves predicted by the equation, just as it does on many other smooth muscle preparations (79).[4]

[3] This equation is derived on the basis of a reversible reaction involving one drug molecule, D, and one receptor, R:



$$R + D \underset{k_2}{\overset{k_1}{\rightleftarrows}} RD \quad \text{and} \quad K_D = \frac{k_2}{k_1}.$$

If the reaction is between n drug molecules and one receptor, then (D) in the equation becomes $(D)^n$. Clark (79) realized that the concentration of drug in the region of the tissue containing receptors might differ from that in the extracellular fluid when equilibrium was reached. Such a situation, however, would not change the basic form of the equation, but K_D would be the true dissociation constant in the "receptor-region" times the distribution coefficient of the drug between the extracellular fluid and that region. (See Section VI, a.)

[4] It should be noted that the assumptions used in the derivation of Clark's equation limit the proper use of it in vascular pharmacology to experiments on isolated strips of large vessels suspended in fluid in a muscle chamber. With such preparations each concentration of drug in contact with the tissue is known and the final "steady state" contractile response to each concentration can be measured directly. The complicated architecture of vascular beds and the fact that resistance to flow in small tubes varies inversely as the fourth

In view of the simplifying assumptions made by Clark in order to derive his equation, it is rather surprising that so much of the data obtained in concentration-action experiments on smooth muscle fit even reasonably well theoretical curves given by the equation. Even if the same dissociation constant applies to the reaction of all of the specific receptors with a specific drug and if maximal response occurs when all receptors are combined with the drug, it still seems impossible that response should be proportional to the fraction of the total receptors combined with the drug. Rather, a lack of proportionality would be expected because the receptor-drug reaction is only the first step in a complex process leading to response. In the case of contraction of smooth muscle it is likely that the primary receptor-drug reaction leads to a chain of reactions which terminates in the activation of the contractile process. In this case response may still be some positive continuous function of the fraction of receptors combined with the drug, but it will no longer be directly proportional to this fraction. It can be shown mathematically (Furchgott, unpublished) that if the reactions in the hypothetical chain have certain characteristics, hyperbolic concentration-action curves may still be obtained, but the K_D value for such curves is not the dissociation constant, but the dissociation constant multiplied by certain other constants. Moreover, there is the possibility, considered by Stephenson (361), that in some smooth muscles there is a large excess of motor receptors and that only a small fraction of these need be combined with a drug to produce a maximal contraction.

In some smooth muscles the situation may be further complicated if there are both motor and inhibitory receptors for the drug used. Such is the case when rabbit aortic strips are treated with epinephrine; but fortunately the masked relaxing effect is relatively small in this case and occurs over the same concentration range as the contracting effect, so that it probably does not alter the shape of the concentration-action curve appreciably.

Finally, the assumption of Clark that the action of different drugs on the same receptors is all-or-none is also open to criticism. As will be shown in part (*e*) of this section, this assumption does not seem to hold good for a number of sympathomimetic amines acting on adrenergic motor receptors of vascular smooth muscle.

b. Epinephrine. The most common effect produced by epinephrine on vascular smooth muscle is contraction. (See earlier reviews by Hess (219); Dale (95); Lands (263).) This may be observed directly in the case of small vessels in tissues suitable for microscopic *in vivo* studies, and with larger vessels prepared for *in vitro* studies. It is also indicated by the increase in resistance to flow in most perfused vascular beds on the introduction of epinephrine. The smooth muscles of different vessels may vary considerably in their sensitivity to epinephrine. For example, in the rat mesentery Zweifach (403) found sensitivity to decrease in the order metarterioles and capillary sphincters, larger arterioles, and venules;

power of the radius of such tubes make any attempt to apply Clark's equation in perfusion experiments utterly useless. Also, the fact that dose-blood pressure response curves frequently take the form of a rectangular hyperbola should not beguile anyone into an interpretation of them on the basis of this equation.

and Brun (45) was able to produce contractions of small arteries in rat muscle with much lower concentrations than in the case of small arteries in rat omentum.

Despite the widespread excitatory action of epinephrine on vascular smooth muscle, it also causes relaxation of the smooth muscle of certain vessels, and under certain conditions will relax the same vascular smooth muscle which it contracts under other conditions. For example, the vessels controlling resistance to flow in the resting limb muscles of man dilate in response to epinephrine infused intra-arterially over a wide range of concentrations, but constrict if the concentration is above this range (111, 387). There are also many reports of decrease in resistance to flow in perfused limbs of cats and dogs with very low concentrations of intra-arterial epinephrine and increase with higher concentrations (*e.g.*, 85, 99, 105, 155, 189). A similar dependence of type of response on concentration has occasionally been reported for other perfused organs (*e.g.*, rat lung (144), and monkey lung (100)). Dale and Richards (99) attributed the decrease in resistance at low concentrations to dilation of the smallest vessels ("capillary bed"), and the increase at higher concentrations to constriction of arterioles. However, the dual effect might also be explained on the assumption that the adrenergic motor receptors in the smooth muscle of small arterioles controlling peripheral resistance are dominant, but that the adrenergic inhibitory receptors, though subordinate, have a greater affinity for epinephrine.[5] Thus, at appropriate low concentrations the fraction of inhibitory receptors combined with epinephrine would be sufficiently greater than the fraction of motor receptors combined with it, so that relaxation of the smooth muscle (vasodilation) would result. However, at higher concentrations the fraction of the dominant motor receptors combined with epinephrine would increase sufficiently, so that even though the fraction of subordinate inhibitory receptors combined was still higher, contraction (vasoconstriction) would result.

In those vascular beds in which epinephrine produces vasodilation at low and vasoconstriction at higher concentrations, and in some in which it produces only vasoconstriction at all effective concentrations, the vasodilating capacity of epinephrine can be readily demonstrated, as was first shown by Dale (93), by selectively blocking the motor receptors of the vascular bed with either natural or synthetic adrenergic blocking agents (310). Among the vascular beds in which clear-cut reversal of response to epinephrine has been observed after such agents are those of the limb muscles, splanchnic region, rabbit ear, and often the lungs (57, 135, 151, 155, 207, 244, 252, 302, 330, 388). In such beds the blocking agent, in effect, unmasks the presence of significant but subordinate inhibitory receptors. On the other hand, there are other vascular beds, such as those of skin and kidney, in which these agents block either largely or completely the vasocon-

[5] "Dominant" and "subordinate" do not necessarily imply more numerous and less numerous. We have no way of determining the relative number of receptors. The dominant type of receptor would be that type which would determine the nature of the response (motor or inhibitory) when both types of receptors were essentially saturated at high concentrations of drug. "Affinity" would be directly proportional to the association constant or inversely proportional to the dissociation constant (K_D of equation 1) of the drug-receptor complex.

stricting action of epinephrine but do not reverse it (155, 207, 208, 244, 360). The smooth muscle of the small vessels in such beds must therefore possess very few if any adrenergic inhibitory receptors. It should be noted that subordinate inhibitory receptors are not restricted to small peripheral vessels, for reversal of the contracting effect of epinephrine after adrenergic blockade has been frequently observed with isolated strips of large arteries and veins (90, 161). With strips of rabbit thoracic aorta the maximal relaxing effect of epinephrine after blockade with Dibenamine is usually of the order of one-tenth of its maximal contracting effect before blockade (168).

Whether adrenergic inhibitory receptors are ever the dominant type in the vessels controlling resistance to flow of any vascular bed is not known. Perhaps this situation exists in the coronary beds of animals (dogs, cats and sometimes rabbits) in which epinephrine causes a decrease in resistance (156, 246, 253, 268, 346, 394); but it is difficult to assess because of the actions of epinephrine on the myocardium and the effects of these actions in turn on coronary flow (383). However, in the case of the main coronary arteries in certain larger animals (beef and sometimes swine, but not human), there is direct evidence that the inhibitory receptors are dominant, for strips or tubular segments from such arteries relax at high as well as at low concentrations of epinephrine (18, 19, 216, 240, 338, 339, 354, 357).

In connection with the relaxing effect of epinephrine, it should be noted that on Dibenamine-treated strips of rabbit aorta this drug is just as potent in reducing tone under anaerobic (N_2 substituted for O_2) as under aerobic conditions (Furchgott, unpublished results). This is strong evidence against the theory of Bacq and Heirmann (15) that the actual relaxant is not epinephrine itself but some oxidized product of it. More recently Mohme-Lundholm (295) has proposed that the relaxation of smooth muscle by epinephrine is due to an increase in intracellular lactic acid resulting from a stimulation of glycolysis by epinephrine. She based her proposal principally on the following evidence: (a) that relaxation of preparations of rabbit gut, guinea pig uterus, and bovine tracheal muscle by epinephrine is associated with an increase in lactic acid production; (b) that certain metabolic inhibitors of glycolysis block the relaxing effect of epinephrine on such preparations; (c) that ergotamine and ephedrine simultaneously block the effect of epinephrine on contraction and lactic acid production in rabbit gut; and (d) that lactic acid, itself, when added to such preparations, can cause relaxation. Undoubtedly epinephrine can stimulate glycolysis in smooth muscle cells just as it can in many other kinds of cells, but it appears unlikely that extra lactic acid production is the direct cause of relaxation. Evidence against the proposal of Mohme-Lundholm has been obtained in experiments (Furchgott, unpublished results) with rabbit intestinal segments in which the smooth muscle was largely depleted of endogenous substrate and dependent on added substrates as sources of contraction energy (171, 173, 174). Epinephrine was found to be just as potent a relaxant on such preparations using non-glycolysable substrates for energy (pyruvate, butyrate or acetate) as on preparations using glucose, or on fresh preparations before depletion of endogeneous

substrate. Even after blocking "glucose contractions" almost completely with the glycolytic inhibitor *d, l*-glyceraldehyde (0.01 M) and restoring contraction with pyruvate or butyrate there was no reduction in the relaxing potency of epinephrine. Similar experiments with rabbit aortic strips largely dependent on added substrates for contraction energy (see Section III) showed that good relaxation with isopropylarterenol occurred regardless of the substrate added.

c. Norepinephrine. Since 1946 when von Euler (121) first presented strong evidence that norepinephrine is the principal sympathetic transmitter, a great many studies have been made of its effects on vascular smooth muscle. (For reviews see 121a, 263.) In perfusion experiments it produces an increase in resistance to flow in the same vascular beds in which epinephrine does (1a, 58, 151, 155, 207, 244, 252). However, norepinephrine injected intra-arterially in low concentrations does not produce a decrease in resistance in vascular beds of skeletal muscle as does epinephrine (21, 86, 155). Also in such vascular beds norepinephrine has been generally found to give either no vasodilation or only very slight vasodilation following complete inhibition of its vasoconstricting effect by adrenergic blocking agents (155, 207, 244). These results seem to indicate that norepinephrine has practically no affinity for the inhibitory receptors in the vascular beds of limb muscles. However, the possibility exists that its affinity is simply so much lower than that of epinephrine, that no appreciable vasodilation could be detected under the experimental conditions used. I would predict that good vasodilation in perfused limb muscles might be obtained with norepinephrine if very high concentrations were given after complete blockade of adrenergic receptors with an irreversible type of blocking agent such as Dibenamine.[6] (With competitive blocking agents very high concentrations of norepinephrine might "break through" the blockade of motor receptors.) This prediction is based on experience with strips of rabbit aorta (168). In early experiments with such strips relaxation with epinephrine but not with norepinephrine was obtained after Dibenamine treatment (167). However, in later experiments in which more intensive treatment with Dibenamine was used, relaxation equivalent to that with epinephrine was obtained with norepinephrine when much higher concentrations of the latter were used. On the basis of the relative concentrations required for half-maximal relaxation (about 2×10^{-8} for epinephrine and about 10^{-6} for norepinephrine), it might be hypothesized that norepinephrine has only about $\frac{1}{50}$ of the affinity of epinephrine for inhibitory receptors in the smooth muscle of the rabbit aorta.

Although the affinity of norepinephrine for inhibitory receptors is probably much lower than that of epinephrine in the case of most vascular smooth muscle, this does not always appear to be the case. For example, in the perfused rabbit ear Burn and Hutcheon (58) usually found norepinephrine to be about one-half as potent as epinephrine in causing vasodilation after blockade with tolazoline. Also in the case of the coronary bed of the dog Folkow *et al.* (156) have reported

[6] For a similar view about the experimental conditions necessary for obtaining reversal of the vasopressor response to norepinephrine in animals, see Nickerson and Nomaguchi (312).

norepinephrine to be almost as potent as epinephrine as a vasodilator, and Schofield and Walker (346) have reported the two drugs to be of equal potency. Indeed, with perfused tubular segments of coronary arteries Smith *et al.* (357) found norepinephrine to be about twice as potent as epinephrine as a relaxing agent. If it is assumed that relative potency is a measure of relative affinity, then norepinephrine would appear to approach and perhaps even exceed epinephrine in affinity for inhibitory receptors in some vascular smooth muscle.

The potency ratio of norepinephrine to epinephrine in producing contraction of vascular smooth muscle also appears to vary somewhat with the vascular bed or blood vessel being studied, as shown by the following examples. In the renal vascular bed in which relaxation receptors are probably too low in concentration to complicate the situation, epinephrine is several times as potent as norepinephrine (1a, 3). In the vascular bed of dog limb muscle norepinephrine is about twice as potent (207, 244), but in this bed the true vasoconstricting potency of epinephrine is obscured because of its simultaneous vasodilating action. On spiral strips of rabbit aorta norepinephrine is a slightly more potent contracting agent, but if allowance is made for the masked relaxing action of epinephrine at low concentrations, then the two would appear to be of about equal potency (168). In perfused rabbit ears, epinephrine is usually several times more potent. Burn and Robinson (59) have postulated that this may be due to a faster rate of inactivation of norepinephrine by monoamine oxidase in the ear vessels, but this seems unlikely (see Section VI). Finally, in the vascular bed of dog skin, epinephrine and norepinephrine are of about equal potency (207, 244). Thus it would appear that the relative affinity of norepinephrine for motor receptors of different vascular smooth muscles is either about equal to or somewhat lower than that of epinephrine.

d. Isopropylarterenol. Isopropylarterenol is probably the most potent vasodilator among the sympathomimetic amines (263). According to Ahlquist (1a) and Lands (265) this can be attributed to its very high affinity (even higher than that of epinephrine) for the inhibitory receptors of vascular smooth muscle. These investigators obtained no direct evidence for any action of this compound on motor receptors; but Kadatz (245) found that when it was administered to the skin by electrophoresis, it was about $1/200$ to $1/300$ as potent as epinephrine in constricting the smallest cutaneous vessels. More recently it has been shown that in addition to its relaxing effects on rabbit aortic strips over a low concentration range (about 10^{-9} to 10^{-6}) isopropylarterenol has a marked contracting effect over a higher concentration range (10^{-6} to 5×10^{-4}) (170). The maximal contracting effect at the highest concentrations used was reported to be about seventy-five per cent of that obtained with epinephrine or norepinephrine; however, in more recent experiments with the levo-isomer of isopropylarterenol (the racemic mixture was used in the work cited) the maximal effect (at 10^{-3}) has been essentially equal to that obtained with epinephrine or norepinephrine (at 10^{-5}). Making use of the ability of a high concentration of a stimulating drug to protect its own receptors against Dibenamine blockade, evidence was obtained in "cross-protection" experiments that the motor receptors with which isopropyl-

arterenol combined were the same ones with which epinephrine and norepinephrine combined (168). It would therefore appear that this drug can activate adrenergic motor receptors as effectively as epinephrine or norepinephrine, but that its affinity for these receptors is very low compared with its affinity for inhibitory receptors. Using the concentrations required for half-maximal contraction as an index, it can be estimated that the relative affinities of the levo-isomers of epinephrine, norepinephrine and isopropylarterenol for motor receptors in rabbit aorta are about 1, 1 and .01 respectively. On the other hand, on the basis of the concentrations required for half-maximal relaxation after complete adrenergic blockade with Dibenamine, the relative affinities of these drugs taken in the same order for relaxation receptors are about 1, .02, and 4. In view of the ability of high concentrations of isopropylarterenol to stimulate contraction in strips of rabbit aorta, it would be of interest to determine whether similar high concentrations might also stimulate vasoconstriction in perfused vascular beds.

Because of the very high affinity of isopropylarterenol for adrenergic inhibitory receptors, it has been used in several recent studies on whole animals (88, 313) and perfused limbs (331) to activate these receptors continuously at the time of test injections of epinephrine or norepinephrine during the course of adrenergic blockade. This procedure, in effect, largely limits the action of the latter drugs to the motor receptors, and thus permits a better evaluation of the true degree of blockade of motor receptors. Harvey and Nickerson (212) have also used the vasodepressor response to isopropylarterenol in rabbits as an index of the vasodepressor response to be expected with epinephrine after full adrenergic blockade.

e. Other sympathomimetic amines. No attempt will be made here to compare quantitatively the effects of the multitude of sympathomimetic amines on vascular smooth muscle. (The reader is referred to the reviews 26, 263, 264 and to the interesting recent papers 1a, 87, 155, 265, 285, 286, 289, 312, 373.) However, a few comments will be made relative to the action of some of these compounds on adrenergic receptors.

It is probable that practically all sympathomimetic amines which are derivatives of phenylethylamine and contain a phenolic hydroxyl group in the meta-position, produce contraction of vascular smooth muscle principally by a direct action initiated by their combination with adrenergic motor receptors. On the other hand, derivatives which have no phenolic hydroxyl group or only one such group in the para-position appear to produce contraction in most cases primarily by an indirect action. Their direct action is much weaker than that of the derivatives containing a meta-hydroxyl group and is often difficult to demonstrate. (For evidence on direct and indirect actions of sympathomimetic amines see the paper of Morton and Tainter (303) and the previous papers of Tainter and others referred to in it; and also see Section VII of this review.) The much weaker direct action of the phenylethylamines lacking a meta-hydroxyl group can probably be attributed both to their lower affinity for motor receptors and to their smaller capacity for activating contraction when combined with a given fraction of these receptors.

New evidence for a smaller capacity for activating contraction in the case of

ephedrine and amphetamine has recently been obtained in experiments on isolated strips of rabbit aorta (Furchgott and Ashby, unpublished results), in which the principal action of these compounds appears to be direct rather than indirect. On such strips these two compounds produce contraction over a concentration range from about 10^{-6} to 10^{-3}, but the maximal contraction height obtainable at the upper limit of this range is only about thirty-five per cent of that obtainable with compounds containing a meta-hydroxy group, such as epinephrine, norepinephrine, phenylephrine, cobefrine, and epinine. A similar low maximal contraction at a concentration of about 10^{-3} is also found in the case of tyramine if strips are used in which the indirect action of tyramine is insignificant (see Section VII, c and e). That ephedrine, amphetamine, and tyramine actually occupy most of the adrenergic motor receptors when exerting their relatively small maximal effect at a concentration of about 10^{-3}, is indicated by the finding that any one of them at this concentration partially or completely blocks the contractile effect of moderate concentrations ($\sim 10^{-7}$) of epinephrine or norepinephrine. This is an example of competition for receptors analogous to competition for an enzyme between two substrates with markedly different maximal velocity constants.

The idea that compounds like ephedrine and amphetamine can compete with epinephrine for adrenergic motor receptors is not new. Gaddum and Kwiatkowski (185), Graham and Gurd (195) and others have used it to explain why such compounds in high concentrations reduce the vasoconstricting action of epinephrine in the whole animal or in perfused vascular beds. However, it should be noted that the idea that different sympathomimetic amines combined with the same fraction of total motor receptors can produce different degrees of contraction is contrary to the assumption of Clark (79) that drugs on combining with receptors produce an all-or-none action on them. Drugs with relatively low capacities for activating contraction when combined with motor receptors would be classified between those with relatively high capacities for activation and true antagonists, which have no capacity for direct activation.

The relative potencies of different derivatives of phenylethylamine as vasodilators vary as widely as do their relative potencies as vasoconstrictors. From coronary perfusion experiments (268), from perfusion experiments on limbs after adrenergic blockade (155) and from blood pressure studies on animals before and after adrenergic blockade (1, 87, 265, 312), it seems likely that a large part of the variation in vasodilator potency with structure of these derivatives is due to variation in affinity for the adrenergic inhibitory receptors of blood vessels. However, the very low or negligible potency of some of these derivatives as vasodilators, especially those with an unsubstituted phenyl ring or with only one phenolic hydroxyl in the para-position, may again be due not only to a low affinity for the receptors in question but also to a low capacity for activating them, when once combined with them.

It is difficult to evaluate from the available literature how much of the vasoconstriction produced by the aliphatic amines usually classified as sympathomimetics is due to a direct action on adrenergic motor receptors and how much is due to a local indirect action. Since there are other distinct types of motor

receptors with which amines such as histamine and 5-hydroxytryptamine react, the possibility exists that some aliphatic amines may cause vasoconstriction by activating more than one type of motor receptor. The fact that certain β-haloalkylamines can inhibit the vasopressor effect of aliphatic amines (87, 312) does not prove that aliphatic amines mediate their effects exclusively through adrenergic motor receptors, for these blocking agents can also block motor receptors for histamine and 5-hydroxytryptamine in blood vessels (118, 134, 136, 162, 168, 308). Likewise it is not certain that the small vasodepressor effects sometimes obtained with aliphatic amines in animals treated with adrenergic blocking agents are due to a direct action of these amines on adrenergic inhibitory receptors (87, 312). Since many aliphatic amines can release histamine from tissues (297), the vasodepressor effect may be due in part to liberated histamine. Also, in view of the relatively high concentrations used, there is a chance that it also may be due in part to nervous reflexes initiated by stimulation of depressor chemoreceptors.

f. 5-Hydroxytryptamine. Page (317) has recently reviewed the literature on the vascular effects of 5-hydroxytryptamine (serotonin). In certain vascular beds this substance is more potent than epinephrine as a vasoconstrictor and in others it is less potent (186). Its direct contracting effect on vascular smooth muscle can be readily demonstrated with isolated strips or rings of large arteries (168, 326, 349, 350, 398). Evidence for specific motor receptors for 5-hydroxytryptamine distinct from adrenergic motor receptors has been obtained by comparing the effectiveness of various blocking agents in antagonizing 5-hydroxytryptamine and epinephrine on several vascular beds. In the perfused rabbit ear, piperoxan and Dibenamine are more effective against epinephrine, while dihydroergotamine and lysergic acid diethylamide are more effective against 5-hydroxytryptamine (186); and in the perfused dog kidney piperoxan and tolazoline are more effective against epinephrine (318). Differentiation of the motor receptors of vascular smooth muscle which react with 5-hydroxytryptamine, epinephrine and histamine respectively has also been accomplished with the use of "protection" experiments on strips of rabbit aorta (168). With such strips the presence of a very high concentration of any one of these drugs (for the purpose of "saturating" its own receptors) during exposure to Dibenamine protects specifically against Dibenamine blockade of the contracting effect of that drug, but not against Dibenamine blockade of the effects of the other two.

The vasoconstricting effect of 5-hydroxytryptamine is undoubtedly due in large part to direct activation of specific motor receptors by this substance; but it is also possible that part of its effect is mediated by a local indirect action (see Section VII, d). Schofield and Walker (346) recently reported that 5-hydroxytryptamine is approximately equal in potency to epinephrine in causing an increase in coronary blood flow on injection into the coronary artery of dogs. This increase may have resulted from a direct activation of specific inhibitory receptors by this substance; but it is also conceivable that it was due to the action of acetylcholine, norepinephrine or epinephrine (or a combination of these) released in the coronary bed following injection of 5-hydroxytryptamine.

g. Pitressin and hypertensin. These two polypeptides are both potent vasocon-

strictors. (For information and references concerning the vascular pharmacology of pitressin see the review by Dale (95) and the book by Hess (219); for hypertensin (angiotonin) see the review by Goldblatt (191).) They do not appear to react with the motor receptors with which epinephrine or 5-hydroxytryptamine or histamine reacts. The possibility exists that they themselves react with a common type of motor receptor, but too few comparative studies on the effects of highly purified pitressin and hypertensin on the same vascular beds or isolated vessels have been carried out to permit any conclusion. Page and McCubbin (318) found that when tachyphylaxis developed to the vasoconstricting effect of pitressin in the renal vascular bed, there was also some degree of depression of the vasoconstrictor effect of hypertensin. On the other hand, hypertensin causes contraction of strips of rabbit aorta, whereas pitressin (commercial) does not (Furchgott, unpublished results). Indeed, on these strips pitressin actually causes relaxation when it is added after tone has been established with stimulating drugs such as epinephrine or histamine. This finding along with that of Kordik (253) that posterior pituitary extract produces coronary vasodilation in the cat heart (but vasoconstriction in dog and rabbit heart), raises the possibility that there may be inhibitory receptors for pitressin in some vascular smooth muscle; but better evidence obtained with highly purified pitressin is required before acceptance of such a possibility.

h. Histamine. The work of Dale and his collaborators (56, 95, 97, 98, 128) firmly established the fact that histamine causes vasodilation of the smaller arterioles and capillaries but vasoconstriction of larger arterioles and small arteries. The net effect of histamine on resistance to flow largely depends on the arteriolar level at which the transition from vasoconstriction to vasodilation occurs. This in turn varies with the species of animal used, and also with the particular vascular bed under study. For example, histamine under the proper experimental conditions causes a marked decrease in resistance in perfused dog or cat limbs (56, 157), but it causes a marked increase in resistance in perfused dog or cat lungs (102, 184). On practically all isolated preparations of large arteries and veins on which histamine has been tested, it has a contracting effect. This is so even in the case of rings or tubular segments of the main coronary arteries of cows, oxen or pigs which epinephrine relaxes (*e.g.*, 216, 338, 339, 351). With reversed perfusion of the cat coeliac venous bed (severed at the level of the very small veins), Domenjoz and Fleisch (104) obtained vasodilation at very small concentrations of histamine (10^{-9} and 10^{-8}), but vasoconstriction at high concentrations (10^{-6} and 10^{-5}).

All of these observations, along with numerous others not cited, indicate the presence of both motor and inhibitory receptors for histamine in vascular smooth muscle. Which type of receptor is dominant depends on the particular blood vessel to which the muscle belongs. It seems probable that individual smooth muscle cells of certain blood vessels may possess both types of receptors, just as they may possess both types of adrenergic receptors. However, proof of this is difficult to obtain because all antihistaminics so far tested, unlike adrenergic blocking agents, are non-selective and block inhibitory as well as motor receptors for histamine (168).

i. Acetylcholine. The marked vasodilating effect of acetylcholine and related parasympathomimetic agents on various vascular beds was thoroughly investigated many years ago (94, 95, 219, 232, 233). According to Dale and Richards (98) acetylcholine dilates not only the smallest arterioles but also those larger arterioles which histamine constricts. Folkow *et al.* (156) have recently found in cross perfusion experiments (carotid of donor dog to coronary of recipient) that acetylcholine is even superior to epinephrine in decreasing resistance to flow in the coronary bed. However, in some perfused vascular beds, such as the pulmonary bed (100, 144, 145, 184) and the coeliac venous bed (139) acetylcholine often causes an increase in resistance, especially when used in high concentrations. Even perfused rabbit ears, which initially undergo vasodilation in response to acetylcholine, undergo vasoconstriction after several hours of use (60). However, Kottegoda (256) has presented strong evidence that this late vasoconstrictor effect in the ear is not due to a direct action of acetylcholine but rather to the action of an "adrenalin-like" substance released by acetylcholine. (See Section VII, b for details.) In view of Kottegoda's finding, it is possible that the vasoconstriction previously observed in perfused lungs was also due to a similar indirect action rather than to a direct action of acetylcholine on motor receptors. Nevertheless, the contraction produced by acetylcholine on isolated strips or segments of large arteries and veins, which has been so frequently reported (*e.g.*, 51, 161, 168, 356, 379), is undoubtedly due to a direct action of this substance on cholinergic motor receptors. This is evidenced by the findings that atropine is an extremely potent inhibitor of this effect (51, 168). Also, it has been recently observed that hexamethonium or phentolamine (in low concentration), either of which blocks acetylcholine vasoconstriction in the rabbit ear, cannot block acetylcholine contraction in the rabbit aortic strip (Furchgott, unpublished results). Thus it may be concluded that vascular smooth muscle may possess both motor and inhibitory cholinergic receptors, just as it may possess two sets of adrenergic receptors and two sets of "histaminergic" receptors.

j. Adenylic acid derivatives. The earlier work on the effect of adenylic acid derivatives on blood vessels has been reviewed by Drury (107). Of these derivatives adenosinetriphosphate (ATP) is the most active vasodilator; however, adenosinediphosphate (ADP) frequently approaches it in activity (142, 147, 228, 395). Duff *et al.* (113) have found the magnesium salt of ATP to be a more potent vasodilator than the sodium salt in the human hand and forearm. On the basis of both flow measurements and photoelectric determinations of "blood content" in the rabbit ear, P. Holton and coworkers (223, 226, 229) have concluded that ATP and ADP in the smallest effective concentrations dilate the capillary bed. At higher concentrations arterioles appear also to be dilated. Because adenylic acid (AMP) and adenosine produced a vasodilation of shorter duration than did ATP and ADP in the rabbit ear, Holton and Holton (229) proposed that the former agents are primarily arteriolar rather than capillary dilators; however, further evidence is needed before this proposal can be accepted. The similarity in structure of these compounds make it likely that all of them react with the same type of inhibitory receptor, whether such receptors be in smooth muscle controlling capillary calibre or in that controlling arteriolar cal-

ibre.[7] On the basis of their studies on the rabbit ear, Holton and coworkers have made the very interesting proposal that ATP may be the neurohumoral transmitter responsible for antidromic vasodilation on dorsal root stimulation. (For details of their evidence the reader is referred to Section V, f, and to the papers cited above.)

Specific inhibitory receptors for adenylic acid derivatives are probably present in the smooth muscle of many larger vessels as well as peripheral vessels. ATP, ADP, and AMP are all relaxants of isolated strips of rabbit aorta (Furchgott and Ashby, unpublished results). ATP, which is the most potent of the three, even exceeds sodium nitrite in relaxation potency in some strips. Whether there are also motor receptors for adenylic acid in vascular smooth muscle is not clear. Both in the whole animal (115) and in perfusion experiments (184), ATP in sufficient concentration causes pulmonary vasoconstriction. There is a chance that this is the result of some indirect action (see discussion of acetylcholine action on pulmonary vessels); but since ATP has a contracting effect on certain visceral smooth muscles (107, 381), the possibility of vasoconstriction by direct action of ATP on motor receptors in pulmonary vessels should not be ruled out.

k. Nitrites, organic nitrates and azide. The ability of sodium nitrite and organic nitrates and nitrites to produce marked vasodilation of peripheral vascular beds is well known. A large number of workers have also demonstrated the ability of these compounds to produce striking relaxation of isolated strips of arteries. In studies on the relaxation of arterial strips, as in studies on peripheral vasodilation, certain organic nitrites and nitrates (*e.g.*, amyl nitrite, octyl nitrite, glyceryl trinitrate, and mannitol hexanitrate) have been found to be many times more potent (100 to 1,000 times) than inorganic sodium nitrite (258, 259; Furchgott, unpublished results). In the case of spiral strips of rabbit aorta the relaxing effects of these compounds can best be shown if the smooth muscle is first brought to a moderate level of tone with a stimulating drug, because such strips usually exhibit very little or no inherent tone (170). In fact, sodium nitrite (or an organic nitrate or nitrite) is a very useful agent for determining the degree of inherent tone present, since it is capable of abolishing such tone completely. Studies on the physiological antagonism between sodium nitrite and epinephrine on strips of rabbit aorta have also clearly demonstrated that at epinephrine concentrations producing maximal contraction (10^{-5} to 10^{-4}), the inhibitory effect of even very high concentrations of sodium nitrite (10^{-4} to 10^{-3}) is greatly reduced (only 5 to 10 per cent reduction of contraction height). In other words, high concentrations of epinephrine can readily "break through" sodium nitrite inhibition. In more recent experiments epinephrine has also been found to break through inhibition due to high concentrations of the much more potent organic nitrates such as glyceryl trinitrate and mannitol hexanitrate (Furchgott, unpublished results).

According to Graham (194) the vasodilating action of sodium azide is very

[7] Holton and coworkers consider the capillary dilation with ATP and ADP to be due to a direct action of these substances on the endothelial cells of the capillaries. However, the action may very well be on the smooth muscles of the smallest arterioles which control blood flow into the non-muscular capillaries (403).

similar to that of sodium nitrite. The similarity of effects of these two agents, both qualitatively and quantitatively, has also been observed in experiments on rabbit aortic strips (Furchgott and Ashby, unpublished results). The relaxation of these strips by azide is apparently not due to an interference with oxidative metabolism through inhibition of the cytochrome-cytochrome oxidase system, since it is equally effective under anaerobic and aerobic conditions, just as are sodium nitrite and organic nitrates. In view of the similarity of their effects it seems likely that sodium azide, sodium nitrite, and the organic nitrates and nitrites have the same site of action in vascular smooth muscle. Some additional evidence for this is afforded by the finding that in the presence of a concentration of glyceryl trinitrate (10^{-4}) sufficient to produce essentially its maximal relaxing effect against high epinephrine-induced tone, additions of high concentrations of sodium azide, sodium nitrite or amyl nitrite give no further relaxation. Whether the site (or sites) of action of these drugs should be termed a "receptor" is arbitrary. However, a consideration of their chemical structures makes one suspect that their site of action may be at a different level of organization of the smooth muscle cells than are the inhibitory receptors for the other relaxants considered in this section.

Krantz *et al.* (257, 261) have found that certain organic nitrates and organic nitrites (but not sodium nitrite) partially inhibit the adenosinetriphosphatase activity of homogenates of rabbit aorta, and that sodium nitrite and glycerol trinitrate at "therapeutic levels" reduce the rate of oxygen consumption of rat aorta. However, it seems unlikely that these compounds cause relaxation by either of these actions. In this connection it should be noted that these compounds are much more potent relaxants of rabbit aortic strips than either cyanide in high concentration or anoxia; and that they are just as effective under anaerobic as under aerobic conditions. More recently, Hunter *et al.* (234) have reported that sodium nitrite and certain organic nitrates are "uncoupling agents" of oxidative phosphorylation in rat liver mitochondria. In view of this finding it would be very interesting to determine whether any fall in the high energy phosphate compounds of smooth muscle occurs during relaxation with these substances.

V. EFFECTS OF SPECIFIC ANTAGONISTS OR BLOCKING AGENTS ON VASCULAR SMOOTH MUSCLE

a. Receptor theory for "reversible competitive antagonism" and its application.[8] Gaddum (179, 180) first pointed out that much of the quantitative data on the response of tissues to specific active drugs (agonists) in the presence of antagonists to these drugs could be explained on the basis of competition between drug

[8] The term "reversible competitive antagonism" is used in this review to designate that type of antagonism in which the antagonist competes with the agonist by reacting reversibly with the same receptors with which the agonist reacts. This type of antagonism is usually designated by the simpler term, "competitive antagonism," but the fuller term, "reversible competitive antagonism," is preferable in the present review in order to differentiate clearly this type of antagonism from "irreversible competitive antagonism," discussed in Section V, b.

and antagonist for the same receptors. Using a mathematical approach similar to that used in deriving the equation for competitive inhibition of enzymes, he obtained the following equation (rearranged and with different symbols),

$$(RD) = \frac{(R_T)\,(D)}{K_D(1 + (I)/K_I) + (D)} \qquad \text{(Equation 2)}$$

where (RD) is concentration of receptor-drug complex, (R_T) is the concentration of total receptors, (D) is the concentration of free drug, K_D is the dissociation constant of RD, (I) is the concentration of free antagonist, and K_I is the dissociation constant of the receptor-antagonist complex. If, as Clark assumed, response A is proportional to (RD), and maximal response, A_M, occurs when (RD) equals (R_T), then equation 2 becomes

$$A = \frac{A_M(D)}{K_D(1 + (I)/K_I) + (D)} \qquad \text{(Equation 3)}$$

This, of course, reduces to Clark's equation (see Section IV, a) when (I) equals zero.

If one plots A of equation 3 against log (D) for increasing values of (I), the symmetrical sigmoid curves obtained are all parallel and approach the same maximum, A_M, and the extent to which each is shifted to higher concentrations along the log (D) axis away from the curve for which (I) equals zero depends directly on the value of $(I)/K_I$. (Shift in log units is equal to log $(1 + (I)/K_I)$.) However, even if response is not proportional to (RD) and even if the original log concentration-action curve is not a symmetrical one, equation 2 predicts that the situation with respect to parallelism and extent to shift of curves with increasing values of $(I)/K_I$ should be the same as with the curves from equation 3 (as long as the same concentration of RD always gives the same response and the antagonism is due only to competition of I and D for the same receptors). Approximately parallel log concentration-action curves shifted along the log concentration axis as theoretically predicted have been obtained in experiments on antagonism of atropine to acetylcholine in frog heart (78, 79, 80), and on antagonism of atropine and antihistaminics to acetylcholine and histamine in segments of guinea pig ileum (340). Similar curves have also been obtained in studies of dihydroergotamine-epinephrine, dihydroergotamine-norepinephrine, and atropine-acetylcholine antagonism in strips of rabbit aorta (Furchgott (169) and unpublished results). Figure 1 is a plot of data obtained in a typical experiment with dihydroergotamine and epinephrine.

From equation 2 for reversible competitive antagonism one can derive other equations useful in quantitative experimental studies. (For examples, see 74, 76, 179, 180, 385.) The derived equations which relate (I), K_I, and the respective concentrations of D necessary to give the same response (*i.e.*, the same (RD)) in the absence and presence of different concentrations of I have the advantage of not being theoretically dependent on the manner in which response varies as a function of (D) or as a function of (RD) in the absence of I. One equation of this

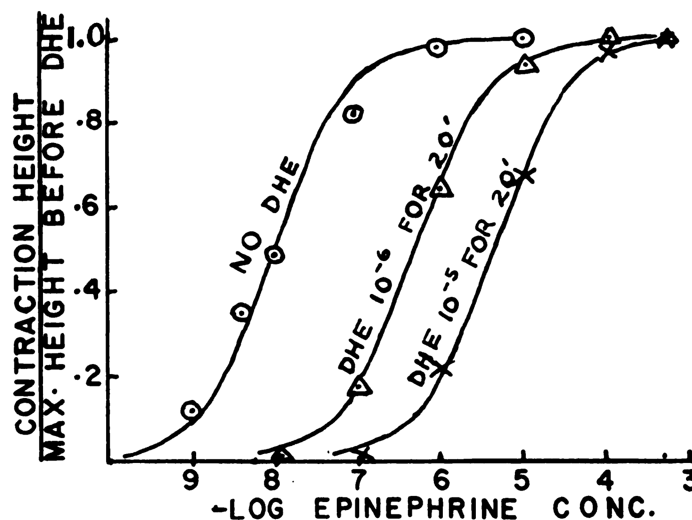


FIG. 1. Effect of a 20 minute exposure to 10^{-6} and 10^{-5} dihydroergotamine methanesulfonate (DHE) on the response of spiral strips of rabbit aorta to epinephrine.

type is:

$$\frac{(D)}{(D') - (D)} = \frac{K_I}{(I)} \qquad \text{(Equation 4)}$$

where (D) and (D') respectively are the concentrations of drug (agonist) necessary to give the same response in the absence and in the presence of antagonist at concentration (I). This equation is obviously useful for obtaining (K_I) values from experiments on isolated muscle preparations. However, it should be emphasized that in order to obtain an actual K_I, "steady state" conditions should prevail with respect to the antagonist at the time responses are determined: that is, sufficient time should be allowed for the antagonist to attain essentially its equilibrium concentration in the receptor region of the tissue (see Section V,c).[9] With many antagonists the approach to equilibrium is so slow that it is impractical to wait for its attainment and one must be satisfied with an "apparent K_I" determined at some time after addition of the antagonist. Apparent K_I values will always be higher than actual K_I values, but they are useful in making comparisons of potency of antagonists. It might be noted that the pA_2 values of Schild (340, 341, 342) for antagonists of acetylcholine and histamine are equivalent to the negative logarithms of apparent K_I values. As Schild has pointed out, there is some variation in pA_2 values for a single antagonist determined in dif-

[9] Strictly speaking, the K_I determined even under "steady state" conditions is not the true K_I, since it is calculated on the basis of concentrations of I in the extracellular fluid (aqueous phase) rather than in that region of the tissue containing the receptors (biophase). It may be considered as equal to the true K_I times the partition coefficient for I between the aqueous phase and biophase.

ferent experiments on the same type of preparation, but this is not surprising in view of the similar variation in sensitivity encountered with agonists. Indeed, the same factors may sometimes determine the sensitivity to both an agonist and its specific antagonist on the same muscle preparation. This is indicated by the finding of Schmiterlöw (344) that the sensitivities of guinea pig ileal strips to histamine and to certain antihistaminics vary in the same direction with different preparations.

Equation 4 (rearranged and with different symbols) or other equations for reversible competitive antagonism have been applied in experiments on whole animals (74, 76, 77, 196, 385). For example, Wells *et al.* (385) obtained data on the blocking action of diphenhydramine against the vasodepressor response of histamine in dogs, which could be very satisfactorily fitted with a theoretical curve based on equation 4. From their data an apparent K_I ($1/\alpha$ in their equation) for diphenhydramine in the whole dog could be calculated. Chen and his co-workers have also attempted to determine apparent K_I values for various adrenergic blocking agents in dogs by application of equations for reversible competitive antagonism to blood pressure responses to epinephrine after injections of different doses of the blocking agents. Their results are interesting, but should be considered with caution, because the blood pressure response to epinephrine is determined not only by its effect on motor receptors of vascular smooth muscle, which are blocked by the agents used, but also by its effect on inhibitory receptors and by its effect on the heart, which are not blocked. From their results with certain β-haloalkylamines, Chen and Russell (76) concluded that these agents are competitive antagonists at low concentrations and non-competitive antagonists at high concentrations. However, the equations used for analysis in their work were the equations for reversible competitive antagonism (equation 3) and for reversible non-competitive antagonism (equation 6), which are derived on the assumption that response to the stimulating drug (in this case epinephrine) is proportional to the number of active receptors with which it is combined. This assumption has proven false in the case of isolated arterial strips (*vide infra*) and undoubtedly cannot be applied in the case of blood pressure studies (see also footnote 4).

I have seen no reports where K_I values or pA_2 values have been calculated for antagonists used in perfusion experiments. However, the experimental data of Fleckenstein (136) on the blockade of epinephrine vasoconstriction by various blocking agents in the perfused rabbit ear permit such calculations to be made with equation 4. This equation has also been used in our laboratory for determining apparent K_I values for a number of antagonists acting on different types of motor receptors in rabbit aortic strips. Some approximate values based on Fleckenstein's data and our own are given in table I.

b. Receptor theory for non-competitive antagonisms and "irreversible competitive antagonism." Hypothetically an antagonist, I, might combine reversibly with some part of the receptor mechanism other than the receptor itself in such a way that while it is combined it prevents the activation of the mechanism result-

TABLE I

Apparent dissociation constants for various receptor-antagonist complexes

Reversible Competitive Antagonist	Agonist Used to Stimulate Specific Receptors	Minutes of Exposure of Tissue to Antagonist	Apparent K_I* in Mols Per Liter
Phentolamine (Regitine)	Epinephrine	30	2×10^{-8} (E)†
	Epinephrine	20	3×10^{-8} (A)
	5-Hydroxytryptamine	20	2×10^{-6} (A)
	Histamine	20	1×10^{-5} (A)
Ergotoxine	Epinephrine	30	4×10^{-9} (E)
Dihydroergotamine	Epi. or Norepi.	30	2×10^{-8} (A)
	Epi. or Norepi.	60	1×10^{-8} (A)
	Epi. or Norepi.	240	5×10^{-9} (A)
	5-Hydroxytryptamine	60	$< 4 \times 10^{-9}$ (A)
Yohimbine	Epinephrine	30	3×10^{-7} (E)
	Epi. or Norepi.	30	2×10^{-7} (A)
	5-Hydroxytryptamine	40	1×10^{-6} (A)
	Histamine	30	3×10^{-4} (A)
Tolazoline (Priscoline)	Epinephrine	30	3×10^{-6} (E)
Atropine	Acetylcholine	30	1×10^{-9} (A)
	Histamine	60	4×10^{-6} (A)
	Epinephrine	30	3×10^{-6} (A)
	Epinephrine	30	6×10^{-6} (E)
Diphenhydramine (Benadryl)	Histamine	40	5×10^{-8} (A)
	Epinephrine	20	2×10^{-5} (A)
Tripelennamine (Pyribenzamine)	Histamine	20	3×10^{-9}(A)
	Epinephrine	30	2×10^{-6} (E)
Phenindamine (Theophorin)	Epinephrine	30	1.5×10^{-7} (E)
Promethazine (Phenergan)	Histamine	30	$< 1.5 \times 10^{-8}$ (E)
	Epinephrine	30	1.5×10^{-7} (E)
Quinidine	Epinephrine	30	3×10^{-6} (E)
2-Methyl-3-ethyl-5-aminoindole	5-Hydroxytryptamine	30	3×10^{-6} (A)
	Epinephrine	30	3×10^{-6} (A)

* Negative logarithm of apparent K_I gives pA_2.

† (E) designates values calculated from data on perfused rabbit ear (Fleckenstein, 1952); (A) designates values calculated from data on spiral strips of rabbit aorta (Furchgott, unpublished).

ing from combination of the receptor with its agonist, D. Such a situation, which will be called "reversible non-competitive antagonism," would be analogous to non-competitive enzyme inhibition. The concentration of receptors activated by the agonist, D, under steady state conditions would be given by

$$(RD) = \frac{\alpha(R_T)\,(D)}{K_D + (D)} \qquad \text{(Equation 5)}$$

where α is the fraction of total receptors, R_T, not inactivated by I and is equal to $K_I/(K_I + (I))$. (For the same equation rearranged and with different symbols,

see Schild (343).) If response is proportional to (RD), then response, A, is given by

$$A = \frac{\alpha A_M(D)}{K_D + (D)} \qquad \text{(Equation 6)}$$

where A_M is the maximal response in the absence of I, and αA_M is the maximal response possible at the particular α determined by the ratio of (I) to its dissociation constant, K_I.

If the antagonist reacts with the receptor mechanism and inactivates it irreversibly, then equation 5 still applies (and also equation 6 if response is proportional to (RD)), except that α is now simply the fraction of the "total receptors still active" (not inactivated) at the time of measuring the response to (D). Conceivably irreversible inactivation might occur either by a reaction of the antagonist with a part of the receptor mechanism with which D does not combine ("irreversible non-competitive antagonism"), or by a reaction with the receptor itself with which D does combine ("irreversible competitive antagonism").

Of the three types of antagonism just described, the only one for which there is actual experimental evidence is "irreversible competitive antagonism". (The fact that the basic equation for non-competitive antagonism (equation 5) applies to it should not be surprising, for the analogous equation in enzyme chemistry applies to the inhibition of cholinesterase by alkyl phosphates which react "irreversibly" with the "active site" of the cholinesterase molecule.) This appears to be the type of antagonism produced by Dibenamine and other β-haloalkylamines. Strong evidence that these blocking agents inactivate adrenergic motor and histaminergic receptors in an essentially irreversible manner has been presented by Nickerson and coworkers (213, 214, 308, 310) and by others (*e.g.*, 196, 197, 273, 274, 363). Evidence that the inactivation should be considered as "irreversible competitive antagonism" was obtained in experiments on rabbit aortic strips (168), in which it was demonstrated that the presence of a very high concentration of an active drug during exposure to Dibenamine could selectively protect the specific motor receptors for that drug against Dibenamine inactivation (successful competition preventing inactivation).

If Dibenamine does act by irreversible competitive antagonism, what should be its effect on the concentration-action curve of a drug whose effect it antagonizes? If it is assumed that response to the drug is proportional to the concentration of the receptors with which it is combined, (RD), equation 6 predicts that in the conventional plot of log concentration against response there will be a proportionate decrease in both maximum and slope of the symmetrical sigmoid curves as inactivation of receptors increases but no shift of the curves along the log concentration axis. (The maximum and slope of each curve will be proportional to α, the fraction of total receptors still active.)

In actual experiments with Dibenamine, however, the log concentration-activity curves obtained with rabbit aortic strips using epinephrine and norepinephrine as stimulating drugs do not behave as predicted by equation 6 (Furchgott, unpublished). The data from one experiment are plotted in figure 2. It will be noted that as the degree of inactivation of adrenergic motor receptors

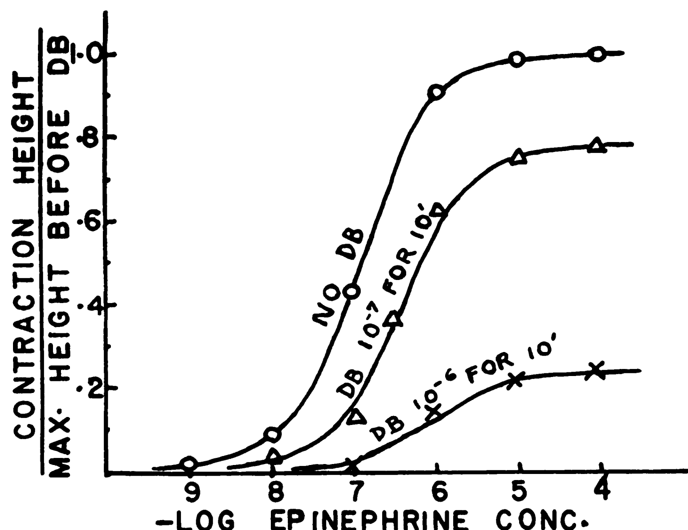


FIG. 2. Effect of a 10 minute exposure to different concentrations of Dibenamine hydro chloride (DB) on response of spiral strips of rabbit aorta to epinephrine. Dibenamine was washed out of muscle chamber at end of exposure period, prior to final testing of response to different concentrations of epinephrine. Exposure to 10^{-5} Dibenamine hydrochloride for 10 minutes completely abolished contractile response to all concentrations of epinephrine.

increases there is both a shift of the curves along the log concentration axis and a decrease in the slope and maximum. Indeed, in some experiments after graded exposures of strips to Dibenamine, the initial shift may be as much as 0.5 log units before there is any appreciable decrease in slope and maximum. One might at first interpret these curves to mean that Dibenamine blocks by reversible competitive antagonism at low concentration (or short exposures), thus causing the shift; and by irreversible competitive antagonism (inactivation) at high concentrations (or longer exposures), thus causing the decrease in slope and maximum. However, since similar sets of curves have been obtained one or two hours after washout of Dibenamine from the muscle bath, and since free Dibenamine does not remain in the tissue for such long periods of time (168, 169), the shift along the log concentration axis cannot be due to reversible competitive antagonism. Apparently the discrepancy between the actual effects of Dibenamine on the concentration-action curves of epinephrine and norepinephrine and those predicted by equation 6 is due to the fact that contractile response is not proportional to the concentration of receptors combined with drug, (RD), as assumed in equation 6. At present there is no way of knowing just how response varies as a function of (RD), but as pointed out in Section IV,a, a hypothetical equation may be derived in which response is no longer proportional to (RD) but is still a hyperbolic function of (D), as it is in equation 1, 3 and 6.[10]

[10] The simplest equation of this type is derived on the assumption that contractile response, A, is proportional to the contracted fraction, (C_c), of the total contractile protein (C_T), so that

$$A/A_T = (C_c)/(C_T) \quad \text{(Equation 7)}$$

Stephenson (362) has also observed that graded exposure of strips of guinea pig ileum to SY28 (N-α-naphthylmethyl-N-ethyl-β-bromoethylamine), a congener of Dibenamine, causes both a shift of the log concentration-action curves to histamine along the log concentration axis and a decrease in the slopes and maxima. The extent of shift prior to any significant decrease in slope and maximum in his experiments is even greater (over 2 log units) than in our experiments on rabbit aortic strips. In view of these results with isolated smooth muscle preparations, it is not surprising that quantitative data on the blocking action of low doses of β-haloalkylamines against epinephrine vasopressor effects in dogs (74, 76, 77, 196) and of higher doses against histamine vasodepressor effects (196) have been satisfactorily fitted by equations for reversible competitive antagonism.

c. Kinetics of the development of and recovery from reversible competitive antagonism. As noted in Section V, a, when a reversible competitive antagonist is added to the extracellular fluid in contact with smooth muscle, time is required for the development of its maximal blocking action. Similarly, after removal of the antagonist from the extracellular fluid, time is required for recovery from the blocking action. Numerous examples of this relationship of blocking action to

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where A_T is the response when all the contractile protein is in the contracted state. At a steady level of contraction the rate of formation of C_c from relaxed contractile protein, C_r, must be equal to its rate of conversion back to C_r. If it is assumed that the rate of formation of C_c is proportional to both (RD) and (C_r) and that the rate of conversion back to C_r is proportional to (C_c), then at the steady state

$$k_c\,(RD)\,(C_r) = k_r(C_c) \qquad \text{(Equation 8)}$$

where the constant k_c may be expected to depend on several factors (including energy immediately available for contraction, etc.) and the constant k_r is the relaxation rate constant for the contractile protein. Substituting $(C_T) - (C_c)$ for (C_r) of equation 8 and rearranging one obtains

$$\frac{(C_c)}{(C_T)} = \frac{(RD)}{K_R + (RD)} \qquad \text{(Equation 9)}$$

where K_R is substituted for k_r/k_c. Substituting for $(C_c)/(C_T)$ from equation 7, and for (RD) from equation 5, and rearranging, one obtains

$$A = \frac{A_T\left\{\dfrac{1}{K_R/\alpha(R_T) + 1}\right\}(D)}{K_D\left\{\dfrac{1}{\alpha(R_T)/K_R + 1}\right\} + (D)}. \qquad \text{Equation (10)}$$

The theoretical curves in figure 2 were obtained with this equation, with $K_R/(R_T)$ equal to 0.153, and α equal to 1.0, 0.33, and 0.039 respectively for the three successive curves. This type of equation is also satisfactory for analyses of reversible competitive inhibition, in which case K_D is simply multiplied by $(1 + (I)/K_I)$ and α remains equal to 1. It also helps explain changes in the concentration-action curve under adverse metabolic conditions which might be expected to raise the value of K_R. If $K_R \ll R_T$, then α may be decreased markedly without causing a significant decrease in maximal response, but only a shift of the log concentration-action curve, resembling the shift in reversible competitive antagonism.

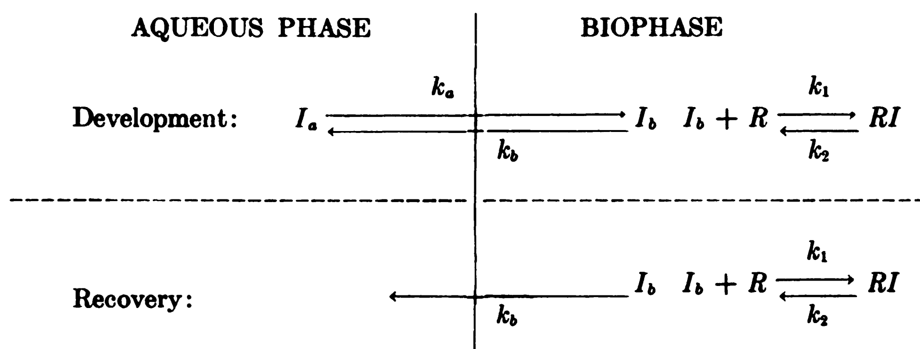
time are to be found in the literature dealing with competitive antagonists acting on either isolated muscle preparations or perfused vascular beds. (See section on kinetics in review of Clark (79) and also more recent papers (25, 126, 136, 169, 302, 327, 328, 330, 334, 340, 341).) In general it has been observed that if a reversible competitive antagonist requires a long period of time for development of its maximal blocking activity against an agonist, a long period is also required for recovery of sensitivity of the tissues to the agonist following removal of the antagonist from the extracellular fluid.

Fleckenstein (136) observed very slow recoveries (1 to 3 hours or more) of the full sensitivity of the perfused rabbit ear to the vasoconstrictor effects of epinephrine and histamine following perfusion of certain potent competitive antagonists of these drugs. He attributed this to the prolonged attachment of the antagonists to the specific motor receptors. This is the same concept which Clark (78) used to explain the slow reversal of atropine antagonism to acetylcholine on the frog heart. According to this concept, the slow rate of recovery is determined principally by the slow rate of dissociation of the receptor-antagonist complex. However, this cannot be correct for reversible competitive antagonism, since it would produce a situation essentially like that of irreversible competitive antagonism. In other words, if the mean life-time of a single receptor-antagonist complex were very great (prolonged attachment), the only receptors with which an agonist could react within a relatively short time after addition would be those receptors not already combined with antagonist at the time of addition. (This is so because an agonist only reacts with free receptors, and does not—as is commonly but erroneously believed—directly displace an antagonist from a receptor-antagonist complex). Such a situation would preclude the possibility of a rapidly developed competitive "break through" following the addition of a high concentration of agonist to a smooth muscle pre-incubated with a high concentration of antagonist ($(I) \gg K_I$). But there are many examples of such "break throughs" even in the case of ergot alkaloids from which recovery is extremely slow. (See 37, 169, 178, 266, 290, and also figure 1.)

Another more promising concept to explain the slow recovery of sensitivity to an agonist following washout of the antagonist was proposed by Fastier and coworkers (124–126), who investigated the time for recovery of sensitivity of various preparations (perfused rat hind quarters, guinea pig ileal strips, and even amine oxidase of liver suspensions) to agonists following exposure to antagonistic S-alkyl-isothioureas with side chains of varying length. They considered rate of recovery to be dependent on the rate of escape of the antagonist from the tissue "biophase" into the aqueous phase. The biophase (a convenient term first used by Ferguson (132) in studies on homologous series of narcotics) might be considered to contain the receptors or to be interposed between the receptors and the aqueous phase (extracellular fluid). Since it seems highly probable that the biophase is the cell membrane itself and that the receptors for the drugs under discussion are in the membrane, the biophase of Fastier and coworkers would be equivalent to what has been called previously in this review the "region of the cell containing the receptors". As pointed out by these workers

the greater affinity a drug has for the constituents of the biophase, the slower will be its diffusion into the aqueous phase from the biophase and the higher will be its partition coefficient between this phase and the aqueous phase.

The relation of the biophase to the kinetics of the development of blockade during exposure to a competitive antagonist and of recovery from blockade after removal of the antagonist is shown in the following scheme:



In this scheme, in which certain simplifying assumptions are made for convenience, k_a and k_b are respectively the rate constants for entry of the antagonist into the biophase and for escape from it (therefore, k_a/k_b = the partition coefficient); and k_1 and k_2 are respectively the rate constants for the formation and dissociation of the receptor-antagonist complex, RI, from free receptors, R, and free antagonist, I_b, in the biophase (therefore $k_2/k_1 = K_I$). Assuming that the concentration of free antagonist in the aqueous phase, (I_a), remains constant (as in experiments on isolated tissues) and that its concentration in the biophase, (I_b), is not appreciably reduced by the formation of RI or by enzymatic inactivation and is essentially uniform throughout this phase, then the following equations may be derived to give (I_b) as a function of time:

Development: $$(I_b) = \frac{(I_a)k_a}{k_b}(1 - e^{-k_b t});$$ (Equation 11)[11]

Recovery: $$(I_b) = (I_b)_0 \, e^{-k_b t};$$ (Equation 12)[11]

where t is the time elapsed from the beginning of exposure during "development", and the time elapsed from the end of exposure (washout) during "recovery", and $(I_b)_0$ is the concentration of antagonist in the biophase at the end of exposure.

It will be noted that the same exponential term determines both the rate of approach to the equilibrium concentration of I_b in development of blockade and

[11] Equation 11 is derived by integrating the differential equation:

$$d(I_b)/dt = k_a(I_a) - k_b(I_b). \qquad [(I_b) = 0 \text{ when } t = 0]$$

Equation 12 is derived by integrating the differential equation:

$$-d(I_b)/dt = k_b(I_b). \qquad [(I_b) = (I_b)_0 \text{ when } t = 0]$$

the rate of approach to complete removal of I_b in recovery. The time for half-completion of both processes ("half-time" or $t_{\frac{1}{2}}$) is equal to $.69/k_b$. If the rate constant k_b is much lower than k_1 or k_2, it will also determine the rate of approach to the maximal reduction of free receptors at constant (I_a) during development, and the rate of approach to complete restoration of free receptors during recovery. Thus, this formulation helps explain the general finding mentioned earlier that the reversible competitive antagonists which require the longest times to produce maximal possible blockade during exposure are the ones which require the longest times for full recovery from blockade after exposure.

It is possible to show mathematically by combining equation 12 with the equation for the concentration of free receptors, (R), as a function of (I_b), namely,

$$(R) = \frac{(R_T)}{1 + (I_b)/K_I}, \qquad \text{(Equation 13)}$$

that the recovery of free receptors following an exposure giving an $(I_b)_0$ about 100 times greater than K_I, will follow a sigmoid curve as a function of time. With this approach it is possible to explain the sigmoid curves which Rocha e Silva and Beraldo (334) and Beraldo and Rocha e Silva (25) obtained on plotting the recovery of response of guinea pig ileal strips to small standard doses of histamine or acetylcholine against time, following exposure to competitive antagonists; and even to calculate k_b and $t_{\frac{1}{2}}$ values with which theoretical curves may be derived to fit their data very satisfactorily (Furchgott, unpublished).

Sigmoid curves similar to those of Rocha e Silva and Beraldo have also been obtained with rabbit aortic strips on plotting the recovery of response to small standard doses of epinephrine against time after exposure to yohimbine, or to small standard doses of histamine after exposure to certain antihistaminics (Furchgott and Ashby, unpublished). However, it has been found more practical in the case of aortic strips, which contract and relax very slowly compared with guinea pig ileum, not to rely on analyses of the sigmoid recovery curves for calculation of k_b or $t_{\frac{1}{2}}$ values, but to make such calculations on the basis of the following equation obtained by combining equation 11 and equation 4:

$$k_b = \frac{.69}{t_{\frac{1}{2}}} = \frac{2.3}{t} \log_{10}\left\{\frac{(D')_0 - (D)}{(D')_t - (D)}\right\} \qquad \text{(Equation 14)}[12]$$

[12] To obtain equation 14, equation 4 is set up to give

$$\frac{(D')_0 - (D)}{(D)} = \frac{(I_b)_0}{K_I}$$

and

$$\frac{(D')_t - (D)}{(D)} = \frac{(I_b)_t}{K_I}$$

Combining these two equations and equation 12, one obtains

$$\frac{(D')_t - (D)}{(D')_0 - (D)} = \frac{(I_b)_t}{(I_b)_0} = e^{-k_b t},$$

which gives equation 14 when solved for k_b.

in which (D), $(D')_0$ and $(D')_t$ are respectively concentrations of agonist giving equal responses before exposure to the antagonist, at the end of exposure to it (zero time), and at time t following the end of exposure.

This equation has been used for analysis of recovery of response of aortic strips to epinephrine and norepinephrine following exposure to various concentrations of dihydroergotamine for various lengths of time. The mean $t_{\frac{1}{2}}$ (half-time) for this antagonist was 83 ± 3 minutes (k_b = .0083 min.$^{-1}$) in seven experiments (Furchgott, unpublished results reported at 1954 meeting of Federated Societies (169)). In two experiments in which development of blockade was followed by testing responses to epinephrine at intervals during exposure to a fixed concentration of dihydroergotamine over several hours (see table 1 for part of data obtained), k_b values of .0084 and .0075 min.$^{-1}$ gave curves fitting the data satisfactorily. Thus, as predicted by equations 11 and 12 the same rate constant limits both the approach to maximal blocking action and the approach to complete recovery after exposure. With a $t_{\frac{1}{2}}$ of about eighty minutes for escape from the biophase, it would take about 8 hours for the $(I_b)/K_I$ ratio of dihydroergotamine to fall to about one per cent of its initial level.

Of the reversible competitive antagonists which we have investigated on aortic strips dihydroergotamine has by far the longest $t_{\frac{1}{2}}$. The $t_{\frac{1}{2}}$ values for some other antagonists in preliminary experiments have been about fifteen minutes for yohimbine (against norepinephrine), twenty minutes for phentolamine (against epinephrine), fifteen minutes for atropine (against acetylcholine), and five minutes for diphenhydramine (against histamine or against norepinephrine). The finding that the same $t_{\frac{1}{2}}$ value is obtained for diphenhydramine in experiments with two agonists (histamine and norepinephrine) of considerably different sensitivities to blockade by this antagonist is further evidence that the rate of recovery from blockade is dependent on the escape of the antagonist from the biophase. The data of Fleckenstein (136) showing that complete recovery of response to histamine is faster than that to epinephrine in the same ear following perfusion of phentolamine or yohimbine, and that it is much slower than that to epinephrine following certain antihistaminics, at first appears contrary to this finding. However, his data are easily explained on the basis of the large difference in the K_I values (see table 1) of the adrenergic and histaminergic receptors for each antagonist which he used.[13]

It should be noted finally that in the case of aortic strips extra washings following the initial washout of a reversible competitive antagonist do not hasten the rate of recovery. Apparently the amount of antagonist taken up by such a strip (weighing 25 mg. and suspended in 20 ml. of medium) during exposure, and subsequently released into the medium during the post-exposure period, is too

[13] It is conceivable that cases may be found in which recovery of sensitivity to an agonist following washout of a reversible competitive antagonist is more dependent on the rate of dissociation of the specific receptor-antagonist complex than on the rate of escape of the antagonist from the biophase. In such a situation k_2, the rate constant for dissociation, would be smaller than k_b, the rate constant for escape. In all of the experiments with reversible competitive antagonists which have so far come to my attention, there have been no quantitative data on recovery which, on analysis, indicated a situation of this type.

small to produce a concentration in the medium sufficient to give a detectable blockade.

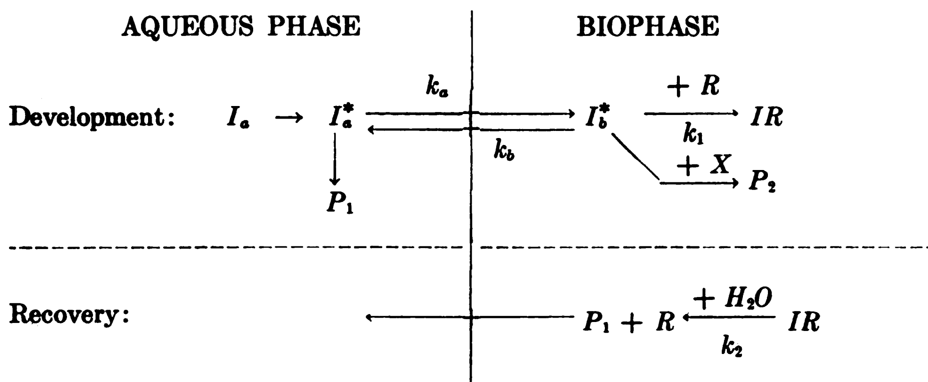
d. Kinetics of the development of and recovery from irreversible competitive antagonism. The only antagonists for which there is strong evidence indicating irreversible competitive antagonism (irreversible inactivation of receptors) as the mechanism of action are the β-haloalkylamines (308). There is some possibility that azopetine (Iladar) (302), with its reactive N-allyl group, may also act by such a mechanism. In whole animal experiments, perfusion experiments and experiments on isolated muscle preparations, the degree of blockade of various types of specific receptors with β-haloalkylamines depends not only on the concentration of the agent used but also on the time of exposure to it (136, 168, 182, 196, 308, 363). It seems highly probable that the actual inactivation of receptors in tissues exposed to these agents is principally due to a reaction of the receptors with the highly reactive cyclic ethyleniminium intermediates formed from these agents (133, 196, 197, 213, 214, 308, 310).

Following full blockade of adrenergic motor receptors by these agents in experiments on whole animals (blood pressure), perfused organs, or isolated arteries, the recovery of sensitivity to the stimulating effects of sympathomimetics is extremely slow, with no significant recovery detectable in one to two hours and only partial recovery even after one or two days (8, 11, 40, 136, 168, 182, 197, 308, 309). This extremely slow recovery is to be expected if the receptors have been inactivated by an irreversible chemical reaction. Brodie and coworkers have proposed that the very slow recovery is largely due to the concentration in and slow escape of these blocking agents from fat depots, but the experiments of some of the other investigators cited above show clearly that it is principally due to the very slow regeneration of active adrenergic motor receptors. This regeneration presumably is the result of either a gradual synthesis of new receptors or a gradual reformation of active receptors from the very receptors which have been inactivated, or of both processes. Evidence from experiments in our laboratory on the recovery of sensitivity of aortic strips to epinephrine and norepinephrine after Dibenamine blockade favors the second process; and it has been suggested that the product (inactivated receptors) of the reaction between the receptors and the ethyleniminium derivative of Dibenamine subsequently undergoes a very slow hydrolytic reaction which liberates active receptors (168). Such a reaction would be analogous to that proposed by Wilson (390) to explain the slow reactivation of cholinesterase after inactivation with tetraethylpyrophosphate.

In experiments on perfused rabbit ears (136, 182) and on isolated strips of rabbit aorta (168), Dibenamine blockade of the constrictor effects of histamine or 5-hydroxytryptamine appears to be just as irreversible over a several hour post-exposure period as blockade of the constrictor effect of epinephrine. However, Graham and Lewis (196) have reported that blockade of the vasodepressor effect of histamine in cats by related β-haloalkylamines undergoes significant reversal in a matter of a few hours. Apparently the rate of regeneration of receptors following inactivation by these blocking agents is dependent on the particular type of receptor inactivated. It may also be dependent on the particular

substituent groups on the amino-nitrogen of the β-haloalkylamine, just as the rate of regeneration of cholinesterase activity after inhibition by alkylphosphates (*e.g.*, diisopropylfluorophosphate and tetraethylpyrophosphate) is dependent on the particular alkyl groups esterified with the phosphate.

On the basis of the present evidence (133, 168, 196, 197, 213, 214, 308, 310), it may be hypothesized that development of blockade during exposure of isolated tissues to a β-haloalkylamine, and the recovery from blockade after removal of such an agent, occur according to the following scheme:



In the scheme for development I_a is the native compound in the aqueous phase; I_a* and I_b* are the cyclic ethyleniminium derivative in the aqueous phase and biophase respectively; R is the specific receptor; IR is the product of the chemical reaction between I_b* and R (inactivated receptor); P_1 is the alcohol analogue of I_a formed by the hydrolysis of I_a* and P_2 represents products formed by the reaction of I_b* with tissue constituents, X, other than the specific receptor. The rate of inactivation of receptors will depend on (I_b*), (R), and the rate constant, k_1. (I_b*) is obviously a complex function of (I_a) added, time of exposure, and the various rate constants, including those controlling the formation of P_1 and P_2. The more rapid blocking activity of certain β-bromo- and iodo-ethylamines as compared with homologous chloro-compounds is apparently due to a much faster rate of conversion to the ethyleniminium intermediates (197). Undoubtedly, the native compound, I_a, also penetrates the biophase (not shown in the scheme), and perhaps some part of the inactivation of receptors is due to a direct reaction with it, especially in the case of those compounds like Dibenamine for which the conversion from I_a to I_a* is relatively slow (213).

In the hypothetical scheme for recovery from blockade, the rate of restoration of active receptors would be dependent on the rate constant, k_2, and would be an exponential function of time. This rate constant would have to be exceedingly small, especially in the case of adrenergic motor receptors. The data on recovery of sensitivity of rabbit aortic strips to epinephrine after exposure to Dibenamine would require a rate constant less than .03 hours^{-1}, or a half-time for restoration of receptors of greater than 24 hours (168).

In the whole animal the situation with respect to the fate of β-haloalkylamines

is undoubtedly more complex than that shown in the scheme. This is indicated by the interesting findings of Brodie and colleagues (11, 40) that the only significant urinary excretion products of Dibenamine and Dibenzyline are their respective de-alkylated amines. Apparently *in vivo* a major portion of these compounds or their products is metabolized to these amines.

e. On the relative specificity of specific antagonists. Many a reversible competitive antagonist has been shown to be effective in reducing the sensitivity to different agonists acting on different types of specific receptors in the same smooth muscle preparation. In many cases the difference in concentrations of the antagonist necessary to produce an equal degree of blockade against two or more different agonists is less than thirty fold. Such is the case, for example, with diphenhydramine antagonism of histamine, 5-hydroxytryptamine and acetylcholine in the guinea pig ileum (324, 340); yohimbine antagonism of epinephrine and histamine in the perfused rabbit ear (136); yohimbine antagonism of epinephrine and 5-hydroxytryptamine in rabbit aortic strips (see table 1); and dihydroergotamine antagonism of epinephrine and 5-hydroxytryptamine in rabbit aortic strips (see table 1) and in the perfused rabbit ear (182). Such antagonists acting on the smooth muscle preparations mentioned have a low relative specificity.

Even those reversible competitive antagonists which are usually considered as specific blocking agents of only one type of receptor in a given type of smooth muscle are actually not completely specific but simply possess a high "relative specificity". In experiments in which the concentration of such an antagonist has been raised anywhere from about a hundred to several thousand times the concentration required to show antagonism against its "specific" agonist or agonists, demonstrable antagonism against other agonists has always occurred. This is the case with such "specific" antagonists as the antihistaminic tripelennamine, the adrenergic blocking agent phentolamine, and the cholinergic blocking agent atropine acting on vascular smooth muscle (see table 1). Many other examples could be cited both in the case of vascular smooth muscle and other types of smooth muscle (*e.g.*, 182, 324).

The lack of complete specificity in the case of any one reversible competitive antagonist is due to its capacity to combine with many types of specific receptors (see Section V,a, and Fleckenstein (136)). The degree of "relative specificity" of such an antagonist will depend, according to receptor theory, on how much lower its dissociation constant, K_I, is for one specific type of receptor than are its dissociation constants for other types. The observation of Burn and Dutta (57) that a very wide variety of substances (atropine, diphenhydramine, meperidine, procaine, and quinidine) were capable of reversing the vasoconstrictor effect of epinephrine in the rabbit ear can be explained on the assumption that sufficiently high concentrations of these substances were used ($(I) > K_I$) to compete successfully with epinephrine for adrenergic motor receptors.

Just as there are no known completely specific reversible competitive antagonists, there are no known completely specific irreversible competitive antagonists. It is well established that all β-haloalkylamines which block adrenergic motor receptors also block motor and inhibitory receptors for histamine (196, 197, 273,

274, 308, 363). Most of them are more potent blocking agents against sympathomimetics but some are more potent against histamine. A number of these agents are also quite potent in antagonizing the vasoconstrictor action of 5-hydroxytryptamine on vascular smooth muscle (118, 134, 162, 182); and one of them, Dibenamine, at a concentration only ten times that needed for adrenergic blockade had been found to be highly effective in blocking irreversibly the contracting effect of acetylcholine on the smooth muscle of rabbit aorta (168). The possibility that some of these agents may even be capable of inactivating cholinergic inhibitory receptors in the smooth muscle of peripheral vessels is indicated by the recent report of Loubatiéres and Bougard (275) that the β-chloro derivative of 883F (2-(diethylaminomethyl)-1,4-benzodioxane) is a potent blocking agent against the vasodepressor effect of acetylcholine.[14] Thus the irreversible competitive antagonists, like the reversible competitive antagonists, possess only varying degrees of relative specificity as far as blocking action is concerned. It is probable that the degree of relative specificity of any one of them for one type of receptor depends on the magnitude of the inactivation rate constant, k_1, for that type of receptor (see diagram in section V,d) relative to the magnitude of the rate constants for other types of receptors.

f. Some comments on the use of antagonists in studies on vascular pharmacology and physiology. Antagonists with relatively high specificity of action have been used extensively in attempts to determine the nature of naturally occurring substances which influence the tone of vascular smooth muscle. Shortly after von Euler (121) brought forward chemical evidence in support of the hypothesis that norepinephrine rather than epinephrine is the principal sympathetic neurohumoral transmitter, Folkow and coworkers provided pharmacological evidence for the same hypothesis. They showed that the vasoconstrictor effects of sympathetic nerve stimulation (direct or reflex) and of norepinephrine on the same atropinized vascular beds were similarly blocked but not reversed by Dibenamine (148, 151, 153, 155). Green and coworkers have also observed that graded doses of various adrenergic blocking agents (tolazoline, phentolamine, azopetine, and Dibenzyline) in dog limb muscle antagonize the vasoconstrictor effect of sympathetic nerve stimulation and that of norepinephrine similarly (200, 203, 209). However, in a recent investigation Green *et al.* (202), using the same blocking agents, found the vasoconstrictor effect of sympathetic stimulation in dog skin to be antagonized somewhat more like that of epinephrine than that of norepinephrine. In particular, they found that phentolamine and Dibenzyline in certain concentrations actually caused a slight reversal of the norepinephrine effect (production of vasodilation), while only blocking but not reversing the effects

[14] In connection with the antagonism of β-haloalkylamines toward acetylcholine, it should be noted that inhibitory effects of acetylcholine on isolated guinea pig auricles are blocked almost completely and irreversibly by exposure of the auricles to 10^{-6} Dibenamine for 30 minutes (168). Moreover SY28 (N-α-naphthylmethyl-N-ethyl-β-bromoethylamine) will not only block the effects of acetylcholine at lower concentrations than Dibenamine, but will also produce a stimulatory effect on rate and height of contraction practically equivalent to that given by concentrations of epinephrine of the same magnitude (Kelch and Furchgott, unpublished results).

of epinephrine and sympathetic stimulation. They cautiously suggest that the sympathetic transmitter to the cutaneous vessels may be an "epinephrine-like" substance; but more work will have to be done to settle this question.

Green and coworkers have also made the interesting observation that in dog limb muscle the adrenergic blocking agents mentioned above are capable not only of reversing the effect of epinephrine to marked vasodilation, but also at concentrations of the order of 10 to 30 times those required for maximal reversal, of blocking the vasodilation of epinephrine and isopropylarterenol (201, 207, 244). This effect, which occurs both in innervated and sympathectomized limbs, is at variance with the general belief that adrenergic blocking agents block only the adrenergic motor receptors and not the adrenergic inhibitory receptors of vascular smooth muscle (308). Before this belief is discarded, however, the situation should be more thoroughly explored. In experiments with isolated strips of rabbit aorta we have obtained no depression of the relaxing effect of epinephrine or isopropylarterenol as a result of long exposures of strips to very high concentrations of either dibenamine (10^{-5}) or phentolamine (10^{-4}) (Furchgott (168) and unpublished results). However, some tachyphylaxis to the relaxing effects of these sympathomimetics is observed with repeated testing on aortic strips even after exposure to smaller concentrations (10^{-6} to 3×10^{-6}) of dibenamine. Perhaps some tachyphylaxis also occurred in the experiments of Green and his colleagues. Another possibility is that in the vascular bed of dog muscle, the arterioles which are ordinarily dilated by isopropylarterenol or by epinephrine following lower concentrations of the blocking agents, are actually so fully dilated by the very high concentrations of these agents themselves that no further vasodilation is detectable. The blood flow data of Green and coworkers do not indicate such a situation; but in view of the observation that most of these agents do produce a transitory vasodilation when injected intra-arterially, and in view of the complicated architecture of a single vascular bed, the possibility should not be dismissed. Perhaps it could be checked by raising the peripheral resistance of the vascular bed with pitressin after it has become irresponsive to epinephrine and isopropylarterenol, and then retesting the effects of these sympathomimetics.

Folkow and Uvnäs (152, 154) and Frumin *et al.* (164), extending the earlier work of Bülbring and Burn (48, 49), have used atropine in conjunction with adrenergic blocking agents to demonstrate the presence of cholinergic vasodilator fibers in the sympathetic trunks innervating the leg muscles of cats and dogs. The vasodilation produced either by acetylcholine or by sympathetic stimulation after adrenergic blockade could be completely blocked by very small doses of atropine. However, Erici *et al.* (117) found that atropine in much larger doses than were necessary to suppress completely the vasodilator effect of acetylcholine in the cat tongue after adrenergic blockade, only depressed to a small degree the vasodilator effects of cervical sympathetic stimulation. Nevertheless, they attributed the vasodilator effect of stimulation to cholinergic dilator fibers in the cervical sympathetic trunk because they were able to potentiate it somewhat with eserin and were able to detect a substance having the pharmacological properties of acetylcholine in eserinized Tyrode's solution perfused through the

tongue during stimulation. They were unable to block the vasodilator effect with the antihistaminic pyrilamine (Neoantergan). However, they did not report on the effects of epinephrine or norepinephrine on blood flow through the tongue after adrenergic blockade, and the possibility remains that one or both of these substances, liberated as the sympathetic transmitter, might have been principally responsible for the vasodilator effect of sympathetic stimulation.

Erici and Uvnäs (116), like a number of earlier investigators (*e.g.*, 96, 218, 232, 233), also found that atropine in much larger doses than were necessary to suppress the vasodilator effect of parasympathomimetic drugs (acetylcholine in this case) in the cat tongue and salivary glands, depressed only to a small extent the vasodilator effect of chorda tympani stimulation. They also demonstrated that the neurogenic vasodilation was not due to antidromic stimulation of afferent fibers and concluded, as did Dale and Gaddum, that it was due to release of acetylcholine by cholinergic fibers. Like Dale and Gaddum (96), they assumed that the inability of atropine to block the neurogenic vasodilation was due to the release of acetylcholine in great intimacy with the receptor mechanism beyond a barrier where atropine blocks the entry of added acetylcholine to the receptor mechanism (see discussion of this concept in Section I,c). More recently, however, Hilton and Lewis (224), studying blood flow through the submandibular gland of cats, presented evidence that the major part of the vasodilation on chorda tympani stimulation is due not to a direct action of released acetylcholine on the blood vessels, but rather to the liberation of a stable vasodilator from the gland by the released acetylcholine. Like Barcroft (20), they assume that such a substance is produced as a result of increased metabolic activity of the gland under stimulation even when its secretion is blocked by atropine. In accord with this concept is their finding that botulinum toxin (which is supposed to block only release of the transmitter from cholinergic nerve endings) prevents vasodilation and secretion due to chorda tympani stimulation but not to pilocarpine. However, their evidence (see original abstract for other details) is still incomplete, for it is probable that when atropine blocks completely the effect of chorda tympani stimulation on glandular secretion, it also blocks completely any increase in glandular metabolism. Their evidence does not as yet rule out the possibility that stimulation of the chorda tympani nerve releases directly another transmitter in addition to acetylcholine, which is not antagonized by atropine and is responsible for a large part of the vasodilation before atropine. Such a situation would be similar to that proposed by Vogt (375, 376) to explain the inability of atropine to block completely the excitatory effect of vagal stimulation on intestinal smooth muscle.

Various antagonists with high relative specificities have also been used in attempts to determine the nature of the substances responsible for vasodilation in post-occlusion hyperemia of muscle, in post-contraction hyperemia of muscle, and on antidromic stimulation of afferent fibers to the skin. Because adrenergic blocking agents, atropine, and antihistaminics do not prevent the transient hyperemia following brief arterial occlusion, as well as for other reasons, a number of recent investigators (112, 146, 221, 319) have proposed that this hyperemia

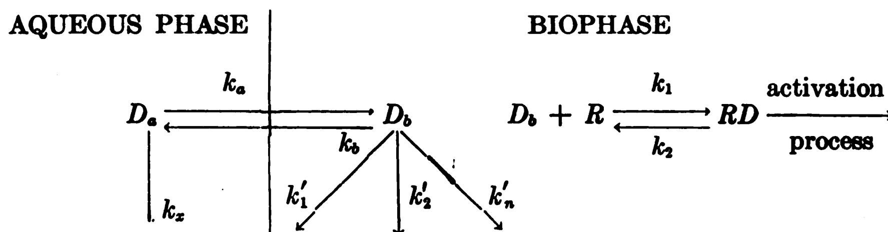
is not due primarily to the action of any liberated substance but rather to a response of the vascular smooth muscle to changes in tension. (See Section II,c, for further discussion.) Hilton (221) has also found that post-contraction hyperemia is not antagonized by pyrilamine, atropine or phentolamine, but that it is reduced or abolished by local injection of cocaine, procaine, or botulinum toxin into the muscle. On the basis of these and other observations (see original paper for details) he has suggested that post-contraction hyperemia is mediated by means of a local axon reflex involving cholinergic fibers in the sympathetic outflow to the muscle vessels.

Most recent workers have also been unable to antagonize the cutaneous vasodilation resulting from antidromic stimulation of dorsal roots with either atropine or potent antihistaminics (69, 70, 164, 223, 226, 229), and have therefore abandoned the old idea that acetylcholine or histamine or both may be the neurohumoral transmitter from the afferent endings. Holton and coworkers, however, have found that physostigmine in high concentrations greatly depresses antidromic vasodilation in the rabbit ear. Because the vasodilation caused by ATP closely resembles that due to antidromic stimulation, and because physostigmine depresses it in a similar manner, these workers have proposed that ATP may be the actual transmitter. (Also see Section IV,j.)

VI. LOCAL POTENTIATION OF DRUG ACTION ON VASCULAR SMOOTH MUSCLE

a. Modes of local potentiation. Local potentiation—that is, potentiation of the response of vascular smooth muscle to one drug as a result of introducing a second drug into the region of the smooth muscle—is a commonly encountered phenomenon. It has been frequently demonstrated in perfused structures as well as on isolated strips or segments of large vessels, and in many cases can account for the potentiation of the blood pressure response to one substance by another in the whole animal. However, because potentiation of the blood pressure response is often due to a remote action of the potentiating drug—that is, action initiated at sites outside the region of the smooth muscle cells—the examples of potentiation discussed in this section will be largely those which have been demonstrated on preparations in which there is little or no possibility for such remote indirect action.

Some possible modes of local potentiation are best illustrated by a consideration of a simplified scheme similar to those used in discussing the kinetics of drug antagonism:



In this scheme D_a and D_b represent the drug being tested (agonist) in the aqueous phase and biophase respectively, R is free receptor and RD is the receptor-drug complex, the formation of which initiates the "activation process" leading to the response. k_a and k_b are the rate constants for penetration into and escape from the biophase by the agonist; while k_1 and k_2 are the rate constants for formation and dissociation of the receptor-drug complex. k_x is the rate constant for inactivation or removal of D_a in the aqueous phase. k_1' is the rate constant for a given enzymatic reaction which converts D_b to an inactive product in the biophase; and k_2' to k_n' are rate constants for any other enzymatic reactions which inactivate D_b in the biophase.

How will the concentration of D_b change as a function of time in an experiment in which D_a is kept at a fixed concentration? If the simplifying assumptions are made that D_a at the time of addition (zero time) is uniformly dispersed throughout the aqueous phase, that all the enzymatic reactions in the biophase are first order reactions at the concentration of D_b attained, and that the concentration of D_b is essentially the same throughout the biophase at any given time, then the following equation may be obtained:

$$(D_b) = \frac{(D_a)k_a}{k_b + k_1' + k_2' \cdots\cdot + k_n'} \{1 - e^{-(k_b + k_1' + k_2' \cdots\cdot + k_n')t}\} \quad \text{(Equation 15)}$$

in which (D_b) and (D_a) are concentrations, and t is the time after addition of the agonist.[15] After attainment of steady state conditions (D_b) would be given by:

$$(D_b) = \frac{(D_a)k_a}{k_b + k_1' + k_2' \cdots\cdot + k_n'} \quad \text{(Equation 16)}$$

The fraction $k_a/(k_b + k_1' + k_2' \cdots\cdot + k_n')$ will be called, for convenience, the "distribution coefficient". It approaches the partition coefficient k_a/k_b when $\sum_{i=1}^{n} k_i'$ approaches zero (no enzymatic inactivation).

By integrating the proper differential equations, equations may also be obtained to give (D_b) as a function of time when (D_a) is not constant, but is also some continuous function of time. For example, if D_a is inactivated or removed from the aqueous phase so that its concentration falls exponentially with time (first order kinetics with rate constant k_x), then with the use of the simplifying assumptions used in obtaining equation 15, the following equation can be obtained:

$$(D_b) = \frac{(D_a)_0 k_a}{k_b + k_1' + k_2' \cdots\cdot + k_n' - k_x} \{e^{-k_x t} - e^{-(k_b + k_1' + k_2' \cdots\cdot + k_n)t}\} \quad \text{(Equation 17)}$$

in which $(D_a)_0$ is the initial concentration of D_a (at $t = 0$). This equation might be applied in experiments with isolated muscle preparations in which the agonist is inactivated by a first order reaction in the fluid of the muscle chamber. In

[15] Equation 15 is obtained by integrating the differential equation:

$$d(D_b)/dt = k_a(D_a) - (k_b + k_1' + k_2' \cdots + k_n')(D_b),$$

setting (D_b) equal to zero when t equals zero.

such experiments the concentration of agonist in the biophase, (D_b), would be expected to rise to a maximum and then decrease again to zero.[16] Equation 16 also might be applied in some perfusion and whole animal experiments in which the concentration of agonist in the perfusion fluid or blood falls exponentially with time.

On the basis of the simplified scheme for reactions of the agonist and the equations given above, the following modes of action of a potentiating agent would be possible:

1) Alteration of the composition of the biophase or the aqueous phase leading to a change in k_a or k_b or in both so as to increase the "distribution coefficient", $k_a / (k_b + k_1' + k_2' \ldots + k_n')$. In experiments on smooth muscle in which the agonist is kept at a fixed concentration, (D_a), in the aqueous phase, equation 16 predicts that after attainment of a steady state, (D_b) will be proportional to the "distribution coefficient" times (D_a). Therefore if this coefficient is increased by a change in k_a or k_b or both, the final level of (D_b) attained at fixed (D_a) will be increased proportionally, and a greater response, that is potentiation, will occur. Whether potentiation will occur at any given time prior to attainment of a steady state will depend on the individual values of k_a and k_b for the smooth muscle cells in the presence and absence of the potentiating agent.

This may be the mode of action when an increase in pH of the aqueous phase potentiates the response to an amine.[17] It is also the mode of action inherent in the concept that potentiation of response to a drug after denervation is due to greater permeability of the cell membrane to the drug (65). However, if "greater

[16] From equation 17 the time, t_m at which (D_b) is at a maximum (*i.e.*, t at which $d(D_b)/dt = 0$) may be determined as a function of the rate constants. It is found that:

$$t_m = \left\{ \frac{1}{k_b + \sum_{i=1}^{n} k_i' - k_z} \right\} \log_e \left\{ \frac{k_b + \sum_{i=1}^{n} k_i'}{k_z} \right\}. \qquad \text{(Equation 18)}$$

And the maximal level of (D_b), designated as $(D_b)_m$, is given by:

$$(D_b)_m = \left\{ \frac{(D_a)_0 \, k_a}{k_b + \sum_{i=1}^{n} k_i'} \right\} e^{-k_z t} \qquad \text{(Equation 19)}$$

[17] In this discussion as well as that concerning the kinetics of antagonism no differentiation has been made between drugs in the ionized and un-ionized form in solution. The term "concentration" has been used to designate the total concentration of both forms, the individual concentrations of which would give a certain ratio depending on the pK of the drug and the pH. It is recognized that un-ionized molecules probably enter the biophase much more readily than ionized molecules; however, the problem of whether the receptors react with the ionized or un-ionized molecules of a given drug, or with both, is far from settled. Presumably if the pH of the aqueous phase were raised so as to increase the relative concentration of the un-ionized form of an amine, the penetration constant k_a, which is defined in terms of the total concentration of the amine, would increase and the "distribution coefficient", $k_a/(k_b + k_1' + k_2' \cdots + k_n')$, would increase accordingly.

permeability" were to produce a proportionate increase in the rate constants for both penetration into and escape of the drug from the biophase, and there was no significant change in the rates of enzymatic inactivation, then even though maximal height of response to a given dose of drug might be increased, the rate of decrease of response from any given height would be more rapid after removal of the drug from the aqueous phase. In other words, the duration of response would not be potentiated relative to the normal duration, but would actually be shortened.

2) Inhibition of inactivation of the agonist in the biophase so as to increase the "distribution coefficient". This mode of action adequately explains the potentiation of the effect of acetylcholine by an anticholinesterase added to a Ringer-type fluid used for perfusion of vascular beds or bathing strips of arteries. It has also been frequently invoked to explain the potentiation of the effects of epinephrine or norepinephrine by various agents known to inhibit the activity of monoamine oxidase (*vide infra*). However, even if an agent markedly inhibits the activity of a given enzyme which inactivates the agonist in the biophase, the agent would be expected to produce detectable potentiation only under certain conditions. The equations above predict that inhibition of a given enzymatic reaction with initial rate constant k_1' will lead to an appreciably higher concentration of agonist in the biophase, (D_b), at any given time after introduction of the agonist into the aqueous phase, only if k_1' is of the same order of magnitude or greater than $(k_b + k_2' \cdots + k_n')$. For example, under steady state conditions, represented by equation 16, complete inhibition of an inactivating enzyme with rate constant k_1', will lead to only a 10 per cent increase in (D_b) if k_1' before inactivation is $1/10$ of $(k_b + k_2' \cdots + k_n')$; and to only a 1 per cent increase if k_1' before inactivation is $1/100$ of $(k_b + k_2' \cdots + k_n')$. Since potentiation by this mode of action depends on the increase in (D_b), no potentiation would be detected if the initial rate constant of the enzymatic reaction inhibited was very much less than the sum of the rate constants for all processes which remove the agonist from the biophase.

3) Inhibition by the potentiator of inactivation of the agonist in the aqueous phase. This mode of action largely accounts for the potentiation of the action of acetylcholine by an anticholinesterase when blood, plasma or serum are used as a perfusion fluid, or as the bathing fluid in the case of an isolated vessel, since the rate of hydrolysis of acetylcholine in the aqueous phase would be inhibited and the concentrations reaching the smooth muscle cells would be elevated. The same mode of potentiation is involved in experiments in which a substance inhibits the oxidation of epinephrine or norepinephrine catalyzed by traces of heavy metals in Ringer-type fluids (*vide infra*) If the agonist, D_a, in the aqueous phase is inactivated by a first order reaction with a rate constant, k_x, equations 17, 18, and 19 would apply. From these equations it is possible to determine theoretically how an inhibition of the inactivation of D_a (reduction in k_x) might be expected to influence the time course of (D_b), and raise the maximal level attained after addition of a given concentration of the agonist.

4) Alteration of the characteristics of the receptor so as to decrease the dissociation constant ($K_D = k_2/k_1$) of the receptor-drug complex, RD. In this situation an added substance in the biophase, perhaps adsorbed at a site next to the receptor site proper, would allow a greater concentration of RD to be formed at a given concentration of D_b. There are no examples of potentiation which can definitely be attributed to this hypothetical mode of action, although Clark (79) speculated that the potentiation of epinephrine by cocaine might be of this sort (*vide infra*).

5) Increasing the over-all rate of the activation process initiated by the formation of the receptor-drug complex. This might conceivably be accomplished by any agent which either increases the forward rate or decreases the reversal rate of any reaction in the chain of reactions set off by the formation of the receptor-drug complex and terminating in the reaction of the contractile protein of the smooth muscle cell. There are no examples of potentiation which can be definitely attributed to this mode of action. In a way, the increased contractile response of rabbit aortic strips brought about by addition of energy-yielding substrates following long incubation with no added substrates, appears to be a potentiation of this sort (see Section III), for these agents undoubtedly furnish energy which increases the forward rate of the contraction reaction. However, this is obviously a special case and on the basis of certain criteria should not be considered as potentiation.

b. A possible source of error in studies on the potentiation of responses to epinephrine and norepinephrine. It is well known that epinephrine and norepinephrine can be rapidly oxidized to adrenochrome and other products by the catalytic action of traces of heavy metals (*e.g.*, Cu^{++} and Fe^{+++}) in oxygenated physiological salt solutions such as those commonly used in perfusion experiments or in experiments with isolated muscle preparations (13, 82). Yet many investigators have overlooked this fact in studies on the potentiation of the responses to epinephrine and norepinephrine. The source of the traces of heavy metals may either be the salts or the water used to make the solution, or some slightly corroded metal fitting in contact with the solution in the apparatus being used (170). If reagent grade salts and glass-redistilled water are used, the concentration of heavy metals may be minimized and the rate of oxidation of epinephrine or norepinephrine greatly reduced; but even with such precautions there is frequently some detectable oxidation of these substances within ten minutes after adding them to a solution. In our laboratory, using the same Krebs solution (made with reagent grade salts and glass-redistilled water) in a number of similar muscle chambers simultaneously, we have observed that the rate of oxidation of low concentrations of epinephrine (as evidenced by the rate of decrease of tone of arterial strips following attainment of maximal tone after addition) is relatively high in certain chambers and practically negligible over as long as twenty minutes in others. This apparently is due to variations in the glass surfaces of the chambers, which somehow influence the rate of catalysis of oxidation. Welch in 1934 also observed that the amount of glass surface exposed to a solution of epinephrine influenced the rate of oxidation.

A great many substances have been found to inhibit the rate of heavy metal catalysis of epinephrine oxidation in oxygenated physiological salt solutions. Among these are various proteins and protein mixtures (*e.g.*, gelatin, serum albumin, plasma proteins, tissue extracts, etc.) amino acids (especially cysteine, cystine and tyrosine), glutathione, phenolic compounds, ascorbic acid and pyrophosphate. (See 13, 82 for earlier references, and also the interesting recent paper of Kitto and Bohr (247)). Probably most of these act as anti-oxidants by complexing with the metal ions, thus preventing them from catalyzing the oxidation of epinephrine. Of the metal complexing agents which we have tested, the most efficient and practical for preventing the oxidation of epinephrine and related catechol derivatives in oxygenated Krebs solution is ethylenediamine tetraacetic acid (EDTA). Usually a concentration of 10^{-5} of this potent metal-chelating agent prevents any detectable oxidation of epinephrine over a thirty minute period even in solutions made with some samples of metal-distilled water in which the oxidation of epinephrine is ordinarily ninety per cent complete within five to ten minutes. In experiments with such solutions the addition of EDTA not only greatly potentiates the duration but also the maximal level of response of aortic strips to small doses of epinephrine or norepinephrine. At the concentration indicated, EDTA removes only insignificant amounts of Ca^{++} and Mg^{++} from the solution and does not of itself directly alter the response of arterial strips to drugs. This has been demonstrated by the finding that EDTA at 10^{-5} concentration neither inhibits nor potentiates the effects of epinephrine, or any other of a variety of drugs, tested on strips suspended in Krebs solution (made with glass-distilled water) in those muscle chambers in which oxidation of epinephrine is negligible over a twenty-minute period even in the absence of EDTA (*vide supra*).

It is probable that a great many examples of potentiation of epinephrine responses by substances added to physiological salt solutions used in perfusion experiments or in experiments on isolated muscle preparations, have been interpreted as being due to some sort of intracellular potentiation (usually mode of action 2), when they have actually been due to inhibition of epinephrine oxidation in the solution itself (mode of action 3). An excellent method for critically determining whether an observed potentiation of epinephrine response by an added substance is due to some mode of action other than inhibition of oxidation in solution is to use sufficient EDTA in the solution to inhibit this oxidation essentially completely, and then to retest the effect of the substance on the epinephrine response. In this way, it was found that what first appeared to be a possible intracellular potentiation of epinephrine response on rabbit aortic strips by the potent amine oxidase inhibitor, iproniazid, was actually nothing more than a partial inhibition by iproniazid of the oxidation of epinephrine in the Krebs solution used (Furchgott *et al.* (177), and unpublished observations).

c) The "monoamine oxidase hypothesis" of potentiation of responses to epinephrine and norepinephrine by various drugs. In 1938 Gaddum and Kwiatkowski (185) introduced the hypothesis that the potentiation of epinephrine responses by ephedrine is due to the inhibitory effect of ephedrine on the oxidation of

epinephrine by monoamine oxidase. Since then a number of investigators have used this hypothesis to explain the potentiation of epinephrine and norepinephrine responses not only by ephedrine but also by numerous other substances that have also been shown to inhibit this enzyme *in vitro* if sufficiently high concentrations of them are used. Among these substances are certain other sympathomimetic amines related to ephedrine, cocaine and certain other local anesthetics, methylene blue and certain antihistaminics. Also the potentiating (or sensitizing) effect of sympathetic denervation on the response of certain smooth muscle effectors to epinephrine and norepinephrine has been attributed by some investigators to a loss of this enzyme. However, the hypothesis has not been generally accepted, and there has been considerable controversy concerning it. The evidence supporting the hypothesis has been recently reviewed by Burn (55); while the opposing evidence has been stressed by Bacq (13) in his argument against the hypothesis. Blaschko in his recent review (27) has presented the experimental evidence both for and against the hypothesis. For details of the evidence and for references the reader is referred to the three review articles cited, but two of the more cogent arguments against the hypothesis may be noted here. One is that the concentrations of drugs such as ephedrine or cocaine or methylene blue required to markedly potentiate the effects of epinephrine and norepinephrine on perfused structures (such as the rabbit ear), on blood pressure, or on the nictitating membrane, are very much lower than the concentrations necessary to inhibit monoamine oxidase activity significantly *in vitro*. This is true not only in the case of monoamine oxidase of liver and kidney, but also in the case of the enzyme in blood vessels (369). A second cogent argument against the hypothesis is that ephedrine and cocaine not only potentiate the responses to epinephrine and norepinephrine but also the responses to cobefrine (1-(3′,4′-dihydroxyphenyl)-2-aminopropanol), a sympathomimetic amine which is not oxidized by amine oxidase (59, 241).

In 1952 Zeller and Barsky (400) showed that the chemotherapeutic agent iproniazid (1-isonicotinyl-2-isopropylhydrazine) is a powerful and essentially irreversible inhibitor of monoamine oxidase (liver and brain) both *in vivo* and *in vitro*. These workers and their colleagues (206) subsequently injected this drug in cats to determine whether it would potentiate the blood pressure and nictitating membrane responses to epinephrine and norepinephrine. They found that it did not do so even though it reduced the monoamine oxidase activity of the livers of the cats used to about one per cent of the normal level. However, they did find that it markedly potentiated the response of the nictitating membrane to phenylethylamine and tyramine. This they attributed to the inhibition of monoamine oxidase, since this enzyme inactivates the latter amines much more rapidly than it does epinephrine and norepinephrine (13, 27). They were unable to demonstrate a significant potentiation of the vasopressor effects of phenylethylamine and tyramine, but considered that this may have been obscured by tachyphylaxis.

Following the appearance of the paper by Zeller and Barsky (400), we tested the effect of iproniazid on isolated strips of rabbit aorta (177). Exposure of such

strips to 10^{-4} iproniazid in oxygenated Krebs solution at 37° for 30 minutes inhibited their monoamine oxidase activity (determined subsequently in Warburg apparatus with 0.01 M tyramine) completely and irreversibly. Such an exposure neither potentiated nor depressed the response of strips to epinephrine, norepinephrine, acetylcholine, or histamine. However, it markedly potentiated the response to tyramine, frequently producing a ten-fold increase in sensitivity to this drug. Our findings are thus in agreement with those of Griesemer *et al.* (206) on the nictitating membrane. The potentiation of the tyramine response by iproniazid on an isolated preparation of vascular smooth muscle is clearly an example of potentiation by mode of action 2). On the basis of the theory given in Section VI, a, the rate constant (k_1') for the enzymatic removal of tyramine by monoamine oxidase in the biophase of the smooth muscle cells must be considerably larger than the sum of the other rate constants for removal (k_b + rate constants for any other enzymatic inactivations). On the other hand, the lack of potentiation of epinephrine (or norepinephrine) by iproniazid would indicate that the rate constant for removal of it by this enzyme is much less than the sum of the other rate constants for removal. That the rate constant for deamination of tyramine at low concentrations (used at 10^{-6} to 10^{-7}) might be expected to be much greater than that of epinephrine (used at 10^{-9} to 10^{-8}), is indicated by the data of Kohn (250) on relative $V_{max.}$ and K_s values (Michaelis constants) of these two amines for pig liver monoamine oxidase. In addition, it appears likely that tyramine, with fewer polar groups than epinephrine, would have a lower rate constant, k_b, for escape from the biophase into the aqueous phase.

The lack of potentiation of responses to epinephrine and norepinephrine by iproniazid on rabbit aortic strips certainly indicates that oxidation of these drugs by monoamine oxidase in this preparation does not detectably limit their effectiveness. This is additional evidence against the monoamine oxidase hypothesis of potentiation, but one must admittedly be cautious in extending the results obtained with this one preparation to other blood vessels of the body. The experiments, however, do indicate how iproniazid can be used as a very useful tool in testing this hypothesis in the case of other blood vessels. Firstly, iproniazid itself might be tried for any potentiating effect on the response of the test preparation (perfused organ or isolated vessel) to epinephrine and norepinephrine. And secondly, substances which have been considered by some investigators to potentiate by inhibiting monoamine oxidase might be tested for their effect on epinephrine and norepinephrine responses before and after inactivation of this enzyme in the test preparation with iproniazid. If the substance potentiates as well after iproniazid treatment as before, then its action cannot be attributed to an inhibition of the enzyme. This has recently been found to be the case with cocaine potentiation of epinephrine and norepinephrine on rabbit aortic strips (Furchgott and Ashby, unpublished results). Therefore in this preparation potentiation by cocaine cannot be explained by the monoamine oxidase hypothesis.

d. Potentiation by cocaine. Since the original report by Fröhlich and Loewi (163) that cocaine potentiated the action of epinephrine on blood pressure and sympathetically innervated effectors, a great number of studies have been made

of this phenomenon. In such studies the nictitating membrane of the cat has been the structure most used for quantitatively measuring the potentiating effect of cocaine and certain other local anesthetics on the response to sympathetic stimulation or to the injection of epinephrine and other sympathomimetic amines (12, 14, 16, 81, 137, 238, 336, 371). However, in a number of studies the potentiating effect of cocaine on the response of blood pressure or of resistance to flow in perfused structures has also been quantitatively investigated (*e.g.*, 42, 62a, 101, 269, 368). The fact that cocaine potentiates the action of epinephrine, norepinephrine and certain related sympathomimetic amines possessing a meta-hydroxyl group, in perfused structures and on isolated arterial strips (see paragraph above) indicates that at least a good part of its potentiation of the blood pressure response in the whole animal is at the level of the vascular smooth muscle cells. However, the mode of action by which cocaine potentiates is still a mystery.

On the basis of the evidence at hand, it seems unlikely that the mode of action is an inhibition of monoamine oxidase. Clark and Raventós (81) concluded that cocaine did not slow the rate of destruction of epinephrine, but in some way sensitized the muscle to it. Such a sensitization could conceivably be brought about by modes of action 1), 4), or 5) discussed in section VI, a. Chen and Russell (75) revived the idea that cocaine and certain other agents, including the antihistaminic, diphenhydramine, potentiated the vasopressor effects of epinephrine and norepinephrine by inhibiting the action of the latter drugs on "vasodepressor effectors" (inhibitory receptors). They based their argument on the observation that these agents were able to block the vasodepressor effect of epinephrine and norepinephrine in dogs under adrenergic blockade with SY28. However, there are several other possible explanations of their observations and similar observations by others. (See, for example, the note by Ahlquist (2), and the paper by Ludwigs (276) on the reversal of the vasodepressor effect of isopropylarterenol by ergotamine in dogs.) On rabbit aortic strips we have been unable to demonstrate any decrease in the relaxing capacity of isopropylarterenol or epinephrine (after full adrenergic blockade with Dibenamine) in the presence of high concentrations of cocaine or diphenhydramine (Furchgott, unpublished results). Hebb and Konzett (217) also did not observe any significant inhibition by cocaine of the vasodilator effect of isopropylarterenol in perfused dog lungs.

A number of investigators have been impressed with the quantitative similarity between the modifying effects of cocaine and chronic denervation on the responses of the nictitating membrane to a variety of sympathomimetic amines (12, 62, 65, 137, 138, 238). Fleckenstein and Bass (137) have developed a hypothesis to explain the similarity of potentiation by denervation and cocaine of the response to epinephrine, norepinephrine and other related sympathomimetic amines possessing a meta-phenolic hydroxyl group. In this hypothesis they assume (a) that in the normal innervated nictitating membrane sensitivity to these amines is limited by accommodation of the smooth muscle to the norepinephrine continuously released from the sympathetic nerve terminals; and (b) that this release is abolished by chronic denervation and blocked by cocaine, so

that accommodation is lost and therefore sensitivity to the sympathetic amines closely related to norepinephrine increases. This is an interesting hypothesis, but it is probably not valid. Strong evidence against it is provided by observations on the potentiating effect of cocaine on the response of rabbit aortic strips (Furchgott and Ashby, unpublished results). On such preparations cocaine alone produces either no response or only a very small and very slowly developing contraction; but at a concentration of about 10^{-5} to 10^{-4} it often approximately doubles the contraction height produced by small concentrations (about 10^{-9}) of epinephrine and norepinephrine. This increase in contraction height is obtained whether one of these drugs is added after cocaine, or cocaine is added after one of these drugs has been present for either short or long periods (up to thirty minutes) in the muscle chamber. Such a potentiation cannot be due to a loss of accommodation of the smooth muscle to small amounts of norepinephrine or epinephrine continuously present, for it occurs rapidly even when cocaine is added after long exposure of aortic strips to concentrations of norepinephrine and epinephrine which maintain a small to moderate degree of tone throughout the "pre-cocaine" exposure period.

Although the potentiating effect of cocaine on the action of epinephrine and other sympathomimetic amines with a meta-hydroxyl group is probably not due to an inhibition of monoamine oxidase, it still is possible that it is due to an inhibition of some other enzyme which very rapidly converts these compounds to inactive or less active products in the biophase of the smooth muscle cells. Such a conversion need not be the result of an oxidation. Indeed, the possibility of a reduction with certain normal constituents of the biophase serving as electron donors is more appealing. The presence of an enzyme-donor system which very actively reduces the meta-hydroxyl group of the amines in question and which is competitively inhibited by cocaine would account very well for the phenomenon of cocaine potentiation. If the activity of such an enzyme-donor system were markedly reduced by chronic denervation, the similarity of potentiation with cocaine and denervation would also be explained.

e. Potentiation by ephedrine and related sympathomimetic amines. In the earlier papers of Burn and Tainter (53, 54, 62a) the marked augmentation of the vasoconstrictor effects of ephedrine and tyramine in perfused limbs when epinephrine was present in small concentrations in the perfusing fluid was considered to be a potentiation by epinephrine of the responses to these less active amines. However, after the report of Gaddum and Kwiatkowski (185) on the augmentation of the epinephrine response in the rabbit ear by ephedrine, and their explanation of this effect by the monoamine oxidase hypothesis, the roles of the amines were reversed. Ephedrine and tyramine now came to be considered as the potentiators of the response to epinephrine by virtue of their ability to inhibit the inactivation of epinephrine by the oxidase. A number of other sympathomimetic amines of relatively low potency (*e.g.*, synephrine, phenylethylamine, amphetamine, paredrine, and certain aliphatic amines) were also found to augment the effect of epinephrine on blood pressure and in some cases, in perfused preparations (193, 241, 271, 284). These drugs were generally classed along with

ephedrine and tyramine as potentiators of the action of epinephrine, although a number of workers did not agree that the potentiation could be explained by the monoamine oxidase hypothesis. Morton and Tainter (303) observed that the vasoconstrictor effects of ephedrine and tyramine were augmented by using defibrinated blood instead of Locke's fluid for perfusion of cat limbs, and attributed this to a potentiation by these substances of the response to small amounts of epinephrine which they assumed to be in the blood. However, it now appears more likely that the substance responsible for the augmentation was 5-hydroxytryptamine; for this substance is released from platelets when blood is defibrinated (183, 317), and Gaddum and Hameed (182) have recently shown that its vasoconstrictor action in the rabbit ear is potentiated by ephedrine as much, if not more, than that of epinephrine.

In perfused structures or isolated preparations of larger arteries, potentiation of the response to epinephrine and norepinephrine by ephedrine (or related sympathomimetic amines) is generally best demonstrated at low concentrations of the latter substance, insufficient in itself to produce more than slight vasoconstriction (9, 59, 185, 195, 355). At much higher concentrations of ephedrine, the responses to epinephrine and norepinephrine are usually depressed, apparently because of competitive antagonism of the much less active ephedrine for adrenergic motor receptors (see Section IV,e). With some vascular preparations it is difficult to demonstrate potentiation with ephedrine and related substances, possibly because the concentration necessary for potentiation is not very different from that for competitive antagonism. On rabbit aortic strips we have been able to demonstrate definite potentiation of the epinephrine response by ephedrine, tyramine, or amphetamine in only a small percentage of experiments (Furchgott and Ashby, unpublished results). Similarly Brun (44) rarely observed potentiation on testing the response of small arteries in muscle, omentum and mesentery of anesthetized rats to topically applied epinephrine following topically applied ephedrine. Yet Kunz *et al.* (262), using the rat mesoappendix preparation of Zweifach (402) consistently obtained marked potentiation of the vasoconstrictor action of topically applied epinephrine on small arterioles and capillary sphincters following intravenous injections of small doses of ephedrine. The difference between the results of Brun and Kunz *et al.* may possibly be due to an additional sensitizing effect of systematically introduced ephedrine, mediated by an action initiated at some site outside the region of the blood vessels under observation. However, it is also possible that the smooth muscle cells of the small arterioles and capillary sphincters are much more susceptible to the direct sensitizing effect of ephedrine than are the small arteries studied by Brun.

As in the case of cocaine, the mechanism by which ephedrine-like substances directly potentiate the action of epinephrine and norepinephrine and closely related potent sympathomimetics on vascular smooth muscle is far from settled. Although the evidence for the monoamine oxidase hypothesis is more impressive in the case of ephedrine than in the case of cocaine, further studies along the lines indicated in Section VI,c, will be necessary before it can be definitely accepted or rejected. As with cocaine, there is still the possibility that direct po-

tentiation by ephedrine-like substances is due to the inhibition of some enzyme system in the biophase of the smooth muscle cells which inactivates epinephrine-like substances much more rapidly than does monoamine oxidase. If cocaine and ephedrine both potentiate by inhibiting enzymes, and if the theoretical approach to mode of potentiation 2) (see Section VI,a) is valid, then the enzyme system inhibited by ephedrine-like substances would have to be the same as that inhibited by cocaine.[18] In the case of cocaine, I speculated that it might potentiate by competitively inhibiting an enzyme-donor system which very rapidly reduced the meta-hydroxyl group of epinephrine, norepinephrine and related sympathomimetics. In this connection, it is interesting that all of the ephedrine-like substances which have been demonstrated to potentiate directly the actions of sympathomimetics possessing meta-hydroxyls have no meta-hydroxyls themselves.

One final note of caution might be introduced with regard to what appears to be direct potentiation of epinephrine-like substances by ephedrine-like substances on certain vascular preparations. It may in the end be discovered that the older concept of Tainter and others is the correct one after all: namely, that the epinephrine-like substances are actually the potentiators and the ephedrine-like substances are the potentiated. (See also Section VII,c.)

f. Potentiation by other drugs. Many other drugs besides those already discussed have been found to potentiate responses to epinephrine, norepinephrine, and related potent sympathomimetic amines. Among them are certain adrenergic blocking agents (at concentrations below the level required for blocking), methylene blue, certain antihistaminics, isothiourea derivatives, some phenolic compounds, indole and imidazole derivatives, and certain aryl ethers of choline. Unfortunately, from the standpoint of the present discussion which is concerned with potentiation by "local action", most of the studies of potentiation with the agents just mentioned have been carried out on whole animals (effects on blood pressure, nictitating membrane, iris, etc.), and therefore involve the possibility of potentiation by "remote action".[19] However, in the case of some of these

[18] The theoretical approach to potentiation by enzyme inhibition developed in Section VI, a, is based on certain simplifying assumptions about the movements and reactions of molecules of a drug in smooth muscle cells. On the basis of these assumptions, if each of two agents potentiate markedly the effect of a single agonist by inhibition of enzymatic inactivation of the agonist in the biophase, then both agents must inhibit the same enzyme system, and inactivation of the agonist by that enzyme system must ordinarily be the principal process for removing the agonist from the biophase. It is realized that other sets of assumptions might be made which would theoretically allow marked potentiation of the effect of a single agonist by each of two agents inhibiting different enzyme systems. For example, if the agonist had to pass through two "barrier layers" in the biophase, before reaching the receptors, and each layer contained a different inactivating enzyme with high activity; then marked potentiation might be obtained by inhibition of either of the two enzymes by added agents. Also, if the inactivation of the agonist in the biophase depended on a reaction series of the following type: $D_b \rightleftarrows P_1 \rightarrow P_2$ in which the first enzymatic reaction was "reversible" and the second was "irreversible"; then marked potentiation might be obtained by inhibition of either reaction by added agents.

[19] An excellent illustration of how potentiation in whole animals may be misinterpreted

agents results have been obtained on perfused structures and isolated preparations of vessels which indicate a direct local potentiating action.

Jang (243) observed a potentiation of the vasoconstrictor action of epinephrine in the perfused rabbit ear following the injection of very low concentrations of ergotoxine, yohimbine, and piperoxan. Fastier and Smirk (127) and Fastier and Reid (125) found that certain S-alkyl thioureas, which are also adrenergic blocking agents at higher concentrations, potentiated at low concentrations the vasoconstrictor response to epinephrine in perfused hind quarters of pithed rats. Potentiation of epinephrine constriction of isolated swine arteries following treatment with diphenhydramine has been reported by Smith (355); and potentiation of epinephrine vasoconstriction in the perfused rabbit ear in the presence of choline-p-tolyl ether, by Gaddum and Hameed (182). All of these drugs (as well as methylene blue, which potentiates the vasopressor and nictitating membrane response to epinephrine (386)) are inhibitors of monoamine oxidase, but the concentrations of each required for significant inhibition vary considerably (see 27, for references). Of them, choline-p-tolyl ether is by far the most potent (41), being about ten times as active as amphetamine and one hundred times as active as ephedrine. Gaddum and Hameed observed that it not only potentiated the response to epinephrine but also the response to 5-hydroxytryptamine. Since the latter substance (as well as tryptamine) is also a substrate for monoamine oxidase (29, 162), these workers considered their finding as further strong evidence for the monoamine oxidase hypothesis of the potentiation of epinephrine. It cannot be denied that this is additional evidence; however, in view of the criticisms of the hypothesis already given, it is best to withhold final judgment on the matter.

It is possible that not all of the substances listed above potentiate the effects of epinephrine and related sympathomimetics by the same mode of action. However, if they do, it seems most likely that their common mode of action would be that of inhibition of the same inactivating enzyme system. As is apparent from the previous discussion, this system may be one other than that of monoamine oxidase.

g. Potentiation by substances of physiological origin. Mylon and coworkers (304–306) reported a marked synergism with respect to vasoconstrictor action in the perfused rabbit ear when epinephrine was injected along with liver and kidney extracts, crude renin, crude hypertensin or tyrosine. Since all of these materials are potent anti-oxidants of epinephrine in the type of physiological salt solution used for perfusion (see Section VI,b), it is possible that a major portion, if not all, of the synergistic effect observed was due to a protection of epinephrine from

as local potentiation by the added agent is to be found in the work of Baez and Shorr (351) on the effect of diphenhydramine on the responses of terminal vessels in the rat mesoappendix to topically applied epinephrine. Cotzias (89) had reported that injections of large doses of this antihistaminic greatly potentiated the response of these vessels to epinephrine. He attributed this potentiation to an inhibitory effect of diphenhydramine on the monoamine oxidase in the vessels under observation. However, Baez and Shorr clearly demonstrated that the potentiation was mediated by VEM (vasoexcitor material) liberated from the rat kidneys as a result of asphyxia due to diphenhydramine-induced bronchial constriction.

metal-catalyzed oxidation in the perfusion fluid itself. More recently Smith (355) has reported on the potentiation of the constricting action of epinephrine on perfused swine carotid arteries in the presence of 5×10^{-8} thyroxin. This is probably a case of true potentiation at the level of the biophase of the vascular smooth muscle, for Smith was careful to use glass-redistilled water in making his perfusion fluid (personal communication). However, since thyroxin has a very high affinity for heavy metals, the possibility of inhibition of epinephrine oxidation in the perfusion fluid should not be overlooked.

Intravenous injection of the renal vasoexcitor material, VEM, markedly potentiates the vasoconstrictor response of terminal arterioles and precapillary sphincters of the rat mesoappendix to topically applied epinephrine or norepinephrine and to splanchnic nerve stimulation (33, 352, 353). The active substance in VEM is probably a polypeptide or a smaller molecule which is readily adsorbed by polypeptides or proteins (172). However, since the material is injected in the Zweifach bioassay procedure, it is not known whether it produces its potentiation by local action on the mesenteric vessels or by some remotely mediated mechanism. Injections of VEM do not potentiate the vasopressor effect of epinephrine; and no potentiation of the epinephrine contraction of rabbit aortic strips has been detected in the presence of VEM (Furchgott, unpublished results). However, the material used in experiments on strips was highly impure, and such experiments would be worth repeating with more highly purified material.

Very recently Akers *et al.* (4) have published a note on a second blood-borne factor, different from VEM, which like the latter material sensitizes the terminal vessels of the rat mesoappendix to topically applied epinephrine. This factor is released into the circulation of rats on stimulation of the splanchnic nerve. Its release is not prevented by acute removal of the kidneys, spleen, or adrenals, but it is prevented by preganglionic blockade with local epidural anesthesia. This would indicate that the factor may be released from the central nervous system as a consequence of the stimulation of afferent fibers in the splanchnic nerve. No reports have yet appeared concerning its chemical properties.

In a discussion of potentiation by physiological substances, it should be noted finally that such relatively simple substances as epinephrine, histamine and 5-hydroxytryptamine frequently can be shown to act synergistically at close to threshold concentrations in causing contraction of isolated arteries (Furchgott, unpublished observations). Also, changes in ionic concentrations (decreased Ca^{++}, increased K^{+} or OH^{-}) of the perfusion fluid, insufficient to cause appreciable changes in vascular tone of themselves, may lead to potentiation of the constricting action of epinephrine (170, 242, 287).

h. Potentiation of responses to drugs following denervation. Chronic sympathetic denervation has generally been found to potentiate the responses of various vascular beds to both vasoconstrictor and vasodilator drugs. (For examples of this phenomenon and references see 9, 65, 98, 110, 199, 269, 287; but also see 207 for a report on lack of potentiation following chronic denervation of dog limbs.) Most of the studies on such potentiation have been carried out with

sympathomimetic amines; and various hypotheses have been proposed to explain the increased sensitivity of the denervated vascular smooth muscle (as well as other types of smooth muscle) to epinephrine-like substances, and the decreased sensitivity to ephedrine- and tyramine-like substances. Burn and Robinson (55, 59–62, 333) have come out strongly for the monoamine oxidase hypothesis—largely because they were able to show a significant correlation between decrease in activity of this enzyme and increase in sensitivity of the nictitating membrane to norepinephrine over the first 8 to 10 days following cervical ganglionectomy. They also obtained a mean decrease in monoamine oxidase activity in arteries of the cat fore limb after denervation. However, Armin *et al.* (10) were unable to show a statistically significant fall in the activity of this enzyme in denervated ear arteries of rabbits, and concluded that the marked increase in sensitivity to epinephrine of these arteries could not be explained by the monoamine oxidase hypothesis. On the other hand, they did find a significant decrease in cholinesterase activity after sympathectomy, and a very marked decrease after both sensory and sympathetic denervation.

Armin *et al.*, in turn, introduced their own hypothesis that potentiation of epinephrine vasoconstriction after denervation is due to a marked decrease in the local concentration of acetylcholine in blood vessels. This hypothesis was based on the following indirect pharmacological evidence: a) that atropine potentiated and eserine usually depressed the epinephrine response in normal but not in denervated ear arteries; b) that an injection of acetylcholine (at a low concentration sufficient to give a small degree of dilation *per se*) along with epinephrine greatly depressed the epinephrine response on denervated arteries; and c) that extracts of denervated arteries appeared to have much less of an acetylcholine-like material than extracts of normal arteries, when assayed on denervated ear arteries before and after injections of either atropine or eserine into such arteries. This is an interesting new hypothesis to explain the greater sensitivity of denervated vessels to epinephrine, but it is not yet convincing. The concentration of atropine used (10^{-6}) is very much greater than that needed to antagonize effectively small concentrations of added acetylcholine, and it could conceivably have potentiated the effect of epinephrine directly. As pointed out in Section V,a, atropine at slightly higher concentrations than this is an effective adrenergic blocking agent, and many such agents have been found to potentiate epinephrine responses at concentrations slightly below the detectable blocking level (Section VI,f). Moreover, if the normal artery contains small concentrations of acetylcholine sufficient to "antagonize physiologically" the effect of added epinephrine, then atropine alone might be expected to cause constriction and eserine alone, to cause dilation; but neither of these effects occurred.

One of the older hypotheses to explain the observed potentiation to many types of drugs after denervation states that the cell membranes become more permeable to drugs in general (65). This might be considered as potentiation by mode of action 1) discussed in Section VI,a. However, as was pointed out in that Section, an increased permeability involving a proportionate increase

in both rate constant, k_a, for penetration of the agonist into the biophase and in rate constant, k_b, for escape from the biophase, would lead to a shorter rather than longer (potentiated) duration of response, provided the rate constants for enzymatic inactivation of the agonist were not changed. In actual experiments denervation has usually been found to potentiate not only degree of response but also duration of response to various drugs. (See, for example, 110 on vasoconstriction by epinephrine in the sympathectomized human hand and 10 on constriction by epinephrine of the central artery of the denervated rabbit ear.) This indicates that if changes in permeability are important in potentiation following denervation, than there is probably not only an increase in the rate constant, k_a, for penetration of the drug into the biophase, but also a decrease in the sum of the rate constants for removal of the drug from the biophase (k_b + rate constants for enzymatic inactivation).

Another hypothesis to explain potentiation after denervation—namely, that of Fleckenstein and Bass (137) on loss of accommodation due to removal of a maintained low concentration of neurohumoral transmitter—has already been considered in the section on potentiation by cocaine. If these investigators are correct in their belief that the mode of potentiation of response to epinephrine-like substances is the same in the case of denervation and cocaine treatment, then the criticism of their hypothesis given in Section VI,d, also applies here. As already noted in that section, the possibility still exists that potentiation of epinephrine-like substances after denervation is due to the loss of some undiscovered enzyme system which normally inactivates these substances in the biophase of the smooth muscle cells much faster than does monoamine oxidase.

Regardless of the mechanism of the marked sensitization of vascular smooth muscle to drugs following denervation, it is very likely that this sensitization accounts for at least part of the restoration of tone which occurs after nerve degeneration. This idea has been discussed by numerous investigators (*e.g.*, 65, 98, 199, 288), who have attributed the restoration of tone to a now effective stimulation of the sensitized, denervated smooth muscle by the normal very low concentrations of vasoconstrictor substances in the extra-cellular fluid of the body.

VII. LOCAL INDIRECT ACTION OF CERTAIN DRUGS ON VASCULAR SMOOTH MUSCLE

a. Possible modes of local indirect action. The term "local indirect action" is used here to indicate an action which is not mediated by a direct activation of the receptors of effector cells by the administered drug, but rather by an activation of the receptors by a second substance which accumulates in the region of the effector cells as a result of localized reactions initiated by the presence of the drug in that region. In a local indirect action the site of formation or release of the second substance which is the actual active agent may either be in the tissues adjacent to the effector cells or in the effector cells themselves. Some possible modes of local indirect action are the following.

1) Competitive displacement of the active agent from

adsorption sites by the added drug: In this case the drug would have a high affinity for the sites in the tissue on which the active agent was adsorbed, and would release it by competing with it for these sites. This may be the mode of action by which certain histamine releasing agents produce part or all of their effect on blood vessels and other smooth muscle structures (*e. g.*, 129–131, 297, 298). In the case of histamine release in the skin, the liberated histamine might act not only directly on the vascular smooth muscle, but also cause additional local vasodilation by the axon reflex mechanism (272). Therefore, an administered drug which released histamine in the skin could provoke a vascular response by both a "one-step" and "two-step" indirect action.

2) Injury by the added drug of cells containing the active agent, leading to escape of the active agent from these cells: This may be the mode of action of the more potent histamine-releasing agents such as 48/80 (a polymeric condensation product of N-methylhomoanisylamine with formaldehyde), certain long chain aliphatic amines, certain amidines, and d-tubocurarine. Some of these have been shown to destroy tissue mast cells, which normally contain high concentrations of histamine (329).

3) Stimulation of nerve terminals by the added drug so as to set off a localized axon reflex which releases the active agent at other terminals: This is the classical mode of indirect action of histamine on afferent terminals, leading to the flare response (272). Fleisch and coworkers (140, 141, 143) have attributed part of the vasodilating effect of acetylcholine and adenylic acid derivatives as well as histamine to an axon reflex of this sort. Brun (43) has even attributed a major part of the vasoconstrictor action of epinephrine on small arteries of the anesthetized rat to a local axon reflex.

4) Stimulation of peripheral nervous synapses by the added drug so as to set off post-synaptic impulses leading to a release of the active agent at post-synaptic terminals: On the basis of pharmacological evidence (inhibition of effect with ganglionic blocking agents) this appears to be the mode of action by which acetylcholine, nicotine and related drugs produce at least part of their actions when applied locally to skin and intestine (*e.g.*, 5–7, 106, 114, 378).

5) Stimulation of local chromaffin cells by the added drug, with the release of epinephrine-like substances from such cells: This mode of action may account for the motor effects of acetylcholine, nicotine and related drugs under certain experimental conditions on autonomic effectors on which their direct effects are inhibitory. The motor effects of acetylcholine and nicotine on the heart, especially after atropinization, have been investigated by a number of workers. (See 187, 188, 255 for the most recent work and for references to previous papers.) Recently the vasoconstrictor effects of acetylcholine and nicotine on certain perfused vascular beds have also been reinvestigated (209, 222, 231, 256). Whether these motor effects on heart and small vessels are due to stimulation by these drugs of local

chromaffin cells or of peripheral sympathetic synapses (mode of action 4)) cannot yet be decided. Ganglionic blocking agents are able to block these effects, but they might be expected to block either type of stimulation.

6) Inhibition by the added drug of an enzyme system which continuously inactivates an active agent which is continuously released locally: This mode of action would allow the active agent to attain higher concentrations locally and so permit it to exert a demonstrable effect. It, of course, accounts for the stimulation of intestine by physostigmine, which inhibits the destruction of locally released acetylcholine. It is the mode of action proposed by Gaddum and Kwiatkowski (185) and adopted by Burn, Tainter and others to explain the vasoconstrictor action of ephedrine, cocaine, tyramine and related substances (see Section VI for references). According to these workers, these drugs inhibit the activity of monoamine oxidase and thus permit a local accumulation of the sympathetic transmitter released by normal sympathetic activity.

7) Local metabolic conversion of the added drug to an active agent: In this situation the added drug at the concentration used would have little if any direct action on receptors, but it would be converted by a local enzyme system to a derivative which would have a greater affinity for and/or greater activating capacity on the receptors. The formation of the derivative (active agent) might occur either in the smooth muscle cells themselves or in adjacent tissue.

b. Local indirect action of acetylcholine, nicotine and related substances. As noted in Section IV,i, acetylcholine does exert a direct action on cholinergic motor receptors in large arteries and veins. However, the vasoconstriction often produced by acetylcholine and nicotine in perfused structures apparently results for the most part not from a reaction of these drugs with motor receptors but from a local indirect action. This vasoconstriction recently has been investigated in the case of perfused rabbit ears (187, 256), perfused dog livers (231), and cat limbs *in situ* (222). Atropine may augment the vasoconstriction (especially that caused by acetylcholine), and ganglionic and adrenergic blocking agents inhibit it. The mode of indirect action, as indicated above, is probably either mode 4) or 5), and the active agent released appears to be norepinephrine or epinephrine. Kottegoda found that the vasoconstrictor effect of acetylcholine in the rabbit ear, which supersedes the vasodilator effect obtained in a fresh preparation after several hours of use, could be reversed to a vasodilator effect with hexamethonium. He also showed that the indirectly mediated vasoconstrictor effect was confined largely to the skin of the ear; for when he removed most of the skin from a perfused ear giving a marked constrictor response to acetylcholine, he obtained in the skinned ear either a much weaker constrictor response or a dilator response.

c. Local indirect action of tyramine, ephedrine and related sympathomimetic amines. The early work of Tainter, Burn and others indicated that a large part of the motor effects of tyramine and ephedrine on sympathetic effectors was due to local indirect action (*e.g.*, see 54, 62a, 366–368). Evidence for this came from the

finding that the effects of these drugs on blood pressure, nictitating membrane, perfused limbs, perfused heart, etc., were partially or completely inhibited by chronic denervation or cocaine treatment; whereas the effects of epinephrine and other potent sympathomimetic amines containing a catechol nucleus were potentiated. The effects of various other weak sympathomimetic amines, related to tyramine or ephedrine in that they possessed only a para-phenolic hydroxyl or no phenolic hydroxyl group, were also found to be inhibited by cocaine and denervation. (See, for example, 303, and references therein to earlier papers.) Various hypotheses about the nature of the indirect action of drugs in the ephedrine-tyramine group were proposed; but the hypothesis which generally supplanted the others, as already indicated, was that of Gaddum and Kwiatkowski (185) that these drugs exerted their action by inhibiting the oxidation of the epinephrine-like sympathetic transmitter by monoamine oxidase.

As noted in Section VI,e, there is good evidence that drugs of the ephedrine-tyramine group may exert a direct action on adrenergic motor receptors when used in high concentrations; however, there can be little doubt that their vasoconstrictor effects at low concentrations are in large part attributable to a local indirect action.[20] That the monamine oxidase hypothesis accounts satisfactorily for this indirect action is questionable; for the same experimental evidence applied against this hypothesis in the case of potentiation by these drugs (see Section VI) can be applied against it as an explanation of their vasoconstrictor effects. As in the case of potentiation, the possibility still exists that they may exert their indirect action at low concentrations by inhibiting some enzyme system which inactivates the sympathetic transmitter much more rapidly than does monoamine oxidase.

In particular, in the case of tyramine there is also the possibility that it exerts a major part of its indirect action by mode of action 7); that is, by being converted by local enzymes to the active agent itself. Such an active agent could be

[20] With rabbit aortic strips, pretreated with iproniazid to block monoamine oxidase, it is possible to demonstrate two modes of action of tyramine leading to contraction. If the strip has a relatively high sensitivity to tyramine, the graded contractions occurring over a concentration range from about 10^{-7} to 10^{-5} can be inhibited completely, or almost completely, by approximately matching concentrations of cocaine. However, the contractions produced by 10^{-4} and 10^{-3} tyramine can only be partially inhibited by matching concentrations of cocaine. The cocaine-sensitive part of the contractions appears to be due to a direct action of a norepinephrine- or epinephrine-like substance accumulating in the presence of tyramine, since it is inhibited by relatively low concentrations of various blocking agents to the same extent as are contractions of similar height produced by low concentrations of norepinephrine or epinephrine. The cocaine-insensitive part of the contractions produced by 10^{-4} to 10^{-3} tyramine appears to be due to a direct action of tyramine on adrenergic motor receptors. In order to inhibit significantly this part it is necessary to use relatively high concentrations of competitive adrenergic blocking agents, such as are required to inhibit significantly the essentially maximal contractions produced by high concentrations ($\sim 10^{-5}$) of norepinephrine and epinephrine. In contrast to the situation with tyramine, local indirect actions of ephedrine and amphetamine on aortic strips cannot be readily demonstrated. Most of the contraction observed with these latter drugs appears to result from their direct action on adrenergic motor receptors (see Section IV, e).

formed in an oxidation which introduced a meta-hydroxyl group. Introduction of a β-hydroxyl group, in addition, would produce an even more active agent, namely, norepinephrine itself. That such a conversion might occur either at the sympathetic nerve terminals or in the biophase of the smooth muscle cells is not inconceivable. The marked inhibitory effect of cocaine treatment or chronic denervation on the response of blood vessels, nictitating membrane, and other sympathetic effectors to tyramine would be explained on the basis of a competitive inhibition of the oxidizing enzyme system in the case of cocaine, and a loss of activity of the enzyme system in the case of denervation.

This is, of course, the counterpart of the speculation advanced in Section VI that many drugs (including cocaine and compounds of the ephedrine-tyramine group) and denervation may potentiate the effects of sympathomimetic amines containing a meta-hydroxyl group by respectively inhibiting or causing a loss of activity of an enzyme-donor system which rapidly reduces the meta-hydroxyl group. To conclude the speculation, the enzyme system which reduces the meta-hydroxyl in epinephrine-like compounds might logically be the same one which introduces the meta-hydroxyl by oxidation in tyramine-like (and possibly also in some ephedrine-like) compounds. In other words, the enzyme would couple the oxidation and reduction of the meta-carbon of the phenyl ring with the reduction and oxidation of some other normally occurring electron acceptor-electron donor system (redox system).

In ending this discussion of the local indirect action of tyramine, it should be noted that in some perfused vascular beds tyramine may possibly exert part of its action by locally releasing histamine as the active agent. This possibility is introduced in view of the reports of Mongar and Schild (297) on the liberation of histamine by aliphatic amines from minced guinea pig lung, of Koch and Szerb (249) on the liberation of histamine by epinephrine from perfused rat lung, and of Baur and Staub (22) on the elevation of blood histamine after synephrine (Sympatol) injections. A release of histamine by tyramine would help explain in part the puzzling finding of Daly *et al.* (101) that in perfused cat lungs the pressor potency of tyramine was often maintained over a long experimental period during which the pressor potency of epinephrine fell markedly to well below that of tyramine.

d. Local indirect action of 5-hydroxytryptamine and tryptamine. Pharmacological investigations of the action of 5-hydroxytryptamine and tryptamine on the isolated guinea pig ileum indicate that part of their excitatory effect on this smooth muscle preparation is indirectly mediated through the release of acetylcholine (182, 332, 335). It has been suggested that this release of acetylcholine is brought about by mode of action 3) or 4). Feldberg and Smith (130) have also demonstrated that these agents have some capacity for releasing histamine from perfused skin and muscle. In the case of vascular smooth muscle preparations, as pointed out in Section IV,f, there is excellent experimental evidence obtained with the use of differential blocking agents that 5-hydroxytryptamine can produce contraction by a direct action on a specific type of motor receptor. Nevertheless, the possibility that part of the vasoconstrictor effect of 5-hydroxy-

tryptamine (and tryptamine) may be the result of a local indirect action should not be overlooked. Some evidence for such an indirect action has been obtained in studies on tachyphylaxis with this substance in strips of rabbit aorta (see next sub-section). It is also possible, as noted in Section IV,f, that the vasodilator effect of 5-hydroxytryptamine on the coronary bed (346) is due to a local indirect action rather than a direct action on inhibitory receptors.

e. The relation between the local indirect action of certain drugs and tachyphylactic phenomena. A steady decline in the blood pressure response to a series of repeated intravenous injections or to continuous systemic administration of certain vasoconstricting agents has been called tachyphylaxis. Some substances including 5-hydroxytryptamine (162, 317) and hypertensin (191, 192) give a somewhat decreased vasopressor response on second injection if the second injection follows the preceding injection at an interval of less than about ten minutes. Such a decreased response appears to be associated with a continued activation of the motor receptors of the vascular musculature by that fraction of the drug from the preceding injection still present in the region of the receptors at the time of the second injection, along with a return of blood pressure to essentially the pre-injection level as a result of compensation of the cardiovascular system under the influence of pressor reflexes. On the basis of receptor theory, the continued activation of receptors by that fraction of drug still present from the preceding injection could decrease the relative vasoconstrictor effect of the drug introduced in the second injection.

Marked tachyphylaxis of the vasopressor response—to the extent of complete abolition of the response for long periods of time following a series of repeated injections—has been frequently observed in the case of ephedrine and related N-phenylisopropylamines and certain aliphatic amines which are also N-isopropyl derivatives. The nature of this phenomenon in the case of three N-phenylisopropylamines (ephedrine, amphetamine, and methamphetamine) has been analyzed by Winder *et al.* (396). (For many references to older work on tachyphylaxis with such amines, the reader is referred to this paper.) These workers simultaneously measured the vasopressor response and the degree of shrinkage of the acutely denervated nasal mucosa of phenobarbitalized, atropinized dogs during a series of injections of a fixed dose of drug at intervals in a pre-determined schedule (successive 50, 40, 30, 20 and several 10 minute intervals). In the case of any of the three drugs indicated above, each injection produced additional shrinkage of the nasal mucosa, and the degree of shrinkage following any one injection was maintained almost without loss up to the time of the next injection. Probit analysis of the results clearly indicated a cumulative dose effect on shrinkage of the denervated mucosa. The degree of shrinkage was taken as a measure of vasoconstriction uncompensated by nervous reflex mechanisms. The vasopressor effect of each repeated dose fell off as the degree of shrinkage of the nasal mucosa increased, and completely disappeared when mucosal shrinkage was close to its maximal level. The authors concluded that the development of such marked tachyphylaxis with respect to vasopressor response represents an increasing occupation of "receptor points" by the drug

with each successive injection. This increasing occupation of "receptor points" leads to increasing activation of vasoconstriction by the drug, so that when occupation of the "receptor points" approaches saturation (maximal activation of vasoconstriction for the drug used), a vasopressor response no longer occurs on injection of the drug.[21] The complete or partial return of blood pressure to the initial level following each injection early in a series of injections is considered to be due to compensatory nervous reflex mechanisms affecting the cardiovascular system.

Winder *et al.* (396), like previous workers, observed cross-tachyphylaxis between the phenylisopropylamines and concluded that they all combined with the same type of receptor points. They preferred to be non-committal as to whether the vasoconstriction with these agents resulted from a direct action on the vascular smooth muscle cells (combination with and activation of motor receptors) or an indirect action, possibly mediated by combination with and inhibition of an enzyme which normally inactivates the sympathetic transmitter. However, a consideration of the experimental evidence of others on the concentrations required for direct and indirect actions of ephedrine-like compounds (Section VII,c) makes it likely that the latter type of action is the predominant if not the only type at the dose levels used in experiments such as those under discussion.

Horita *et al.* (230) clearly demonstrated in a series of well designed experiments with amphetamine on dogs that the accumulation of this drug leading to tachyphylaxis of the vasopressor response must be in the region of the receptors with which the drug combines rather than in the body fluids generally. Working with the perfused rabbit ear these workers also showed that the presence of 1:50,000 amphetamine in the perfusion fluid for 30 minutes led to a vasoconstriction which approximately halved the flow rate and persisted for as long as twelve hours after return to drug-free perfusion fluid. During the post-treatment period an injection of 0.05 micrograms of epinephrine was still able to cause temporarily an almost complete cessation of flow, just as in ears untreated with amphetamine. This is further evidence that the receptors occupied by amphetamine during the period of maintained vasoconstriction are not the adrenergic motor receptors, for if they were, then vasoconstriction with small doses of epinephrine would have been antagonized. (See Section, IV,e.) The prolonged activation of vasoconstriction which accounts for tachyphylaxis to phenylisopropylamines may therefore result from prolonged inhibition by these agents of some enzyme which ordinarily inactivates norepinephrine or epinephrine continuously released from sympathetic nerve terminals. This enzyme, according to the hypothesis of Gaddum and Kwiatkowski (185), would be monoamine oxidase; however, as

[21] Winder *et al.* (396) explained the symmetrical sigmoid curves obtained on plotting degree of vasoconstriction against logarithm of the cumulative dose on the basis of the theory that the probability of occupation of a receptor point by the drug depends on the logarithm of the cumulative dose. However, the curves may just as readily be explained on the basis of receptor theory (Sections IV and V), according to which occupation is determined by the law of mass action.

previously noted in this review, the possibility exists that another enzyme system inactivates norepinephrine and epinephrine much more rapidly than does monoamine oxidase.

In contrast with the prolonged indirectly mediated vasoconstriction following administration of phenylisopropylamines in experiments with animals or perfused structures is the contraction produced by these drugs on isolated strips of rabbit aorta (Furchgott, unpublished results). Such strips give small graded contractions over a concentration range from about 10^{-6} to 10^{-3} of ephedrine or amphetamine. These contractions, which are apparently due primarily to a direct action of these drugs on adrenergic motor receptors (see Sections IV,e, and VII,d) are well maintained as long as the drug is present; but relaxation quickly sets in after washout of the drug, and, although slower than after epinephrine, is practically complete within about thirty minutes. These results indicate that ephedrine and amphetamine escape from the biophase of the smooth muscle cells of rabbit aorta fairly readily. If the same situation prevails in the case of the muscle cells of peripheral vessels, how can these drugs produce such prolonged, indirectly mediated vasoconstriction of such vessels? One possibility is that they are adsorbed almost irreversibly on the enzyme which inactivates the sympathetic transmitter, and so inhibit this enzyme for very long periods. A second possibility is that they escape much more slowly from the structures which contain the enzyme responsible for inactivating most of the sympathetic transmitter than they do from the smooth muscle cells. Such structures might conceivably be the sympathetic nerve fibers themselves.

Tachyphylaxis of the vasopressor response to certain vasoconstrictor drugs in the whole animal should be clearly differentiated from tachyphylaxis of the contractile response to certain drugs sometimes observed in *in vitro* studies with preparations of smooth muscle. Whereas the former phenomenon is associated with a maintained activation of vasoconstriction by the administered drug, the latter phenomenon is associated with a specific loss of sensitivity of the isolated smooth muscle preparation to the drug used and to related drugs which mediate contraction by the same mode of action.[22] In this second type of tachyphylaxis the isolated smooth muscle is unable to maintain the maximal height of contraction which it attains during continued exposure to a high concentration of the drug, but instead relaxes partially or completely; and even after washout of the high concentration, its sensitivity to subsequent additions of the drug used and related drugs is markedly reduced for long periods of time. This second type of tachyphylaxis, which might better be called "specific desensitization", has been observed primarily in the case of drugs acting on smooth muscle preparations on which they presumably produce part or all of their effects by means

[22] The phenomenon of tachyphylaxis in the case of isolated smooth muscle preparations should not be confused with the generalized partial loss of sensitivity to all stimulating drugs often observed after exposure of such a preparation to one stimulating drug at a sufficiently high concentration to cause an essentially maximal contraction (66, 103, 170). In true tachyphylaxis the loss of sensitivity is specific for the drug used and closely related drugs.

of a local indirect action. It therefore seems likely that such desensitization represents an inhibition or exhaustion of the mechanism which mediates the local indirect action—an inhibition or exhaustion resulting from a continuous, near maximal activation of the mechanism by the high concentration of drug added.

The best known example of this phenomenon in smooth muscle is the specific desensitization to the motor effect of nicotine on the gut following applications of high concentrations of this drug. This is commonly attributed to excessive, prolonged depolarization of the peripheral ganglion cells on which nicotine acts in initiating its local indirect action in this structure (mode of action 4)). A similar situation has been reported by Hilton (222) in the case of nicotine vasoconstrictor and vasodilator effects in the cat hind limb. Marked specific desensitization has also been observed by Gaddum (181) in the case of tryptamine and 5-hydroxytryptamine added in high concentrations to segments of guinea pig ileum. Recovery of the contractile response of the ileum to these drugs following washout of the high concentrations was very slow, and incomplete even after one hour. As already noted, these drugs are thought to exert part of their effect on this preparation by mode of indirect action 3) or 4). The nature of the inhibition of the mechanism mediating the action following high concentrations is not understood.

Tachyphylaxis of the desensitization type has also been observed frequently in recent experiments on the contracting effect of tyramine on rabbit aortic strips (Furchgott and Ashby, unpublished results). This is best demonstrated with strips which have a relatively high initial sensitivity to tyramine. As has already been pointed out in Section VII,c, the effect of tyramine on such strips is in large part due to a local indirect action, probably mediated by mode of action 7). The maximal height of contraction of such strips with tyramine, obtained at a concentration of 10^{-4} to 10^{-3}, is often as much as seventy-five per cent of that with epinephrine. This is in contrast to the behavior of strips with an initial low sensitivity to tyramine, which often give a maximal height at the same high concentrations of no more than thirty-five per cent of that with epinephrine. However, in the case of the strips with high initial sensitivity, the contractions produced by high concentrations of tyramine usually are not maintained but gradually fall off to a lower level which is approximately the same as that obtained with strips of low initial sensitivity. This appears to be due to an exhaustion of the mechanism which mediates the indirect action of tyramine. Additional evidence for this is that that portion of the contraction height which is not maintained is approximately the same portion as is sensitive to cocaine antagonism (see footnote in Section VII,c). After washout of high desensitizing concentrations of tyramine, strips which originally had a high initial sensitivity now respond to smaller concentrations like strips with low initial sensitivity.

A specific desensitization very similar to that described for tyramine has been found to occur frequently when high concentrations of 5-hydroxytryptamine are added to aortic strips (168). This is considered as evidence that this drug also produces part of its contracting effect on this vascular smooth muscle preparation by means of a local indirect action. The nature of this indirect action is

not clear, but preliminary experiments indicate that it is not similar to the indirect action of tyramine, for there does not appear to be cross-tachyphylaxis between the two drugs, and cocaine does not significantly antagonize the response to 5-hydroxytryptamine.

CONCLUDING REMARKS

In this review I have taken the liberty of indulging in considerable speculation about the actions of various drugs on vascular smooth muscle. In so doing, I have frequently introduced new or modified hypotheses. These hypotheses at the moment appear more satisfactory—at least to me—than do the ones for which they have been substituted; but I have little doubt that they in turn will either have to be discarded or markedly modified as new experimental observations are made.

Most of the hypotheses discussed in this review—whether new or old—have been concerned in one way or another with the reactions of drugs or natural agents with specific receptors of smooth muscle. Indeed, most of this review might be considered an essay on the application and extension of "receptor theory" in the field of vascular pharmacology. Receptor theory has proven very useful as a basis for classifying the modes of action of various drugs on vascular as well as other smooth muscle. However, receptor theory, as it now stands, falls short of explaining the mechanisms of action of various drugs on smooth muscle. This failure, of course, stems in the first place from our lack of knowledge about the nature of receptors. What is their role in the structural and functional organization of the cell? Are they parts of enzyme systems? How do they influence the exchange of ions across cell membranes? What is the nature of the reactions which take place once a drug molecule has been adsorbed on a specific receptor? Questions such as these cannot at present be answered. They make us realize that our general use of the term "receptor" is in a way an acknowledgement of our ignorance of the very nature of those components of the cells to which we apply this term.

If our understanding of the actual mechanisms of drug action on smooth muscle cells, as well as on other cells to which receptor theory is applied, is to be advanced, working hypotheses on the nature of receptors and receptor-drug reactions will have to be proposed and tested experimentally. This is the direction for the future development of receptor theory.

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Fuchgott (Review)

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