Microvascular Res. 3, 361-384 (1971). BOL 284/LSD 2247/SYT 1242/VAP 380

Chemical and Humoral Regulation of Blood Flow Through the Precapillary Sphincter[1, 2]

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Received May 15, 1971

INTRODUCTION

According to current concepts, local tissue homeostasis is maintained by autonomous chemical and humoral regulatory mechanisms of the microcirculation (Lewis, 1927 Krogh, 1929; Dale, 1929; Zweifach, 1957, 1968; Altura, 1967; Haddy and Scott, 1968; Mellander and Johansson, 1968; Siggins, 1970). Local regulation of microcirculatory blood flow, and more specifically blood flow through the precapillary sphincters, where they exist, is thought to be largely dependent upon the liberation of chemical and humoral (i.e., blood-borne) mediators either in the parenchymal tissues in the immediate vicinity of these small microscopic blood vessels or possibly in the vascular smooth muscle–endothelial cell complexes. These chemicals are thought to modify the vasomotor tone of the muscular components of the capillary bed by directly producing constriction or dilation, thus tempering the local inactivation of blood-borne constrictor or dilator substances, or by altering the reactivity of the muscular microvessels and precapillary sphincters to endogenous humoral stimuli.

It is important to realize at the outset that no one explanation for the maintenance of local tissue homeostasis and the phenomenon of vasomotion is proven for the circulation to any one bodily tissue. Although the myogenic response to stretch probably contributes to different degrees to local tissue homeostasis in different vascular beds (Baez, 1968a; Johnson, 1968; Mellander and Johansson, 1968; Wiedeman, 1968), the most prevalent explanations for local control of blood are based upon an interaction of chemicals produced for the most part by tissue metabolism (Berne, 1964; Haddy and Scott, 1968; Mellander. 1970; Skinner and Costin, 1968), with humoral or blood-borne substances (Lewis, 1927; Krogh, 1929; Dale, 1929; Zweifach, 1957, 1968).

Some investigators think that local control of blood flow resides solely in local chemicals produced by tissue metabolism (Berne, 1964; Haddy and Scott, 1968; Skinner and Costin, 1968). For example, when tissue cells depart from the resting state or are deprived of an adequate blood supply resulting from local constriction of arterioles, metarterioles, and/or precapillary sphincters, substrate concentrations are thought to fall and certain metabolite or chemical concentrations may thus increase in the tissue fluids surrounding the microscopic blood vessels. Either or both changes should, at

[1] Presented at the 19th Annual Conference of the Microcirculatory Society, Symposium on the Precapillary Sphincter, April 16, 1971, Chicago, Ill.

[2] Supported by United States Public Health Service Grants HE-12462 and HE-11391.

[3] Recipient of Public Health Service Research Career Development Award 5-K-3-GM-38, 603.

least in theory, act to inhibit or attenuate the normal activity of the smooth muscle cells in the walls of the microvessels and bring about vasodilatation or increased local blood flow. For example, a decrease in oxygen tension in the vascular beds of skeletal and cardiac muscle is thought to act as the stimulus for vasodilatation (Berne, 1964; Guyton *et al.*, 1964; Haddy and Scott, 1968). Whatever the chemical change may be, more blood flows to the tissue and/or increased cellular metabolism subsides. Thus, changes in substrate and certain metabolite or chemical concentrations are reversed, inhibition or attenuation to the microvascular smooth cells is removed, and an appropriate local vasoconstriction then takes place restoring blood flow to normal. This idea

TABLE 1

HUMORAL AND CHEMICAL SUBSTANCES CURRENTLY IMPLICATED IN REGULATION OF BLOOD FLOW IN THE MICROCIRCULATION

Humoral (blood-borne) substances		Chemicals (locally produced-metabolic)	
Agent	Response	Agent	Response
Catecholamines		Adenosine and adenine	
Epinephrine	vcs, vdil[a]	nucleotides	vdil, vcs
Norepinephrine	vcs[b]		
Dopamine	vcs, vdil	Hypoxemia	vdil
Amines		H^+	vdil
Serotonin	vcs, vdil		
Histamine	vdil	K^+	vdil[c]
Acetylcholine	vdil	Inorganic phosphate	vdil
Polypeptides			
Angiotensin	vcs	Hypercapnea	vdil
Kinins	vdil		
Vasopressin	vcs, modifier	Kreb's cycle intermediates	vdil
Oxytocin	vcs, vdil, modifier		
		G-I tract polypeptides	
Glucocorticoids	Modifiers	Glucagon	vdil
		Cholecystokinin	vdil
Estrogens	Modifiers	Secretin	vdil
Plasma factors	Modifiers, vcs	Prostaglandins	vcs, vdil, modifiers
		Hyperosmolarity	vdil

[a] vcs = vasoconstriction; vdil = vasodilation.
[b] Thought to promote coronary vdil as well.
[c] High concentrations promote vcs.

has led to the concept that local regulation of blood flow is dependent upon a balance between vasodilator and vasoconstrictor factors. The search for identification of such naturally occurring chemical and humoral substances has continued for more than 75 years. A list of possible naturally occurring substances that may play some role in regulation of blood flow in the microcirculation is presented in Table 1 as compiled from the literature.

The humoral or blood-borne substances include *catecholamines* (Zweifach, 1957, 1961, 1968; Altura, 1967; Mellander and Johansson, 1968; Abboud, 1968; Berman and Siggins, 1968; Duling *et al.*, 1968), *other amines* (Zweifach, 1957, 1968; Illig, 1961;

Altura, 1967; Abboud, 1968; Berman and Siggins, 1968; Schayer, 1968; Wurzel and Zweifach, 1966), *polypeptides* (Zweifach, 1966, 1968; Lewis, 1968; Gross, 1963; Altura, 1967, 1970a; Altura and Hershey, 1967; McGiff, 1968; Altura *et al.*, 1970; Abboud, 1968), *glucocorticoids* (Zweifach *et al.*, 1953; Schayer, 1964; Altura, 1966a), *sex hormones* (Lloyd, 1959a, 1959b; Pickford, 1966; Lim and Walters, 1970), and *plasma factors* (Bohr and Johansson, 1966; Wurzel *et al.*, 1964, 1967; Bohr and Sobieski, 1968). Most of these blood-borne substances act directly to initiate either vasoconstriction or vasodilation. Others such as the neurohypophyseal hormones vasopressin and oxytocin as well as glucocorticoids, estrogens, and miscellaneous plasma factors may modify blood flow by potentiating or inhibiting the constrictor or dilator action of certain humoral substances on the muscular microvessels (Zweifach and Metz, 1956; Zweifach, 1961; Wurzel *et al.*, 1964; Altura *et al.*, 1965; Bartelstone and Nasmyth, 1965; Altura, 1966a; Pickford, 1966; Altura and Hershey, 1967, 1968; Yano *et al.*, 1968).

The chemicals listed in the right column of Table 1 are mainly produced locally and also act solely as vasodilators. Although all of these humoral and chemical substances have been shown experimentally to affect in one way or another certain muscular microvessels and precapillary sphincters from one or more regional vascular beds, the vasotropic actions could be merely coincidental and not necessarily indicative of involvement in local regulation of blood flow, tissue homeostasis, and transcapillary exchange.

In order for any of these substances to qualify as a candidate or mediator in regulation of capillary blood flow several postulates must be satisfied:

1. The substance must act directly on one or more components of the regional microvasculature in order to affect blood flow through the true capillaries. That is, the chemical or humoral substance must alter the caliber or diameter of a particular muscular microvessel (i.e., arteriole, metarteriole, precapillary sphincter, and/or muscular venule) directly and not through release of an intermediary substance(s).
2. The substance must be identified by chemical means or bioassay as being in the vicinity of the affected muscular microvessels (Haddy and Scott, 1971).
3. The substance must be vasoactive over the range of plasma concentrations found in the particular vascular bed in question (Haddy and Scott, 1968).
4. The magnitude of the vasotropic response must be sufficient to account for the changes seen during local regulation of blood flow in the regional vascular bed in question (Haddy and Scott, 1968).
5. The duration of action must follow a time course similar to that for local regulation (Haddy and Scott, 1968).
6. The substance, if humoral, should have an associated counterpart for metabolic or physiologic inactivation, preferably in the vicinity of action.
7. Specific pharmacologic blocking drugs for the proposed humoral or blood-borne substance should unmask or reveal its presence by an opposite action.

Using some of these criteria, Haddy and Scott (1968) evaluated a number of substances with respect to local regulation of blood flow. Although several of the vasodilator metabolites such as oxygen, hydrogen, and potassium ions as well as adenosine and some adenine nucleotides satisfied certain criteria and thus may be directly or indirectly involved in local regulation, it seems highly unlikely that any one of these vasodilator metabolites can account completely for local tissue homeostasis in any one regional vascular bed. Haddy and Scott concluded that several vasodilator metabolites

could act in concert, but the types and combinations of such substances probably vary with the vascular bed.

Hyperosmolarity may be implicated as a prime regulator of capillary blood flow and transcapillary exchange in exercise hyperemia in animals and man (Mellander *et al.*, 1967; Gray *et al.*, 1968; Lundvall, 1969; Lundvall *et al.*, 1969). Mellander and co-workers have detected a venous plasma hyperosmolarity as high as 40 milliosmols/kg above the control resting level after exercise, and have mimicked the pattern of vasomotor responses seen after exercise by intraarterial infusions of hypertonic glucose, sucrose, or xylose solutions. According to their concept any tissue metabolic by-product that is osmotically active (e.g., most of those listed in the right column of Table 1) could contribute to the overall plasma hyperosmolarity and hence to the local vasodilator effect. Gray (1971) has recently presented direct *in vivo* microscopic evidence that such hyperosmolar solutions dilate primarily arteriolar and precapillary resistance vessels when applied topically to the rat spinotrapezius muscle preparation. Nevertheless, Mellander and Lundvall (1971) do not think that hyperosmolarity by itself completely accounts for exercise hyperemia.

Inorganic phosphate may be the prime mediator of functional vasodilatation in fast skeletal muscles (cat), since venous blood levels were increased with electrical stimulated ion (Hilton and Vrbová, 1970; Hilton, 1971). Hilton and co-workers reported that intraarterial infusions and injections of isosmotic NaH_2PO_4 and Na_2HPO_4 solutions potently increase blood flow in the fast skeletal muscle beds and that vasodilator effects of fast skeletal muscle contractions could be mimicked by infusions of NaH_2PO_4 producing final venous blood concentrations around 20 μmoles/ml. However, these latter amounts of venous blood inorganic phosphate concentrations are higher than those found during fast skeletal muscle contractions (Hilton, 1971).

The gastrointestinal tract hormones (glucagon and secretin) appear to increase blood flow rather selectively in the feline splanchnic vascular bed and may do so in very low physiologic concentrations (Ross, 1970a, b, 1971).

Although many substances listed in Table 1 evoke vasodilator effects in certain vascular beds and at plasma concentration levels near physiologic, thus satisfying several of the seven postulates, knowledge of their discrete sites of action in the microcirculation is for the most part based upon indirect measurements such as gross changes in flow and/or resistance across a vascular bed, and/or clearance of locally injected solutes across a vascular bed. Such measurements do not allow accurate determination of the type(s) of microvessel (precapillary sphincter, metarteriole, arteriole, or venule) that may be responding at any one instant. Precise information is vital to our understanding of chemical and humoral regulation of local blood flow and can be obtained through the use of direct microscopic observation of a living microvascular bed such as the rat mesocecum preparation of Chambers and Zweifach (1944), with documentation by quantitative measurements of microvascular diameters and lumen sizes (Baez, 1961; Altura, 1967, 1970b), and responses of single vascular smooth muscle cells demonstrated by an image-splitter television microscope technique (Baez, 1966, 1968b).

For the past several years this author has been making attempts to evaluate and identify by pharmacologic means the other side of the coin, namely, the possible humoral (or blood-borne) substances that may play a role in local regulation of blood flow in one vascular bed, namely, the rat mesocecum. Although our findings may not necessarily

apply to other vascular beds in the rat or other species, the approaches that we have taken probably can, with some modification, be used to assess humoral factors in other vascular areas. Studies on the terminal vascular bed of the rat mesocecum were designed to evaluate further the role of some of the humoral (or blood-borne) substances in microcirculatory regulation, namely, the catecholamines epinephrine, norepinephrine, and dopamine, amines such as serotonin, histamine, and acetylcholine, polypeptides such as the neurohypophyseal hormones vasopressin and oxytocin as well as glucocorticoids and estrogenic hormones. The approach consists of (1) quantitatively assessing the effects of topically applied, and intraarterially administered, graded doses of the proposed humoral substances on microvascular diameters or lumen size of the various muscular microvessels, i.e., precapillary sphincters, metarterioles, arterioles and venules, and trying to determine how these dose levels compare with known circulating rat plasma levels; (2) determining what effect proposed modifiers such as the neurohypophyseal hormones, glucocorticoids, and estrogens have on vascular reactivity to other endogenously circulating substances; (3) using differential systemic and local blockade with specific pharmacologic antagonists of norepinephrine, epinephrine, serotonin, histamine, and acetylcholine; and (4) determining the effects of tissue depletion of catecholamine stores with agents known to interfere with sympathetic transmitter mediation or release.

METHODS AND MATERIALS

Animals and Microscopy

All studies were made on the mesocecum of pentobarbital-anesthetized female and male rats (120–250 g in wt). The rat mesentery was prepared and kept under physiologic conditions according to procedures described previously (Zweifach and Metz, 1956). *In vivo* microscopic observations for discrete quantitative changes in microvascular diameters or lumen size of the different types of microvessels were made at magnifications up to 1500 times with a stage-calibrated optical micrometer (Altura, 1967, 1970b) or up to approximately 4000 times using the image-splitting television microscope recording system of Baez (1966). High, dry long-working distance objectives (Leitz) up to 32× and Leitz-Ultrapak water immersion objectives, 55× and 75×, were used in conjunction with 10× wide-field Bausch and Lomb oculars on a Bausch and Lomb Dyna-Zoom microscope equipped with a trinocular head.

Drugs and Solutions

All topically applied vasoactive test substances were freshly prepared daily in a Ringer solution containing 1% gelatin buffered to pH 7.2–7.4 with $NaHCO_3$ (Zweifach and Metz, 1956) and administered to the surface of the rat mesentery in fixed volumes of 0.1 ml. All pharmacologic antagonists and catecholamine depletors, except for reserpine and diethylstilbestrol proprionate, were prepared daily in sterile isotonic saline. The alkaloid reserpine was dissolved in a few drops of glacial acetic acid and diluted with sterile isotonic glucose for systemic (im) administration. Diethylstibestrol proprionate was freshly prepared in oil and administered subcutaneously. The dose of each drug (topically or systemically administered iv, im, or sc) is referred to below as the salt except

for the pure synthetic neurohypophyseal polypeptides, oxytocin and 8-lysine vasopressin, and the catecholamine depleting agents reserpine and alpha-methyl dopa.

Since some pharmacologic antagonists can be inhibitors of more than one type of agonist (Ariëns, 1964; Furchgott, 1955), pharmacologic doses of blocking agents were chosen for their specificity for antagonizing a specific vasoactive substance. As a precaution all so-called specific pharmacologic antagonists were tested for cross-blocking effects against the microvascular actions of other vasoactive agents that might have been also antagonized, e.g., a selected dose of phenoxybenzamine was tested for its alpha-adrenergic blocking effects not only against norepinephrine and epinephrine but against other constrictor substances such as serotonin and angiotensin. The latter precaution was necessary in order to rule out nonspecific blocking effects.

The following vasoactive substances were tested: acetylcholine chloride (Nutritional Biochemicals Corp.), histamine dihydrochloride (Nutritional Biochemicals Corp), l-epinephrine hydrochloride (Adrenalin Chloride, Parke, Davis and Co.), l-norepinephrine bitartrate (Levophed bitartrate, Winthrop Labs.), dopamine hydrochloride (Sigma Chemical Co.), angiotensin II amide (Hypertensin, Ciba Pharmaceuticals),[4] serotonin creatinine sulfate (Nutritional Biochemicals Corp.), synthetic oxytocin,[5] synthetic 8-lysine vasopressin,[5] isoproterenol hydrochloride (Isuprel, Winthrop Labs.). Alpha-adrenergic blocking agents used were: phenoxybenzamine hydrochloride (Dibenzyline, Smith, Kline and French Labs.), chlorpromazine hydrochloride (Smith, Kline and French Labs.), and yohimbine hydrochloride (Sigma Chemical Co.). Catecholamine depletors were used: guanethidine sulfate (Ismelin, Ciba Pharmaceuticals),[4] reserpine (Sigma Chemical Co.), and α-methyl dopa (Aldomet, Merck, Sharp and Dome). Antihistamines utilized included diphenhydramine HCl (Benadryl HCl, Parke, Davis and Co.), chlorpheniramine maleate (Chlor-Trimeton maleate, Schering Corp.), promethazine HCl (Phenergan HCl, Wyeth Labs.), and pyrilamine maleate (K&K Labs.). Beta-adrenergic blockers were dichloroisoproterenol (DCl)–HCl (Aldrich Chemical Co., Inc.) and propranolol–HCl (Aldrich Chemical Co., Inc.). Glucocorticoids utilized were dexamethasone-21-phosphate (Merck, Sharp and Dome), prednisolone-21-phosphate (Hydeltrasol, Merck), and cortisone acetate (Cortone, Merck). Anti-serotonins were lysergic acid diethylamide (LSD-25)[5] and 2-bromolysergic acid diethylamide (BOL).[5] Drug-induced changes from control diameters or initial lumen sizes (before drug application) were assessed for statistical significance, where appropriate, by either paired *t* tests or Students's *t* test (Bancroft, 1962).

RESULTS AND DISCUSSION

Effects of Topically Applied Graded Vasoconstrictor Doses of the Catecholamines Epinephrine, Norepinephrine, and Dopamine on Rat Mesenteric Microvessels

Relative Sensitivity of Precapillary Sphincters. At the outset it was important to determine the quantitative vasoconstrictor effects of the naturally occurring catecholamines epinephrine, norepinephrine, and dopamine on the precapillary sphincters (Altura, 1971b). This was essential for two reasons: First, to verify the assumption that

[4] Dr. R. Gaunt of Ciba Pharmaceuticals kindly supplied synthetic angiotensin II (Hypertensin).
[5] The late Dr. R. Bircher of Sandoz Pharmaceuticals kindly supplied these drugs.

precapillary sphincters may act in an all-or-none fashion (Zweifach, 1961; Cobbold *et al.*, 1963; Folkow, 1964; Winsor and Hyman, 1965); that is, a threshold concentration of a vasoconstrictor drug or hormone might cause either closure or no effect. And second, to determine the relative quantitative sensitivity of precapillary sphincters to the three naturally occurring catecholamines. The data shown in Fig. 1, obtained with the image-splitter television microscope recording system, indicate that the precapillary sphincters not only contract in graded dose-dependent fashion to all three catecholamines but are extremely sensitive to epinephrine and norepinephrine, responding to threshold doses of 10^{-5} or 10^{-4} μg/ml. Rat plasma epinephrine and norepinephrine levels measured recently by Chin and Evonuk (1971) average 6–8 $\times$ 10^{-3} μg/ml. Consequently, precapillary sphincters may respond to circulating plasma catecholamine

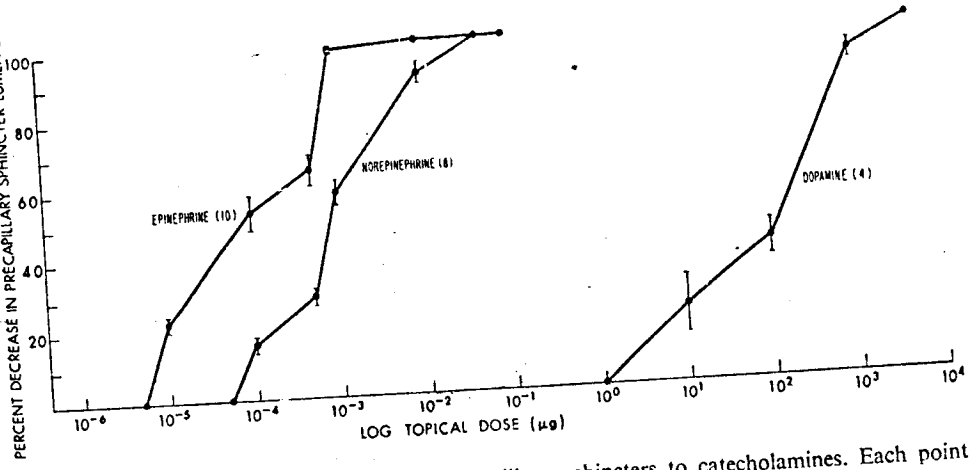

FIG. 1. Graded contractile responses of precapillary sphincters to catecholamines. Each point represents the mean value obtained from measurements on vessels of different male rats (indicated by numbers in parentheses). Only one type of catecholamine was tested on each rat mesentery. The bars represent the SEM. The mean control lumen sizes for the precapillary sphincters were: epinephrine (6.4 $\mu \pm 0.3$ SEM), norepinephrine (7.4 $\mu \pm 0.6$ SEM), and dopamine (5.6 $\mu \pm 0.4$ SEM). These data were obtained with the image-splitter TV microscope recording system.

levels, and preliminary observations indicate an even greater sensitivity to intraarterially administered catecholamines (unpublished observations). Although precapillary sphincters may also contract to closure in a graded fashion in response to dopamine, they are relatively insensitive to this catecholamine. Dopamine did not elicit vasodilatation of the precapillary sphincters. This finding is of interest in view of recent reports that dopamine is a vasodilator (based on resistance and flow measurements) in the splanchnic area in dogs (Eble, 1964; Yeh *et al.*, 1969).

Relative Sensitivity of Precapillary Sphincters, Arterioles, and Venules to Epinephrine and Norepinephrine. The precapillary sphincters were, on the average, 500–1000 times more sensitive to the vasoconstrictor action of epinephrine and norepinephrine than the arterioles. The quantitative data shown in Fig. 2 confirm and extend the qualitative and semiquantitative observations of previous workers (Zweifach and Metz, 1956;

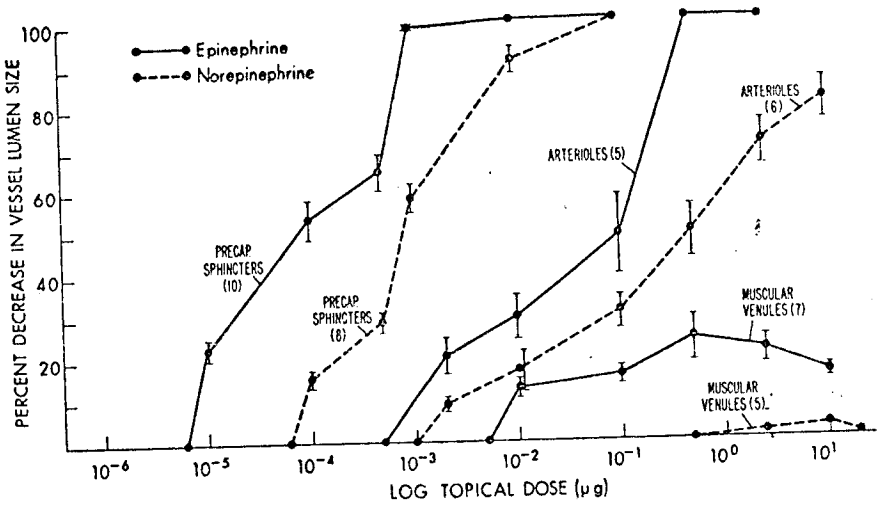

FIG. 2. Relative sensitivity of rat mesenteric microvessels to the constrictor action of epinephrine and norepinephrine. Each point represents the mean value obtained from measurements on vessels of different male rats (as in Fig. 1). Only one type of catecholamine was tested on each rat mesentery. The bars represent the SEM. The mean control lumen sizes for the precapillary sphincters are in Fig. 1 legend. The mean control lumen sizes for the arterioles were: epinephrine (21.6 $\mu \pm 2.9$ SEM); norepinephrine (28.8 $\mu \pm 1.4$ SEM). The mean control lumen sizes for the venules were: epinephrine (59.5 $\mu \pm 7.3$ SEM); norepinephrine (49.5 $\mu \pm 4.7$ SEM). These data were obtained with the image-splitter TV microscope recording system.

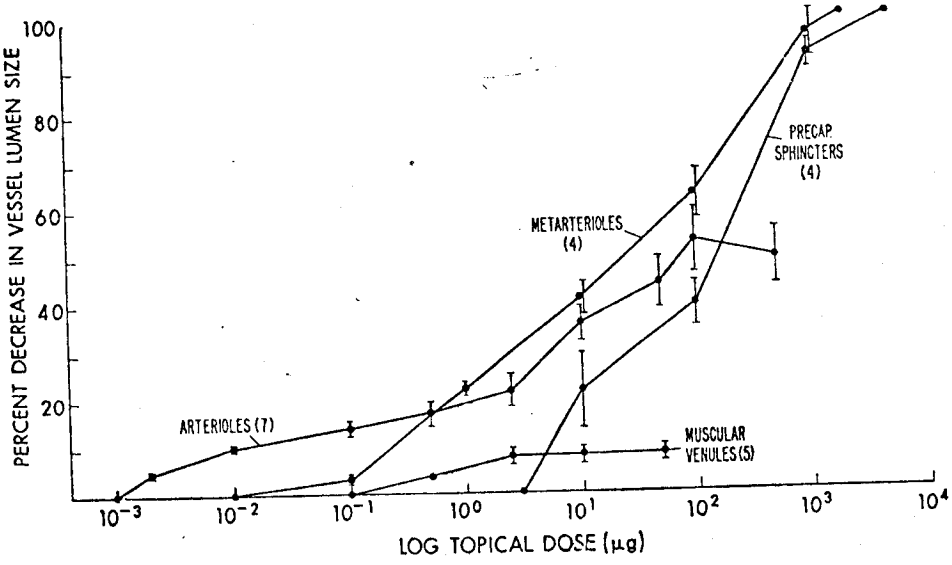

FIG. 3. Relative sensitivity of rat mesenteric microvessels to the constrictor action of dopamine. Each point represents the mean value obtained from measurements on vessels of different male rats (as in Fig. 1). The bars represent the SEM. The mean control lumen sizes were: precapillary sphincters (see Fig. 1 legend); metarterioles (9.4 $\mu \pm 0.3$ SEM); arterioles (24.1 $\mu \pm 1.1$ SEM); and venules (48.8 $\mu \pm 5.0$ SEM). These data were obtained with the image-splitter TV microscope recording system.

Baez, 1959, 1961; Zweifach, 1961; Altura and Zweifach, 1965a; Altura, 1967). Although the muscular venules may exhibit a 20% closure with epinephrine, they hardly respond to norepinephrine (Fig. 2). This is of interest in view of the proposed involvement of sympathetic nervous innervation in normal regulation of the microscopic capacitance vessels (see review by Mellander and Johansson, 1968). These data seem to contradict such a concept and afford support for the electronmicrographic findings of Rhodin (1968) indicating a lack of nervous innervation to these channels. Furthermore, the possibility must be considered that a part or all of these very small venular constrictions, seen after topical application of epinephrine and norepinephrine, might be passive and not active in nature. This can be resolved either by using microiontophoresis techniques (Duling *et al.*, 1968) or micropipettes (Zweifach, 1953). However, our results strongly support the notion that a gradient of sensitivity to constrictor catecholamines exists in the rat mesenteric microvessels proceeding from the precapillaries to arterioles to venules (Zweifach, 1961).

Relative Sensitivity of Rat Mesenteric Microvessels to Dopamine. In view of the effects of epinephrine and norepinephrine (Figs. 1 and 2), an assessment of the relative sensitivity of the microvessels to the catecholamine dopamine became significant. Although dopamine is capable of inducing contractile responses of all four types of muscular microvessels (Fig. 3), the pattern of responsiveness is quite different from that exhibited by epinephrine and norepinephrine. First, the arterioles and metarterioles show a greater sensitivity than the precapillary sphincters. And second, the arterioles cannot be induced to narrow down more than 50% with this catecholamine; epinephrine and norepinephrine induce complete arteriolar closure (Fig. 2). This seems to support the concept that the peripheral vascular constrictor actions of dopamine are not completely due to effects at alpha-adrenergic receptors. Experiments with adrenergic blocking agents should clarify this possibility.

Local and Systemic Adrenergic Blockade

If catecholamines such as epinephrine and norepinephrine are important in controlling blood flow in the microcirculation, and through precapillary sphincters, then alpha-adrenergic blocking and catecholamine-depleting agents should be of value in unmasking their presence, and action, in various sections of the microvascular tree.

Locally applied alpha-adrenergic blocking agents such as phenoxybenzamine, chlorpromazine, and yohimbine and local anesthetics like procaine and lidocaine (see Fig. 4), as well as systemically administered adrenergic blockers and a ganglionic blocker hexamethonium (Fig. 4), promote relaxation or dilation of arterioles and precapillary sphincters with little or no action on the venules. All locally and systemically administered alpha-adrenergic blocking agents specifically and completely blocked topically applied epinephrine and norepinephrine from exerting any constrictor action on the mesenteric microvessels at doses 10–20 times threshold, while the local anesthetics rather specifically and transiently potentiated the constrictor actions of topically applied catecholamines.[6] These are the microvascular effects to be expected if the pharmacologic antagonists are specifically unmasking the presence of endogenous circulating epinephrine and norepinephrine.

[6] This work is based on material presented in detail elsewhere (Altura, 1967).

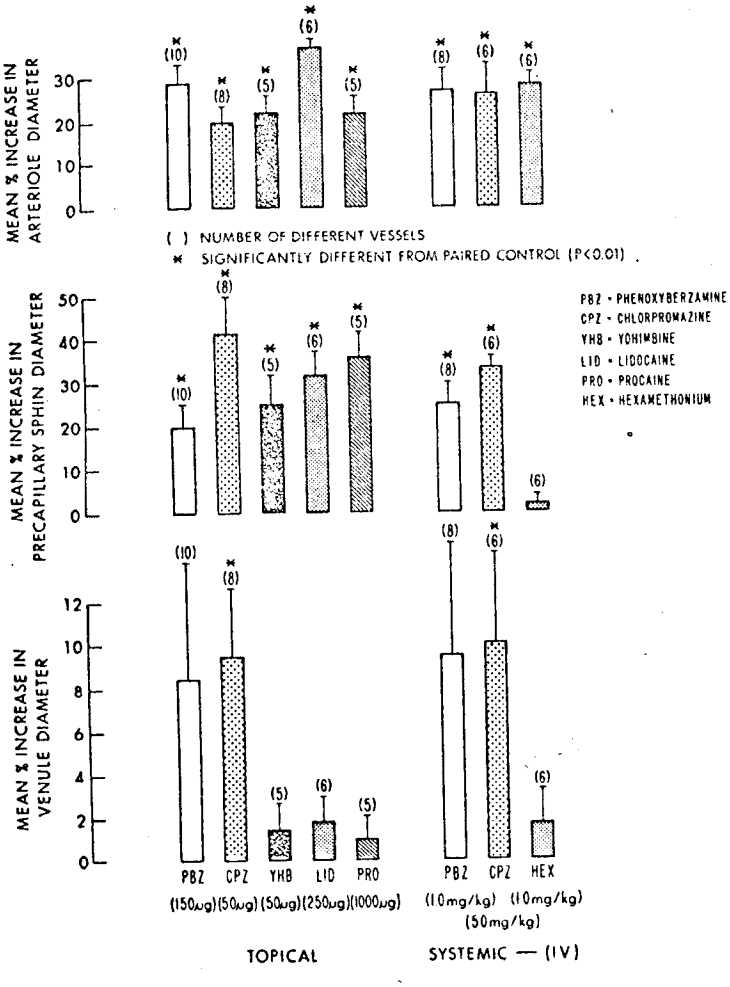

FIG. 4. Effects of adrenergic blocking drugs and local anesthetics on rat mesenteric microvessels. Each mean percentage increase in vessel diameter represents the mean value obtained from measurements on vessels of different female rats. The small bars represent the SEM. Only one type of autonomic blocking drug was examined in each rat. The numbers in parentheses represent the total number of vessel types examined in different rats.

Catecholamine Depletion

Quantitative observations were made (Fig. 5) on mesenteric microvessels of rats pretreated systemically with a variety of pharmacologic agents known to cause tissue depletion of peripheral catecholamine stores in one way or another (Burn, 1963; Burn and Rand, 1958a, b; Carlsson and Lindqvist, 1962; Day and Rand, 1963; Rand and Trinker, 1966). All three agents, namely reserpine, guanethidine, and alpha-methyl dopa, produced almost identical microvascular effects,[6] that is, primarily dilatation of precapillary sphincters, metarterioles, and arterioles. Overall, the data reported in Fig. 5 and that in Fig. 4, involving the utilization of specific alpha-adrenergic antagonists

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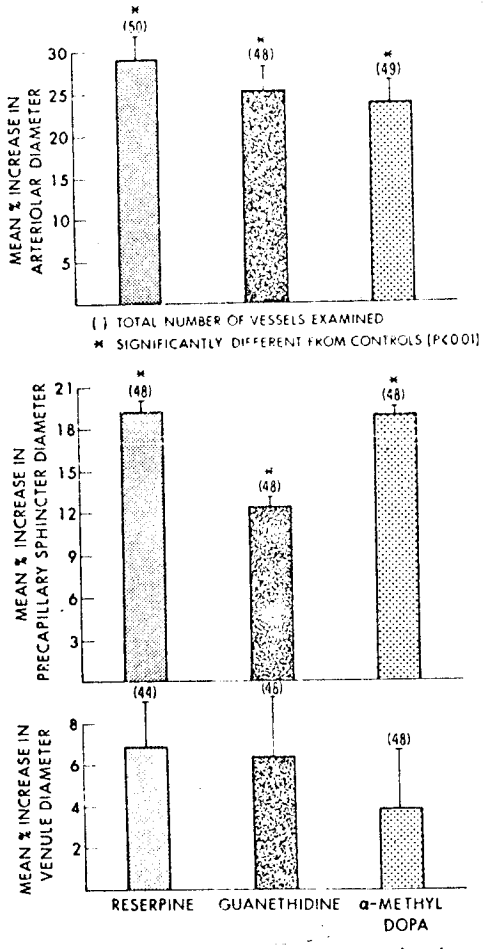

FIG. 5. Effects of catecholamine-depleting agents on rat mesenteric microvessels. Each mean percentage increase in vessel diameter for each drug was derived from a minimum of 12–15 random measurements per mesentery. Numbers in parentheses represent total number of measurements. Eight female rats were utilized for reserpine and eight for guanethidine while 12 were utilized for α-methyl dopa. Control rats received the vehicle diluents (see Materials and Methods).

and local anesthetics,[7] show in almost every case identical vasomotor effects, i.e., dilatation of precapillary sphincters, metarterioles, and arterioles, together with a suppression of the phenomenon of vasomotion. The microcirculatory observations with these pharmacologic antagonists and tissue catecholamine depletors suggest, in each case, an interference with the eventual constrictor action of endogenous constrictor catecholamines on the effector microvascular smooth muscle cells. In addition, these data, taken together with the graded dose-response relationships exhibited by the microvessels in response to locally administered constrictor catecholamines, suggest a preponderance of alpha-adrenergic receptors on the precapillary and arteriolar side of the rat mesenteric microcirculation.

[7] The significance of the findings with local anesthetics and the ganglionic blocker, hexamethonium, is discussed in detail elsewhere (Altura, 1967) and therefore will not be elaborated upon further.

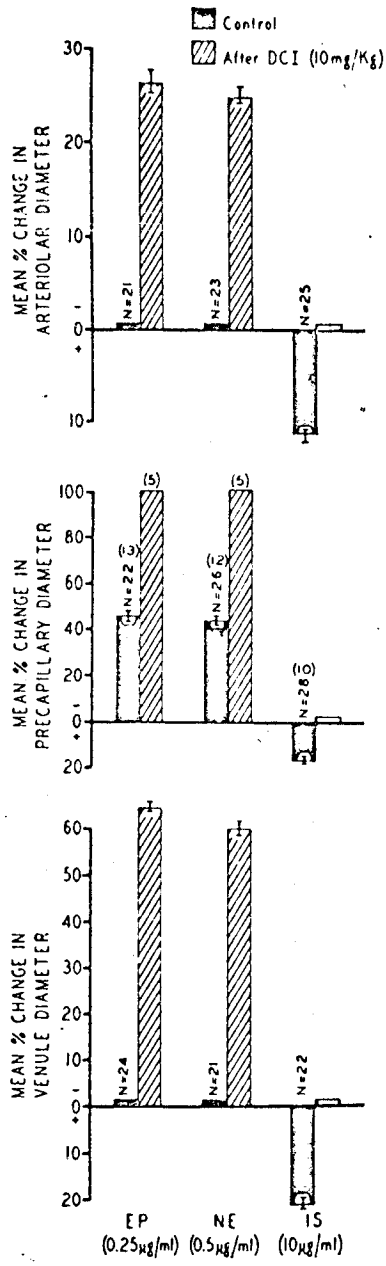

FIG. 6. Influence of a beta adrenergic blocker, dichloroisoproterenol, on vascular reactivity in rat mesenteric microvessels. Vascular responses were observed over a period of 5–35 min after iv administration of DCI. Brackets = ±SEM. Vertical bars above zero represent percentage decrease (contraction) in vessel diameter, whereas those below represent percentage increase (dilation) in vessel diameter. Open horizontal bar (⇨) represents zero response. All experimental values (i.e., after DCI) are significantly different from their paired controls ($p < .01$). EP = epinephrine; NE = norepinephrine; and IS = isoproterenol. N = number of vessels measured. Numbers in parentheses above each bar represent mean onset time for precapillary sphincters to contract or relax. Seven female rats were in each group.

Recent electronmicrographic evidence, histochemistry, and previous histologic evidence from several laboratories show no or sparse nervous innervation of the metarterioles or precapillaries (Chambers and Zweifach, 1944; Falck and Howman, 1966; Rhodin, 1962, 1967; Zweifach, 1961) or an intermittent innervation (Girgor'eva, 1962; Rhodin, 1967; Berman and Siggins, 1968; Berman and McNary, 1971). The pharmacological data reported here show: (1) suppression of vasomotion after either catecholamine depletion or blockade and (2) differential, relatively high sensitivity of precapillary sphincters to epinephrine and norepinephrine. Therefore, it appears that normally occurring sympathetic stimulation at the arterioles may release norepinephrine and/or some related constrictor catecholamine (dopamine ?) that is not rapidly metabolized and can, therefore, act more peripherally on microvessels such as the metarterioles and precapillary sphincters.

Beta-Andrenergic Blockade

Other experiments were designed to determine (1) whether there are beta-adrenergic receptors that subserve relaxation in the microvessels and (2) the anatomical sites of their location in the microcirculation. Intravenous administration of two different beta-adrenergic blockers, namely, dichloroisoproterenol and propranolol, in doses of 5–10 mg/kg produced a marked transient venular constriction (persisting only 2–5 min) and rather selectively blocked the local vasodilator effect of isoproterenol in the arterioles, precapillary sphincters, and venules (Altura and Zweifach, 1965c; Altura, 1970b). In addition, these beta-adrenergic blockers rather selectively potentiated the constrictor action of epinephrine and norepinephrine on the microvessels (Fig. 6) and failed to alter microvascular contractions induced by vasopressin, angiotensin, and serotonin (Altura, 1970b). Furthermore, the constrictor actions of epinephrine as well as norepinephrine (the catecholamine that usually fails to contract the muscular venules) became potentiated in the microscopic capacitance vessels. Data of this nature, as suggested previously (Altura and Zweifach, 1965c; Altura, 1967), are strongly indicative of a preponderance of beta-adrenergic receptors in the muscular venules in the rat mesentery. This may imply that the microscopic capacitance vessels are, to some degree, normally dilated by circulating epinephrine.

Serotonin Blockade

According to some workers, serotonin might be a candidate for local regulation of blood flow (Wurzel and Zweifach, 1966; Page, 1968). Experiments with antiserotonins administered both topically to the rat mesentery and intravenously were therefore performed. Two different antiserotonins, BOL and LSD, were administered locally in specific serotonin blocking doses and both failed to produce noticeable effects on the tonal appearance or vasomotion of the rat mesenteric microvessels (Fig. 7), confirming earlier qualitative findings (Altura and Zweifach, 1965b). Similar results were obtained with systemic administration of BOL and LSD.

In rat mesentery, the constrictor action of serotonin is more prominent on the muscular venules than the precapillary vessels (Altura *et al.*, 1965; Altura, 1967; Weiner and Altura, 1967). In addition, although the muscular venules are sensitive to as little as 5–6 μg of serotonin (Altura, 1967), rat blood levels of serotonin are much less than

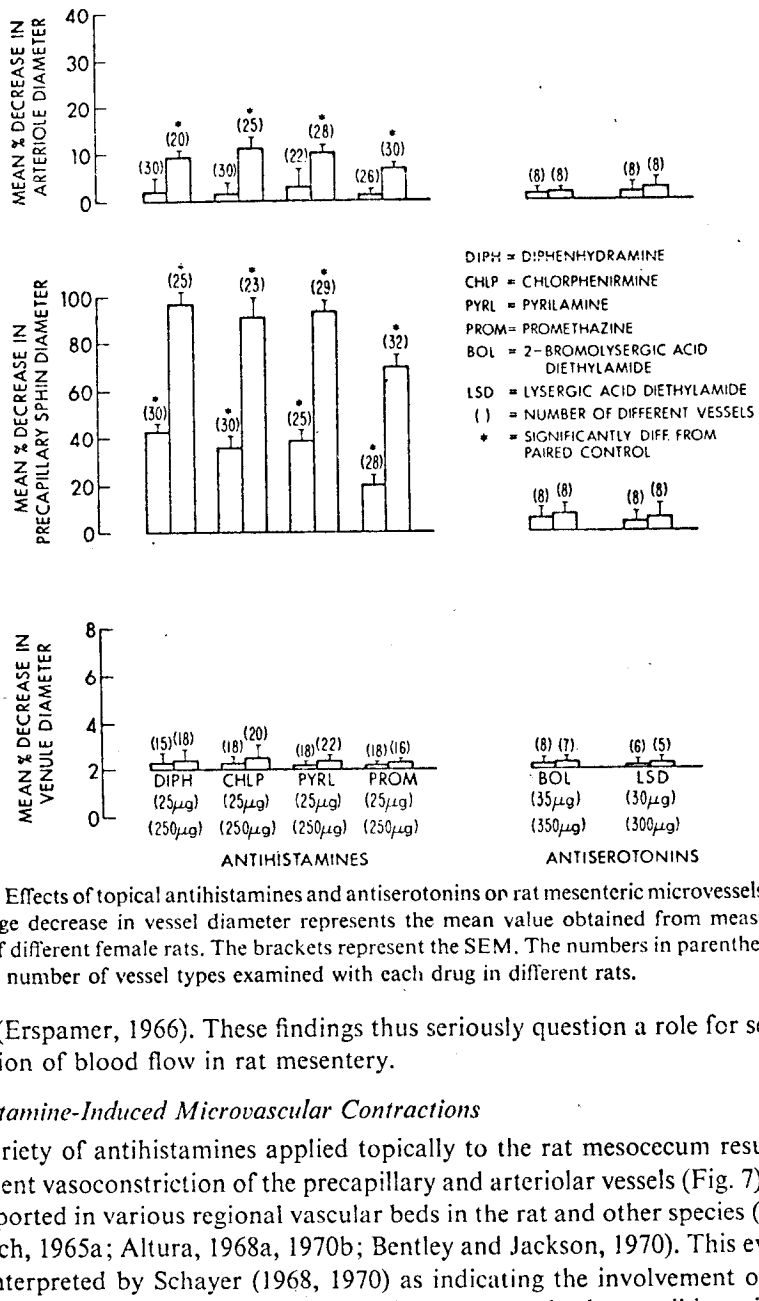

FIG. 7. Effects of topical antihistamines and antiserotonins on rat mesenteric microvessels. Each mean percentage decrease in vessel diameter represents the mean value obtained from measurements on vessels of different female rats. The brackets represent the SEM. The numbers in parentheses represent the total number of vessel types examined with each drug in different rats.

1.0 μg (Erspamer, 1966). These findings thus seriously question a role for serotonin in regulation of blood flow in rat mesentery.

Antihistamine-Induced Microvascular Contractions

A variety of antihistamines applied topically to the rat mesocecum result in dose-dependent vasoconstriction of the precapillary and arteriolar vessels (Fig. 7), similar to that reported in various regional vascular beds in the rat and other species (Altura and Zweifach, 1965a; Altura, 1968a, 1970b; Bentley and Jackson, 1970). This evidence has been interpreted by Schayer (1968, 1970) as indicating the involvement of "intrinsic histamine" in local tissue regulation. Schayer contends that antihistamine-induced microvascular contractions result only from a simple suppression of locally synthesized histamine and an unmasking of an opposing constrictor mechanism.

At first glance this concept does appear to be very convincing. In other extensive experiments detailed elsewhere (Altura and Zweifach, 1965b, 1966; Altura, 1968b, 1969, 1970b), antihistamine-induced microvascular contractile responses are the result

of a direct constrictor action of these compounds. Such results point out the dangers of injudicious use of certain pharmacologic antagonists to reveal or unmask the presence of a humoral substance. In addition, these findings must be considered in using antihistamines to define a physiologic or pharmacologic response as histaminic in the cardiovascular system. Moreover, recent preliminary data, obtained utilizing a variety of isolated vascular smooth muscles, indicate that many antihistamines are not specific antagonists of histamine and exhibit *noncompetitive* antagonism with histamine (Altura, 1968b, 1971c).

In addition, we found that procedures known to induce activation of histidine decarboxylase, such as chronic pretreatment with compound 48/80, injection of epinephrine in oil, and systemic administration of bacterial endotoxin, were not regularly associated with a parallelism between changes in microvascular tone (e.g., vasodilatation) and microvascular smooth muscle reactivity (Altura and Zweifach, 1967). However, our data do not necessarily conflict with the *in vitro* biochemical findings obtained by Schayer. Histidine decarboxylase formation as measured by an *in vitro* chemical analysis or even a radioactive histamine analysis on selected tissues taken from an animal may not in actuality reflect histamine formation in the microcirculation and/or *in vivo* pharmacologic histamine action on the smooth muscle cells of the blood vessels of the microcirculatory system (Altura and Zweifach, 1967). Previous *in vivo* observations (Altura, 1970b) indicated that the assayed *in vivo* free histamine blood concentrations are much less than those needed to elicit vasodilatation in the rat mesentery.

Recently, Baez and co-workers (1970, 1971) have presented *in vivo* data to suggest that small nonvasodilator doses of histamine can dose-dependently attenuate drug-induced arteriolar contractions in rat mesentery. These findings may be of physiologic significance if it can be demonstrated that circulating free histamine levels can also inhibit drug-induced contractions. However, Baez and co-workers (1970, 1971) used histamine diphosphate exclusively (i.e., the hydrochloride salt was not tested) and the possibility must be entertained that the diphosphate part of the histamine salt is responsible for a part of the observed antagonism at the microcirculatory level. Thus, Hilton (1971) has suggested that inorganic phosphate is a potent vasodilator; Goodford (1967) has demonstrated that phosphate may precipitate microamounts of calcium; and calcium is an essential requirement for all drug-induced contractions on smooth muscle (Somlyo and Somlyo, 1968, 1970). This conception may aid in explaining why histamine diphosphate appears to be more potent than betahistine hydrochloride (Baez and coworkers, 1971) as a nonspecific microvascular smooth muscle antagonist.

Cholinergic Blockade

Acetylcholine has been shown to be a potent vasodilator in several vascular beds (Bean and Sidky, 1958; Illig, 1961; Zweifach, 1961; Skinner *et al.*, 1970) including the rat mesentery and therefore might be a humoral candidate for control of blood flow (Zweifach, 1961; Altura, 1967).

Locally applied atropine, a potent and selective cholinergic blocking drug, leads to transient dose-dependent constrictor actions on precapillaries and arterioles (Table 2), confirming earlier results (Altura, 1967). The dose regimens of atropine specifically antagonized the vasodilator responses induced by topical acetylcholine, i.e., the vasodilator actions of histamine, bradykinin, or isoproterenol were not antagonized by the

TABLE 2

EFFECTS OF TOPICAL ATROPINE SULFATE ON RAT MESENTERIC MICROVESSELS

Atropine (μg)	N	% Decrease in arteriole diam[a]	% Decrease in precapillary sphincter diam	% Decrease in venule diam
30	5	2.2 ± 1.8[b]	48.0 ± 6.7[c]	0
300	5	25.3 ± 5.8[c]	96.8 ± 12.5[c]	2.7 ± 2.3

[a] Mean control arteriole diam (for 30 μg) = 35.6 ± 1.8 μ; mean control precapillary sphincter diam (for 30 μg) = 10.8 ± 0.6 μ; for venules (30 μg) = 52.3 ± 3.5 μ; mean control arteriole diam (for 300 μg) = 37.5 ± 2.1 μ; mean control precapillary sphincter diam (for 300 μg) = 10.5 ± 0.5 μ; for venules (300 μg) = 48.6 ± 3.2 μ.

[b] Values are means ± SE.

[c] $p < .01$ (when compared to the paired control utilizing a paired t test).

atropine (Altura, 1967). When atropine was administered intravenously in doses sufficient to selectively block locally applied acetylcholine, some of the precapillary sphincters, metarterioles, and arterioles became moderately narrowed, producing results very similar to those seen on local application (Altura, 1967). The microvascular contractions after local atropine might reflect the presence of endogenous acetylcholine or some related choline-ester molecules in the microcirculation (Altura, 1967). A direct constrictor action of atropine is possible but highly unlikely in view of the low doses used, the fact that only acetylcholine-induced dilations were blocked upon testing for microvascular relaxation with topical dilators (i.e., histamine, bradykinin, isoproterenol, and acetylcholine), and that atropine in concentrations up to 10 mg/ml fails to contract *in vitro* a variety of isolated vascular smooth muscles (Altura, unpublished observations), unlike the antihistamines (Altura, 1968b, 1970b). Further work will be required, however, to assess the role of acetylcholine or a related molecule in regulation of blood flow in rat mesentery. Anticholinesterases, administered locally, should be helpful in shedding more light on the problem.

Glucocorticoids—Modifiers of Vascular Reactivity

Over the years glucocorticoids have been shown to modify small blood vessel behavior through effects believed to be mediated by both the smooth muscle cell and the endothelial wall (Fritz and Levine, 1951; Ashton and Cook, 1952; Zweifach *et al.*, 1953; Zweifach, 1961). However, these early studies employed crude adrenal cortical extracts and not pure glucocorticoids. Therefore, experiments were initiated to assess the possible role of pure, synthetic steroids on the regulation of the microcirculation (Altura, 1966a). Topically applied cortisone, hydrocortisone, as well as dexamethasone failed to exert visible effects on the diameter of the rat mesenteric microvessels. Baez (personal communication), however, recently found that dexamethasone, a synthetic glucocorticoid 30 times more potent than cortisone, will increase microvascular tone in 20–25% of the rat mesenteries so pretreated. Systemic administration of either prednisolone or dexamethasone potentiates the constrictor action of the catecholamines epinephrine and norepinephrine on all types of muscular microvessels including venules (Fig. 8), vessels which normally fail to respond to norepinephrine. Similar results are obtained after topical application of glucocorticoids (Altura, unpublished findings).

FIG. 8. Influen
Vascular respon
female rats com
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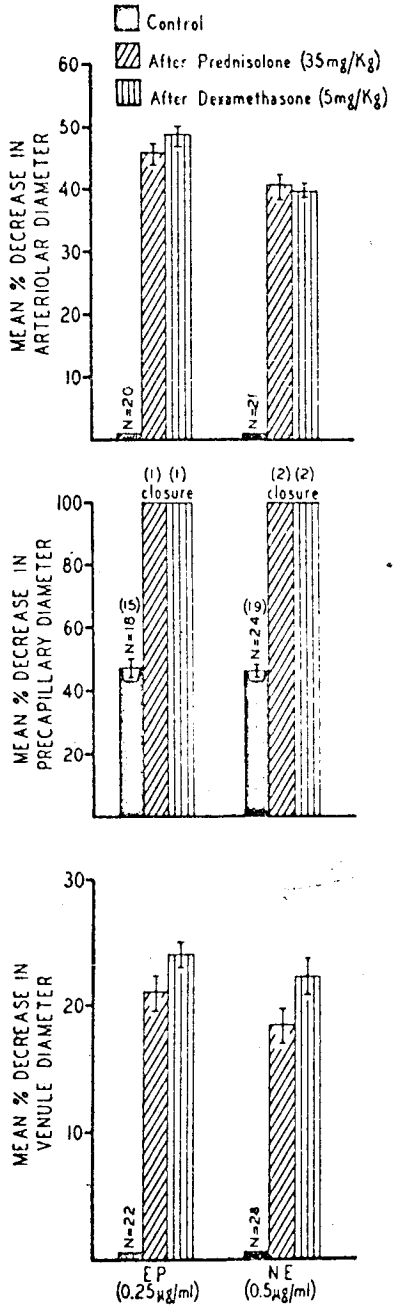

FIG. 8. Influence of systemic glucocorticoids on vascular reactivity in rat mesenteric microvessels. Vascular responses were observed over a period of 10–40 min after iv administration of steroids. Six female rats comprised each group. Brackets = ±SEM. N = number of vessels measured. All experimental values are significantly different from their paired controls ($p < .01$). EP = epinephrine; NE = norepinephrine. Numbers in parentheses above each bar represent mean onset time for precapillary sphincters to contract.

Baez and co-workers (1970) have recently reported that dexamethasone can potentiate the constrictor action of vasoxyl, a noncatecholamine, on rat mesenteric arterioles. Multiple systemic doses of these steroids can also potentiate the constrictor actions of serotonin and vasopressin (Altura, 1966a). Furthermore these steroids will restore vascular reactivity in response to a variety of constrictor substances with peripheral actions that have been suppressed or are absent in animals depleted of their mast cells (Altura, 1966a). This seems to reinforce the hypothesis that glucocorticoids may serve a basic function in local regulation of blood flow through the response of vascular smooth muscle to other endogenous humoral agents (Fritz and Levine, 1951; Zweifach, 1961; Altura, 1966a). Since the present data and that of Baez indicate that glucocorticoids can potentiate microvascular responses induced by agents other than catecholamines, one is hard put to accept the thesis that these steroids solely potentiate vasoactive substances by inhibiting catechol-*O*-methyl transferase (Kalsner, 1969).

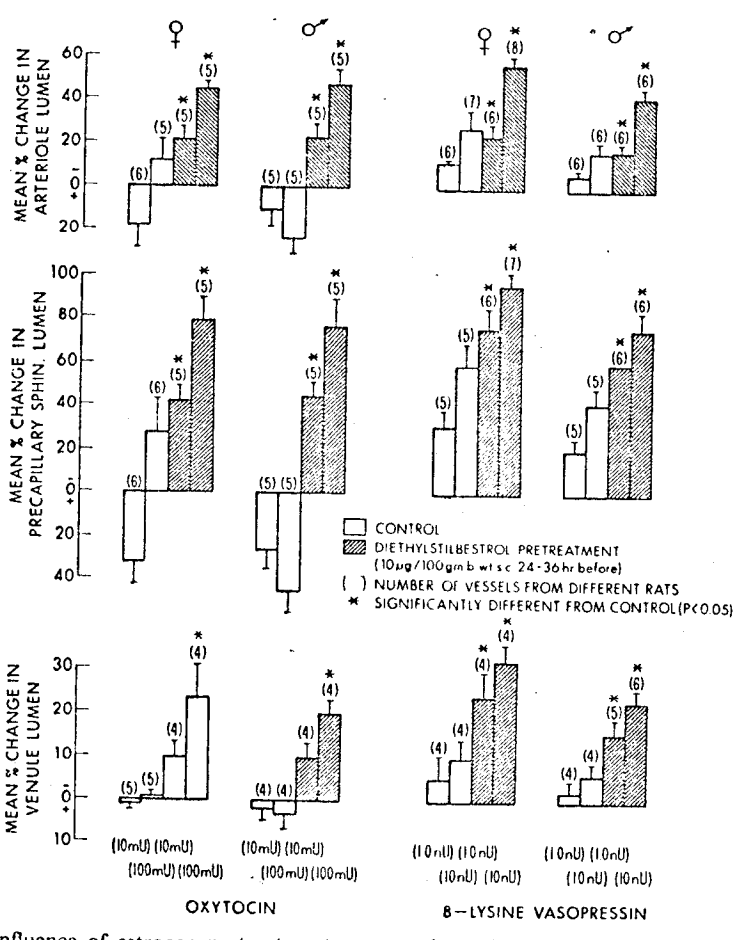

FIG. 9. Influence of estrogen pretreatment on neurohypophyseal hormone action in mesenteric microvessels of female and male rats. Vertical bars above zero represent percentage decrease (contraction) in vessel diameter, whereas those below represent percentage increase (dilation) in vessel diameter. Note that oxytocin was applied in milliunits while 8-lysine vasopressin was applied in nanounits. The numbers in parentheses represent the total number of vessel types examined in different rats.

Influence of Estrogenic Hormones on Neurohypophyseal Hormone Action

A review of the literature suggests that estrogens may play an indirect role in the regulation of blood pressure and blood flow by exerting influences on the renin–angiotensin, adrenergic, cholinergic as well as the oxytocin–vasopressin systems (Lloyd, 1959; Lloyd and Pickford, 1961, 1962; Pickford, 1966; Lim and Walters, 1970). In 1959 Lloyd presented evidence that oxytocin, normally without effect on rat blood pressure, becomes a pressor substance during the period of natural estrus and also temporarily after the administration of estrogenic hormones. In view of the paucity of data concerning the possible relationships between sex hormones, vascular reactivity, and blood flow, studies were initiated to determine the effects of oxytocin and vasopressin on the mesenteric microcirculation of female and male rats before and after treatment with the estrogen diethystilbestrol proprionate.

Low doses of oxytocin (syntocinon) elicit relaxation or constriction in precapillary and arteriolar vessels of female rats, depending upon dose, and relaxation only in male rats (Fig. 9). However, high doses can produce contraction (Altura, unpublished findings) suggesting a permissive effect of estrogenic hormones on the vasotropic action of oxytocin. Vasopressin, on the other hand, elicits only vasoconstriction in both male and female rats. A small subcutaneous injection of an estrogen strongly potentiates the constrictor action of the neurohypophyseal hormones and also changes an oxytocin vasodilator action into one of constriction (Fig. 9). These results thus confirm and extend the findings of Lloyd (1959a, 1959b). Although further studies are needed to elucidate the permissive and modulating effect of sex steroids on microcirculatory dynamics, these experiments using doses of estrogen that are near physiologic could be interpreted as support for a role for these steroids in local control of blood flow through a modifying action similar in many respects to that of glucocorticoids.

CONCLUSIONS

Overall, our *in vivo* data may be used to reinforce the concept of local regulation of blood flow by humoral or blood-borne substances, at least for the rat mesentery. This statement is meant to be cautious with respect to extrapolation for other vascular beds within the rat or other species. It is becoming almost axiomatic that pharmacologic receptor sites or reactive smooth muscle cells for certain endogenous vasoactive substances may be heterogeneous and even different in response among different vascular beds (Altura, 1970a; Bohr, 1965), within adjacent microvascular smooth muscle cells (Folkow *et al.*, 1961; Altura and Hershey, 1967; Altura, 1971a), as well as between segments within one and the same bed (Somlyo *et al.*, 1965; Altura, 1966b; Altura and Altura, 1970). The impression is derived from our experiments with humoral substances, and the work of others, that no one substance is responsible for control of blood flow through the precapillary vessels, but different combinations of chemical and humoral substances, probably differing with the vascular bed, may interact with one another to ultimately control nutritive blood flow in the microcirculation. Bioassay and chemical analyses of venous blood in exercise hyperemia, reactive hyperemia, and autoregulatory conditions strongly support the latter tenet (Haddy and Scott, 1971).

ACKNOWLEDGMENTS

The author gratefully acknowledges the excellent technical assistance of Mrs. Zona Laidlaw and Mr. R. William Burton.

REFERENCES

ABBOUD, F. M. (1968). Vascular responses to norepinephrine, angiotensin, vasopressin and serotonin. *Fed. Proc. Fed. Amer. Soc. Exp. Biol.* **27**, 1391–1395.

ALTURA, B. M. (1966a). Role of glucocorticoids in local regulation of blood flow. *Amer. J. Physiol.* **211**, 1393–1397.

ALTURA, B. M. (1966b). Differential actions of polypeptides and other drugs on coronary inflow vessels. *Amer. Heart J.* **72**, 709–711.

ALTURA, B. M. (1967). Evaluation of neurohumoral substances in local regulation of blood flow. *Amer. J. Physiol.* **212**, 1447–1454.

ALTURA, B. M. (1968a). Antihistamine constriction in mouse skin microcirculation. *J. Pharm. Pharmacol.* **20**, 71–72.

ALTURA, B. M. (1968b). Effects of antihistamines on isolated arterial smooth muscle. *Pharmacologist* **10**, 164.

ALTURA, B. M. (1969). Insight into the contractile actions of antihistamines on micro-vascular smooth muscle. *Bibl. Anat.* **10**, 349–354.

ALTURA, B. M. (1970a). Significance of amino acid residues in vasopressin on contraction in vascular muscle. *Amer. J. Physiol.* **219**, 222–229.

ALTURA, B. M. (1970b). Contractile responses of microvascular smooth muscle to antihistamines. *Amer. J. Physiol.* **218**, 1082–1091.

ALTURA, B. M. (1971a). Pharmacology of neurohypophyseal hormones and analogs on isolated vascular muscle and in the terminal vascular bed. "Proceedings of the Symposium on *Physiology and Pharmacology of Vascular Neuroeffector Systems*." (J. A. Bevan, R. F. Furchgott, R. A. Maxwell and A. P. Somlyo, eds.), pp. 274–290. Karger, Basel.

ALTURA, B. M. (1971b). Do precapillary sphincters respond to vasoactive substances in an all-or-none manner? *Proc. Soc. Exp. Biol. Med.*, Oct. 1971, in press.

ALTURA, B. M. (1971c). Histamine-antihistamine antagonism in isolated arterial smooth muscle. "Proceedings XXV International Congress of Physiological Sciences." Münich, 1971, Abstracts, vol. IX, p. 15. German Physiological Soc., München.

ALTURA, B. M., AND ALTURA, B. T. (1970) Heterogeneity of drug receptors in different segments of rabbit thoracic aorta. *Eur. J. Pharmacol.* **12**, 44–52.

ALTURA, B. M., AND HERSHEY, S. G. (1967). Pharmacology of neurohypophyseal hormones and their synthetic analogues in the terminal vascular bed: Structure–activity relationships. *Angiology* **18**, 428–439.

ALTURA, B. M., AND HERSHEY, S. G. (1968). Structure–activity relationships of neurohypophyseal polypeptides in the microcirculation. A molecular basis for shock therapy. *In* "Intermedes Proceedings 1968: Combined Injuries and Shock" (B. Schildt and L. Thorén, eds.), pp. 247–259. Försvarets Forskningsanstalt, Stockholm.

ALTURA, B. M., HERSHEY, S. G., AND ALTURA, B. T. (1970). Microcirculatory actions of polypeptides and their use in the treatment of experimental shock. *Advan. Exp. Med. Biol.* **8**, 239–247.

ALTURA, B. M., HERSHEY, S. G., AND ZWEIFACH, B. W. (1965). Effects of a synthetic analogue of vasopressin on vascular smooth muscle. *Proc. Soc. Exp. Biol. Med.* **119**, 258–261.

ALTURA, B. M., AND ZWEIFACH, B. W. (1965a). Antihistamines and vascular reactivity. *Amer. J. Physiol.* **209**, 545–549.

ALTURA, B. M., AND ZWEIFACH, B. W. (1965b). Pharmacologic properties of antihistamines in relation to vascular reactivity. *Amer. J. Physiol.* **209**, 550–556.

ALTURA, B. M., AND ZWEIFACH, B. W. (1965c). Evidence for beta receptors in the rat mesenteric microvasculature. *J. Pharmacol. Exp. Ther.* **150**, 23–25.

ALTURA, B. M., AND ZWEIFACH, B. W. (1966). Influence of reserpine and guanethidine on vascular-reactivity and antihistamine constrictor action in the microcirculation. *Angiology* **17**, 493–502.

ALTURA, B. M., AND ZWEIFACH, B. W. (1967). Endogenous histamine formation and vascular reactivity. *Amer. J. Physiol.* **212**, 559–564.

ARIËNS, E. J. (1964). "Molecular Pharmacology," Vol. 1. Academic Press, New York.
ASHTON, N., AND COOK, C. (1952). In vivo observations of the effects of cortisone upon the blood vessels in rabbit ear chambers. *Brit. J. Exp. Pathol.* **33**, 445–450.
BAEZ, S. (1959). Microcirculation in the intramural vessels of the small intestine in the rat. *In* "The Microcirculation" (S. R. M. Reynolds and B. W. Zweifach, eds.), pp. 114–128. Univ. of Illinois Press, Urbana.
BAEZ, S. (1961). Response characteristics of perfused microvessels to pressure on vasoactive stimuli. *Angiology* **12**, 452–461.
BAEZ, S. (1966). Recording of microvascular dimensions with an image-splitter television microscope. *J. Appl. Physiol.* **211**, 299–301.
BAEZ, S. (1968a). Bayliss response in the microcirculation. *Fed. Proc. Fed. Amer. Soc. Exp. Biol.* **27**, 1410–1415.
BAEZ, S. (1968b). Vascular smooth muscle: Quantification of cell thickness in the wall of arterioles in the living animal in situ. *Science* **159**, 536–538.
BAEZ, S., LORENZO, J., AND ORKIN, L. R. (1970). Interference by histamine and betahistine HCl with hydrocortisone potentiation of responses to epinephrine, norepinephrine and methoxamine in single smooth muscle cell in situ. *Physiologist* **13**, 140.
BAEZ, S., ORKIN, L. R., AND LORENZO-LAGISQUET, J. A. (1971). Antagonism of some vascular smooth muscle agonists by histamine and betahistine. *Microvasc. Res.* **3**, 170–182.
BANCROFT, H. (1962). "Introduction to Biostatistics," pp. 172–181. Harper and Row (Hoeber), New York.
BARTELSTONE, H. J., AND NASMYTH, P. A. (1965). Vasopressin potentiation of catecholamine actions in dog, rat, cat, and rat aortic strip. *Amer. J. Physiol.* **208**, 754–762.
BEAN, J., AND SIDKY, M. (1958). Intestinal blood flow as influenced by vascular and motor reactions to acetylcholine and carbon dioxide. *Amer. J. Physiol.* **194**, 512–518.
BENTLEY, A. J., AND JACKSON, R. T. (1970). Changes in the patency of the upper nasal passage induced by histamine and antihistamines. *Laryngoscope* **80**, 1859–1870.
BERMAN, H. J., AND MCNARY, W. F. (1971). Innervation of precapillary sphincters and their regulation. *Microvasc. Res.*, This issue.
BERMAN, H. J., AND SIGGINS, G. R. (1968). Neurogenic factors in the microvascular system. *Fed. Proc. Fed. Amer. Soc. Exp. Biol.* **27**, 1384–1390.
BERNE, R. M. (1964). Regulation of coronary blood flow. *Physiol. Rev.* **44**, 1–29.
BOHR, D. F. (1965). Individualities among vascular smooth muscles. *In* "Electrolytes and Cardiovascular Diseases" (E. Bajusz, ed.), pp. 342–355. Karger, Basel.
BOHR, D. F., AND JOHANSSON, B. (1966). Contraction of vascular smooth muscle in response to plasma. Comparison with response to known vasoactive agents. *Circ. Res.* **19**, 593–601.
BOHR, D. F., AND SOBIESKI, J. (1968). A vasoactive factor in plasma. *Fed. Proc. Fed. Amer. Soc. Exp. Biol.* **27**, 1396–1398.
BURN, J. H. (1963). "The Autonomic Nervous System," p. 96. Davis, Philadelphia.
BURN, J. H., AND RAND, M. J. (1958a). Noradrenaline in artery walls and its dispersal by reserpine. *Brit. Med. J.* **1**, 903–908.
BURN, J. H., AND RAND, M. J. (1958b). The action of sympathomimetic amines in animals treated with reserpine. *J. Physiol. London* **144**, 314–336.
CARLSSON, A., AND LINDQVIST, M. (1962). In vivo decarboxylation of α-methyl DOPA and α-methyl metatyrosine. *Acta Physiol. Scand.* **54**, 94–97.
CHAMBERS, R., and ZWEIFACH, B. W. (1944). Topography and function of the mesenteric capillary circulation. *Amer. J. Anat.* **75**, 173–205.
CHIN, A. K., AND EVONUK, E. (1971). Changes in plasma catecholamine and corticosterone levels after muscular exercise. *J. Appl. Physiol.* **30**, 205–207.
COBBOLD, A., FOLKOW, B., KJELLMER, I., AND MELLANDER, S. (1963). Nervous and local chemical control of pre-capillary sphincters in skeletal muscle as measured by changes in filtration coefficient. *Acta Physiol. Scand.* **57**, 180–192.
DALE, H. H. (1929). Some chemical factors in the control of the circulation. *Lancet* **1**, 1179–1183.
DAY, M. D., AND RAND, M. J. (1963). A hypothesis for the mode of action of α-methyldopa in relieving hypertension. *J. Pharm. Pharmacol.* **15**, 221–224.

DULING, B. R., BERNE, R. M., AND BORN, G. V. R. (1968). Microiontophoretic application of vasoactive agents to the microcirculation of the hamster cheek pouch. *Microvasc. Res.* **1**, 158–173.

EBLE, J. N. (1964). A proposed mechanism for the depressor effect of dopamine in the anesthetized dog. *J. Pharmacol. Exp. Ther.* **145**, 64–70.

ERSPAMER, V. (1966). Occurrence of indolealkylamines in nature. In "Handbook of Experimental Pharmacology XIX. 5-Hydroxytryptamine and Related Indolealkylamines" (V. Erspamer, ed.), p. 133. Springer-Verlag, New York.

FALCK, B., AND HOWMAN, C. (1966). Histochemistry of monoaminergic mechanisms in peripheral neurons. In "Mechanisms of Release of Biogenic Amines" (U.S. von Euler, S. Rosell, and B. Uvnas, eds.), p. 66. Pergamon, Oxford.

FOLKOW, B. (1964). Description of the myogenic hypothesis. *Circ. Res.* **14** and **15**, Suppl. 1, 279–287.

FOLKOW, B., JOHANSSON, B., AND MELLANDER, S. (1961). The comparative effects of angiotensin and noradrenaline on consecutive vascular sections. *Acta Physiol. Scand.* **53**, 99–104.

FRITZ, I., AND LEVINE, R. (1951). Action of adrenal cortical steroids and norepinephrine on vascular response of stress in adrenalectomized rats. *Amer. J. Physiol.* **165**, 456–465.

FURCHGOTT, R. F. (1955). The pharmacology of vascular smooth muscle. *Pharmacol. Rev.* **7**, 183–265.

GOODFORD, P. J. (1967). The calcium content of the smooth muscle of the guinea-pig taenia col. *J. Physiol. London* **192**, 145–157.

GRAY, S. D. (1971). Effect of hypertonicity on vascular dimensions in skeletal muscle. *Microvasc. Res.* **3**, 117–124.

GRAY, S. D., LUNDVALL, J., AND MELLANDER, S. (1968). Regional hyperosmolarity in relation to exercise hyperemia. *Acta Physiol. Scand.* **73**, 11A-12A.

GRIGOR'EVA, T. A. (1962). "The Innervation of Blood Vessels." Pergamon, London.

GROSS, F. (1963). Angiotensin. *Naunyn-Schmied. Arch. Exp. Pathol. Pharmakol.* **245**, 196–229.

GUYTON, A. C., ROSS, J. M., CARRIER, O., JR., AND WALKER, J. R. (1964). Evidence for tissue oxygen demand as the major factor causing auto-regulation. *Circ. Res.* **14** and **15**, Suppl. 1, 60–69.

HADDY, F. J., AND SCOTT, J. B. (1968). Metabolically linked vasoactive chemicals in local regulation of blood flow. *Physiol. Rev.* **48**, 688–707.

HADDY, F. J., AND SCOTT, J. B. (1971). Bioassay and other evidence for participation of chemical factors in local regulation of blood flow. *Circ. Res.* **28**, Suppl. 1, 86–92.

HILTON, S. M. (1971). A new candidate for mediator of functional vasodilatation in skeletal muscle. *Circ. Res.* **28**, Suppl. 1, 70–72.

HILTON, S. M., AND VRBOVÁ, G. (1970). Inorganic phosphate—a new candidate for mediator of functional vasodilatation in skeletal muscle. *J. Physiol. London* **206**, 29P.

ILLIG, L. (1961). "Die terminale Strohmbahn. Capillarbett und Mikrozirkulation." Springer-Verlag, Berlin.

JOHNSON, P. C. (1968). Autoregulatory responses of cat mesenteric arterioles measured *in vivo*. *Circ. Res.* **22**, 199–212.

KALSNER, S. (1969). Mechanism of hydrocortisone potentiation of responses to epinephrine and norepinephrine in rabbit aorta. *Circ. Res.* **24**, 383–395.

KROGH, A. (1929). "The Anatomy and Physiology of Capillaries." Yale Univ. Press, New Haven.

LEWIS, G. P. (1968). Pharmacologically active polypeptides. *In* "Recent Advances in Pharmacology" (J. M. Robson and R. S. Stacey, eds.), pp. 213–246. Churchill, London.

LEWIS, T. (1927). "The Blood Vessels of the Human Skin." Shaw, London.

LIM, Y. L., AND WALTERS, W. A. W. (1970). Oestrogens and the maternal circulation. *Austr. N. Z. J. Obstet. Gynaecol.* **10**, 61–69.

LLOYD, S. (1959a). Changes in the vascular responses of the rat during pregnancy. *J. Physiol. London* **149**, 586–592.

LLOYD, S. (1959b). The vascular responses of the rat during the reproductive cycle. *J. Physiol. London* **148**, 625–632.

LLOYD, S., AND PICKFORD, M. (1961). The action of posterior pituitary hormones and oestrogens on the vascular system of the rat. *J. Physiol. London* **155**, 161–174.

LLOYD, S., AND PICKFORD, M. (1962). The effect of oestrogens and sympathetic denervation on the response to oxytocin of the blood vessels in the hindlimb of the dog. *J. Physiol. London* **163**, 362–371.

LUNDVALL, J. (1969). Further studies on regional hyperosmolality in relation to exercise hyperemia. *Acta Physiol. Scand.* Suppl. 330, 98.

LUNDVALL, J., MELLANDER, S., AND WHITE, T. (1969). Hyperosmolality and vasodilatation in human skeletal muscle. *Acta Physiol. Scand.* **77**, 224–233.

MCGIFF, J. C. (1968). Tissue hormones: angiotensin, bradykinin and the regulation of regional blood flows. *Med. Clin. N. Amer.* **52**, 263–281.

MELLANDER, S. (1970). Systemic circulation: Local control. *Ann. Rev. Physiol.* **32**, 313–344.

MELLANDER, S., AND JOHANSSON, B. (1968). Control of resistance, exchange, and capacitance functions in the peripheral circulation. *Pharmacol. Rev.* **20**, 117–196.

MELLANDER, S., JOHANSSON, B., GRAY, S., JONSSON, O., LUNDVALL, J., AND LJUNG, B. (1967). The effects of hyperosmolarity on intact and isolated vascular smooth muscle. Possible role in exercise hyperemia. *Angiologica* **4**, 310–322.

MELLANDER, S., AND LUNDVALL, J. (1971). Role of tissue hyperosmolality in exercise hyperemia. *Circ. Res.* **28**, Suppl. 1, 39–45.

PAGE, I. H. (1968). "Serotonin," pp. 44–50. Year Book Med. Pub., Chicago.

PICKFORD, M. (1966). Neurohypophysis and vascular reactions. In "The Pituitary Gland" (G. W. Harris and B. T. Donovan, eds.), vol. 3, pp. 399–413. Univ. California Press, Berkeley.

RAND, M. J., AND TRINKER, F. R. (1966). Pharmacological agents affecting the release and activity of catecholamines. *Brit. J. Angaesth.* **38**, 666–689.

RHODIN, J. A. G. (1962). Fine structure of vascular walls in mammals. *Physiol. Rev.* **42**, 48–81.

RHODIN, J. A. G. (1967). The ultrastructure of mammalian arterioles and precapillary sphincters. *J. Ultrastruct. Res.* **18**, 181–223.

RHODIN, J. A. G. (1968). Ultrastructure of mammalian venous capillaries, venules and small collecting veins. *J. Ultrastruct. Res.* **25**, 452–500.

ROSS, G. (1970a). Regional circulatory effects of pancreatic glucagon. *Brit. J. Pharmacol.* **38**, 735–742.

ROSS, G. (1970b). Cardiovascular effects of secretin. *Amer. J. Physiol.* **218**, 1166–1170.

ROSS, G. (1971). The regional circulation. *Ann. Rev. Physiol.* **33**, 445–478.

SCHAYER, R. W. (1964). A unified theory of glucocorticoid action. *Perspect. Biol. Med.* **8**, 71–84.

SCHAYER, R. W. (1968). Histamine and a possible unity of autonomous microcirculatory dilator responses. *Med. Col. Virginia Quart.* **4**, 101–106.

SCHAYER, R. W. (1970). Biogenic amines and microcirculatory homeostasis. *In* "Biogenic Amines as Physiological Regulators" (J. J. Blum, ed.), pp. 239–251. Prentice-Hall, Englewood Cliffs, N.J.

SIGGINS, G. R. (1970). Some aspects of humoral and neuromotor control of the microvascular system. *In* "Microcirculation, Perfusion, and Transplantation of Organs" (T. I. Malinin, B. S. Linn, A. B. Callahan, and W. D. Warren, eds.), pp. 59–74. Academic Press, New York.

SKINNER, N. S., JR., AND COSTIN, J. C. (1968). Tissue metabolites and regulation of local blood flow. *Fed. Proc. Fed. Amer. Soc. Exp. Biol.* **27**, 1426–1429.

SKINNER, N. S., JR., COSTIN, J. C., AND WEBSTER, M. E. (1970). Acetylcholine and functional vasodilatation in the submaxillary gland of the cat. *Eur. J. Pharmacol.* **12**, 271–275.

SOMLYO, A. P., AND SOMLYO, A. V. (1968). Vascular smooth muscle. I. Normal structure, pathology, biochemistry, and biophysics. *Pharmacol. Rev.* **20**, 197–272.

SOMLYO, A. P., AND SOMLYO, A. V. (1970). Vascular smooth muscle. II. Pharmacology of normal and hypertensive vessels. *Pharmacol. Rev.* **22**, 249–353.

SOMLYO, A. V., SANDBERG, R. L., AND SOMLYO, A. P. (1965). Pharmacologically heterogeneous smooth muscle cell distribution in blood vessels. *J. Pharmacol. Exp. Ther.* **149**, 106–112.

WEINER, R., AND ALTURA, B. M. (1967). Serotonin-bradykinin synergism in the mammalian capillary bed. *Proc. Soc. Exp. Biol. Med.* **124**, 494–497.

WHELAN, R. F. (1967). "Control of the Peripheral Circulation in Man." Thomas, Springfield.

WIEDEMAN, M. P. (1968). Blood flow through terminal arterial vessels after denervation of the bat wing. *Circ. Res.* **22**, 83–89.

WINSOR, T., AND HYMAN, C. (1965). "A Primer of Peripheral Vascular Diseases." Lea and Febiger, Philadelphia.

WURZEL, M., BACON, R. C., KALT, R. B., AND ZWEIFACH, B. W. (1964). Vasoactive properties of plasma protein fractions. *Amer. J. Physiol.* **206**, 923–925.

WURZEL, M., BLAIR, D. C., ZWEIFACH, B. W., CRAIG, L. C., AND TAYLOR, W. I. (1967). Blood-borne factors affecting vascular tone. *Experientia* **23**, 486–492.

WURZEL, M., AND ZWEIFACH, B. W. (1966). Contracting principles of arterial smooth muscle in rabbit and dog plasma. *Arch. Int. Pharmacodyn. Ther.* **162**, 1–19.

YANO, S., KUMAGAI, A., AND YAMAMURA, Y. (1968). "Integrative Mechanism of Neuroendocrine System," Vol. 1, pp. 165–174. Hokkaido Univ. Med. Library Series, Japan.

YEH, B. K., MCNAY, J. L., AND GOLDBERG, L. I. (1969). Attenuation of dopamine renal and mesenteric vasodilation by haloperidol: Evidence for a specific dopamine receptor. *J. Pharmacol. Exp. Ther.* **168**, 303–309.

ZWEIFACH, B. W. (1953). Circulatory changes in the peripheral vascular bed following local injury. *In* "Mechanism of Inflammation" (E. Bajusz, ed.), pp. 77–84. Acta, Montreal.

ZWEIFACH, B. W. (1957). General principles governing the behavior of the microcirculation. *Amer. J. Med.* **23**, 684–696.

ZWEIFACH, B. W. (1961). "Functional Behavior of the Microcirculation." Thomas, Springfield.

ZWEIFACH, B. W. (1966). Microcirculatory effects of polypeptides. *In* "Hypotensive Polypeptides" (E. G. Erdös, N. Back, and F. Sicuteri, eds.), pp. 451–461. Springer-Verlag, New York.

ZWEIFACH, B. W. (1968). Vasoactive agents in the microcirculation. *Fed. Proc. Fed. Amer. Soc. Exp. Biol.* **27**, 1399–1402.

ZWEIFACH, B. W., AND METZ, D. B. (1956). Rat mesoappendix procedure for bioassay of humoral substances acting on peripheral blood vessels. *Ergeb. Anat. Entwicklungsgesch.* **35**, 176–239.

ZWEIFACH, B. W., SHORR, E., AND BLACK, M. M. (1953). The influence of the adrenal cortex on behavior of terminal vascular bed. *Ann. N. Y. Acad. Sci.* **56**, 626–633.