THE GRADIENT OF VASCULAR PERMEABILITY*

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Plates 14 to 17

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The main purpose of this paper and of others which follow is to bring proof of the existence in certain situations of a gradient of permeability along the capillaries which makes for the equalization of opportunity within the tissues served by them. Means are described whereby this equalization is achieved in different fashion elsewhere in the body, and evidence is brought forward of a significant permeability of the arterioles and venules.

The Distribution of Vital Stains within Skeletal Muscle

The sheet muscles of guinea pigs and young rabbits are so transparent that the distribution of innocuous dyes can readily be followed during life.

The smallest vessels of voluntary muscle are arranged almost diagrammatically (1), with arterioles and venules disposed alternately in the same plane, at approximate right angles to the muscle fibres (Figs. 1, 2, and 3; Schema I, A). The numerous, parallel capillaries which bridge the gaps between are, in the rabbit, often more than 1 mm. in length. Certain of them pass the nearest venule and course to that next beyond. All lie next the fibres which they nourish and adjoining ones are linked by occasional cross connections; but the existence of these does not significantly affect the rule that the blood leaving an arteriole finds its way back to the large vessels by way of the next venule.

To witness the distribution of vital dyes a window can be inserted over the external oblique, a "white" muscle. The rabbit is etherized after a fast of 18 to 24 hours, laid—not stretched—on its back, an oval piece is snipped with scissors

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from the shaved skin of the side of the belly, the subcutaneous layer is parted, and a plate of clear, dry mica, ½ to 1½ cm. in greatest diameter, is rapidly inserted over the muscle. It should be devoid of scratches, thin enough to be pliable, and of a size to fit snugly beneath the opening. Abnormal tensions and pressures must be avoided. In rabbits of 700 to 1500 gm. the fascia is glassy and so loose that the window set upon it can be pushed here and there to obtain a view of regions not directly exposed. In old individuals it is somewhat opaque, not infrequently contains fat, is fixed to a murky muscle; and more than one window must frequently be inserted to bring to view a field showing arteries and veins in the characteristic relation.

Brilliant pictures are obtained by underlaying the muscle with a curved strip of white celluloid inserted into the peritoneal cavity through a slit in the mid-line. But this complicating procedure is for special occasion.

As soon as the window has been placed the dye is injected into a vein while the muscle is watched through a binocular dissecting microscope, with the animal upon a warmed platform that can be tilted. The light from a Leitz carbon arc lamp, cooled by filtration through Magnus' fluid (2), is reflected upon the field by a plane mirror. The transverse venules appear broad and stumpy, the arterioles thread-like, and often indistinctly visible until the blood is stained.

To procure specimens at any special stage of the staining, the carotids are severed for exsanguination, the skin and cutaneous muscle are dissected away together, the oblique is cut through on three sides of a square (the fourth being toward the back), the free edge is seized with a clip of the sort used to hold photographic films while drying, and by gentle traction and dissection the piece of muscle is separated from the underlying tissue, everted upon a glass plate, covered with another, and cut loose. The location of the stain is best seen when the specimen is placed upon a white surface and viewed on its inner side; for the vessels mostly ramify near this side. The cutaneous muscle, internal oblique and transversalis also yield instructive pictures, while the serratus magnus, gracilis, adductors and pectorals provide corroboratory data.

The tissue of guinea pig muscle is so thin and clear that high magnifications can be used; but the external obliques of the cat and rat are ruddy and relatively opaque; and the abdominal wall of the mouse is so thin that its individual layers cannot easily be separated. In all these animals, however, the distribution of the dye takes the same course.

The staining is most easily followed with dyes which pass very slowly through the vascular walls. The findings with Chicago blue 6B (No. 518, Colour Index (3)) are typical.

Chicago blue 6B (General Dyestuff Corporation) has a molecular weight of 992 and is so slightly diffusible that dialysis can be utilized to purify the commercial preparation. A saturated watery solution of it is placed in the receptacle made

by clamping together the corners of a square of "diphtheria parchment paper" (Reeve Angel and Company), and this is immersed for 4 days, first in running tap water, then in distilled. Thymol must be added to prevent bacterial growth. It is removed later from the purified and dried dye by prolonged shaking with absolute alcohol. As thus prepared Chicago blue dissolves readily, an 8 per cent solution in water being isotonic with blood and having a pH of 8.3.* The intravenous injection at body temperature of 5 cc. for each 1600 gm. of rabbit causes no symptoms in unanesthetized animals, when it is given in the course of 4 minutes; but the kymograph discloses in etherized ones a slight, almost momentary drop in blood pressure. A considerable interval elapses before the dye begins to escape from the vessels.

Following the injection the skin becomes only light blue; but it gradually darkens during the next 2 hours, while the muscle, lighter to begin with, becomes darker still. The external oblique watched through the mica shows a swift bluish shadowing as the stain reaches the viscera beneath, and a few seconds afterwards its arterioles and venules stand out in blackish blue to their least ramifications. The region between them now has an indistinct, azure striation owing to dye in the capillaries. These can be readily made out with the high power. The extravascular tissue itself is unstained.

The period before Chicago blue begins to leave the vessels varies with the age and state of the animal. In rabbits of 1600 gm. under light ether a gradually intensifying blue mist appears after 5 to 15 minutes, in the region served by the last third of each set of capillaries connecting transverse arteriole with transverse venule. With a low magnification the arterioles are easily recognizable in the midst of completely unstained tissue, whereas each of the venules alternating with them is surrounded by a blue cloud, or plume, with fading margins (Fig. 9). The result viewed in the gross is a fern-like marking of the external oblique (Fig. 8). With a watchmaker's eye-piece (magnification $\times 1\frac{1}{2}$) one can see that the center of each blue plume is separated from the next by $1\frac{1}{2}$ to 2 mm., by twice the gap between arteriole and venule that is to say; but there are some larger unstained spaces through the center of each of which a solitary arteriole courses. Here the distance to the nearest collecting venule is often $1\frac{1}{2}$ mm. at the least. In long muscles,—gracilis, pectorals, and quadratus lumborum,—zigzag bars of color, suggestive of the markings upon a mackerel, lie transverse to the fibres (Fig. 11), as mentioned in a previous paper (4).

What is the cause for this barred coloration? It does not occur until after the dye has circulated for some minutes; and it persists when

* The hydrogen ion concentration of the various dyes was determined with the glass electrode by Dr. Dole or by Dr. Mirsky, to both of whom we feel greatly indebted. The figures on pH and tonicity may hold only for the dye specimens with which we have dealt.

the vessels are washed out with salt solution. Evidently the stain has escaped into the tissues. This cannot have resulted from the injury of exposure, for it has happened everywhere through the muscle. Nor is it due to the ether. The typical barring comes about relatively soon in unanesthetized animals (rabbit, mouse), being found when they are decapitated at a blow. Local decolorization will not account for it, since the dye retains its character in the organism, being gradually stored in cells with result that the tissues appear dark blue even after many weeks. However, the amount of Chicago blue we have employed causes the blood to become completely incoagulable for some hours, and though producing no immediate symptoms may prove fatal after 4 or 5 days. Some of the animals succumbing had marked ascites, a development which suggests that the dye may injure the endothelium selectively, with the coloration in the neighborhood of the least venules as result.

It has seemed better to broaden our observations with the aid of other poorly diffusible dyes than to study the effects of a single one intensively. Pure trypan red, trypan blue, and trypan violet (Grueb-ler) have proved suitable for the purpose. Though so like in name these differ greatly in constitution; but all have large molecules and escape from the vessels slowly. All give rise to bars of color situated like those of Chicago blue in the region traversed by the last third of the muscle capillaries. Each bar intensifies toward its middle where lies a transverse venule.

A 4 per cent solution of trypan red (No. 438, Colour Index) is isotonic with the blood and has a pH of 9.21. Added in a 1 to 20 proportion to blood at pH 7.44 under paraffin oil it causes no change in reaction perceptible with the glass electrode. To effect a sharp staining 2½ cc. must be injected for 400 gm. of guinea pig. The dye leaves the vessels nearly as slowly as Chicago blue, so its relation to the small vessels can be studied at leisure.

Trypan violet (5) is isotonic in 3.75 per cent solution and has a pH of 8.67. We have injected 1.25 cc. for 400 gm. of guinea pig. It passes from the vessels *in vivo* at approximately the rate of trypan red and gives especially beautiful color pictures.

Trypan blue (No. 477, Colour Index) has been used merely in corroboration, as an isotonic solution (6 per cent, pH 8.92) in the same amount per kilo as trypan red. Like the latter it does not notably alter the reaction of blood when added in 1 to 20 proportion. For reasons as yet undetermined some dye specimens fail to stain intensely.

The possible influence of toxicity, staining affinities or pharmacological action to cause the barred staining with the dyes thus far mentioned can be dismissed from account. For not only do they all yield essentially the same findings as Chicago blue, but this has proved true as well of two highly diffusible, innocuous, dyes, namely patent blue V and brom phenol blue (6).

The diffusibility of the dyes has been determined by the method of Northrop and Anson (7). The following table shows the relative rates through water and gelatin, respectively. The two columns of figures are independent of each other.

Dye	Water	Gelatin
Chicago blue 6B	1.0	—
Trypan blue	1.17	0.74
Trypan red	1.34	0.86
Trypan violet	1.70	1.09
Brom phenol blue	*3.2*	*3.2*
Patent blue V	6.2	6.3
Dextrose	7.1	20.3
Phenol red	7.6	7.8
Urea	12.4	41.7

For the determinations in the second column the porous disc was filled with 8 per cent isoelectric gelatin. The amounts passing at 0°C. in successive periods of 30 minutes in the case of water, and 6 hours in that of gelatin, were quantitated against standard solutions in a colorimeter.

The speed with which brom phenol blue diffuses has been taken as the standard for each tabulation. One sees that in water it spreads 3.2 times as fast as Chicago blue 6B. The latter dye did not pass through gelatin in a measurable quantity in 6 hours. Dextrose spreads through water only a little more rapidly than patent blue V but through gelatin more than three times as quickly.

Patent blue V passes out into the muscle everywhere at once and special means are needed to demonstrate the barring when it is given in large quantity. Either the distribution must be slowed in some way or else checked at an early period. We have resorted to both procedures.

Patent blue V has been utilized in our previous work on tissue maintenance. Three cc. of an isotonic solution (8 per cent, pH 6.0) will color deeply a 1600 gm. rabbit, and even when injected very rapidly it produces no untoward effects. When it is given in the course of a minute the external oblique, watched through a

window, seems to turn greenish blue throughout, as if drenched; and drenched indeed it has been from the capillaries throughout their length. There is not the least indication of a barring with color then, or when the muscle is removed 3 minutes after the injection. But if the dye is placed abruptly in circulation and the carotids are cut the moment it is seen to color the blood in the least venules of the muscle, most informative specimens are got. Superimposed upon the diffuse coloration in far deeper blue is the familiar barring. The bars have as axes the collecting venules, but are broader than those caused by the poorly diffusible trypan dyes and by Chicago blue. They occupy about half the tissue between transverse venule and arteriole, fading off in the direction of the latter.

The suffusion is so rapid and deep as in many instances to obscure the barring which becomes plainly visible only when the sheet of muscle is stripped back. Fortunately the conditions in animals depleted by bleeding favor observations *in vivo*. By three or four large hemorrhages from a carotid cannula, at intervals of 5 to 10 minutes, the blood pressure is lowered to about 30 mm. Hg; a window is inserted as usual into the abdominal wall; and the stain thrown forthwith into circulation.

The veins are still prominent in the muscle of the bled animal but very few arterioles can be seen. The generality stand forth as the stain reaches them, which may not happen for nearly a minute; and 3 further minutes may elapse before the color of the venous blood notably alters. One perceives a dark line of blue, as if from an unseen pencil, glide smoothly along the arteriole and trace to the tip each least twig of an arborization only guessed at before. Then rapidly a mist of blue forms about the arterial twigs while the rest of the muscle is still without color (Schema I, B). Already the dye is escaping. The localized mist, which in the gross appears as a blue barring of the tissue about the arterioles, does not spread but instead is lost after some seconds in an even, general staining of the muscle beyond; and upon this as a background a new and broad pattern asserts itself secondarily, namely that of the familiar barring about the transverse venules (Schema I, B). During the few minutes while this develops by a passage of dye out of the slowed stream but little reaches the venous blood. Rapidly the diffuse staining between the bars grows deeper and soon they are lost in it. When they are most intense the picture observed *in vivo* is like that in a normal animal killed at once after injection. The secondary barrings are spaced at the same distance as those of Chicago blue, which could not be the case unless the early, arterial, barrings had completely disappeared.

Though patent blue V stains the tissue all along the muscle capillaries, it does so with increasing intensity as the transverse venules are approached (Schema I, B). In the experiments just described the bars of color were broader than those of Chicago blue or of the trypan stains. When the circulation has been slowed by bleedings the tissue first met is first stained from the feeble current of blood, as does not happen

when the pressure is normal and the stream swift; and in consequence a blue barring occurs about the arterioles (Schema I, B). But the law of first come first served in the depleted animal holds for a brief period only. The entire region traversed by the capillaries undergoes a rapid, even suffusion with color, and upon this is superimposed secondarily the barring about the venules.

The conditions are such as should lead, one would think, to an especially pronounced spread of dye from the first portion of the capillaries; for the dye reaches this portion first, is most abundant there, and there the blood is under most pressure. It loses so much dye in passing through the muscle that the venous blood appears dirty brown, or green at most, while the bars are developing, never a sharp blue like that of the arterioles. The greatest staining takes place precisely where the known conditions seem most unfavorable thereto.

Brom phenol blue yields corroboratory results.

Etherized rabbits receiving intravenously 5 cc. of an isotonic solution of brom phenol blue (4 per cent, pH 7.24) in the course of 7 to 10 seconds show a gradual, diffuse staining of the external oblique muscle, and a superimposed pronounced barring round about the transverse venules, which endures for 2 minutes or more. The bars extend with diminishing intensity nearly half way to the arterioles. In animals with the local circulation slowed by bleeding no staining like that with patent blue V takes place about the distributing arterioles before the dye has gone further, but on the other hand the diffuse coloration is so slight as to render the venous barring especially vivid (Schema II, D). As with patent blue V the staining is greatest where the blood is poorest in dye. While the bars are developing the blood in the collecting venules appears but little stained, whereas that in the arteries is intensely blue.

The findings (Schema II, D) are precisely what one would expect with a coloring matter greatly more diffusible than Chicago blue but considerably less so than patent blue V, if one assume that the same general laws govern the distribution of all three dyes.

The Gradient of Distribution

Why were the muscles so singularly barred with color? Their fibres were little if at all stained, and though the interfascicular connective tissue became gradually and diffusely blue, neither it nor the contents of the vessels was responsible for the markings. These were traceable

to extravascular dye lying between the fibres of the individual muscle bundles in the regions supplied from the further portion of the parallel capillaries (Fig. 10). There are no interstitial structures in this situation which stain especially well and which recur with each set of the little vessels serving the fibres. The inequalities of hue were limited to the period when the dyes were first being distributed and hence must have resulted from local differences in the ease with which these passed from the blood, differences so great that most of the coloring matter got out precisely where the known conditions appeared least favorable.

As already stated, the dye of greatest diffusibility, patent blue V, can be seen in bled animals to pass into the tissue about the terminal arterioles before the slow stream can carry it further; but soon there occurs a blueing along the entire capillary way. One must conclude that the little vessels are permeable to this dye throughout their length. And it escapes from some larger vessels as well. In animals killed within a few seconds after injection one often finds a narrow zone of blue next the arteries from which the transverse arterioles are given off, vessels which can at this time be absolutely identified because the blood within the venules is uncolored as yet. Later, as the tissue generally becomes suffused with blue, the zone can no longer be discerned, but soon an intense, localized coloration develops next the veins which receive the transverse venules. These vessels and the arteries about which staining occurs are close to the limit of visibility for the unaided eye, and injections with gelatin-carmine prove them to be devoid of *vasa vasorum*. So rapid is the spread of the dye in tissue manipulated post mortem that reliable photographs of the color phenomena have not been obtainable.

With brom phenol blue (Schema II, D) no staining takes place by direct passage through the wall of arterioles, but after some minutes a blue zone develops next those veins receiving blood from the transverse venules. The broad blue bars which appear early about the latter vessels do not spread and disappear by merging with one another, but, like those of patent blue V, remain unchanged while a more gradual, even staining takes place elsewhere along the capillary; and as this staining intensifies their margins are lost in it, so that they appear to be narrower, and soon they are no longer to be recognized. It is certain that brom phenol blue, while escaping most readily near the venules, emerges everywhere along the capillaries.

The passage into the tissues of Chicago blue and the trypan stains is so slow that in animals with thin, nearly transparent, abdominal musculature (guinea pigs, young rabbits, kittens) one can tell precisely where dye first leaves the vessels (Schema II, C). It is where the capillaries enter the transverse venules directly, or unite near them to form tiny radicles (Figs. 9 and 10). Here a mist of blue, red or violet forms and envelops the transverse trunk in color. The outline of each

bar of mist is step-like because the dye extends further back along some capillaries than along others. Not infrequently these enter one side only of the venous trunk, and the staining is then confined to this side (Fig. 10). None occurs about the venous trunk beyond the region where capillaries enter it (Fig. 10), and none about the larger vein into which it gives, nor about any of the arterioles.

Instead of spreading and merging with one another the colored bars retain their size while an even staining gradually takes place between them. Evidently the capillaries are somewhat permeable throughout their length even to the most poorly diffusible of the dyes we have used. As the general staining intensifies the boundaries of the bands disappear in it, and at last they are totally obscured. In young etherized rabbits injected with Chicago blue this may take more than 4 hours.

These facts prove that the dyes pass out all along the capillaries, but most readily in the region where they unite into venules. From their proximal portion Chicago blue and the trypan dyes escape very slowly. Brom phenol blue and patent blue V pass out of the capillaries everywhere, though with special ease at their end; and the small venules are permeable to them as well. Patent blue V penetrates even the wall of small arterioles, staining the tissue next them.

The progressive increase in intensity of the staining as the venule is approached suggests the existence of a gradient of distribution along the capillaries. But an alternative explanation is possible, namely that a secondary dispersion occurs of dye escaping only at the veno-capillary junction or from the least venules. In such event one should see the dye emerge like smoke from a leaky stovepipe, and spread backwards in the direction of the arterioles. The phenomenon could not be overlooked with patent blue V or brom phenol blue which give rise quickly to intense, broad bars. It never occurs. Furthermore the mist of Chicago blue remains of the same dimensions for hours, proving that secondary spread of the dye through the tissues is extremely slow. True, extravascular color is first noted where the capillaries enter the venules; but it does not spread thence. The bar of stain materializes throughout its eventual situation as a mist of graded intensity from center to margin; and when first perceptible it has nearly its eventual breadth, enlarging later only to the extent that might be expected from increased visibility. One is forced to conclude that some gradient affecting the distribution of vital dyes exists along the further portion of the capillaries.

Factors Influencing the Gradient

The gradient is but little affected by drastic circulatory changes.

Ordinary muscular activity does not essentially disturb it. The external oblique and leg muscles of a rabbit injected with Chicago blue 6B, which wandered about the room for a few minutes before being killed, and struggled when picked up, showed the characteristic picture. So too with an animal that had repeated convulsions owing to air embolism. If the external oblique of etherized rabbits is directly stimulated to rapid contraction by 60 to 120 induction shocks per minute diffuse staining takes place; but the conditions have little relation to those of life.

Often plethora was produced incidentally, as when the blood volume was increased one-seventh by the injection of isotonic trypan red solution. In some tests the animal was bled nearly to exsanguination, the systemic blood pressure being reduced to the tolerable lower limit before the dye was injected, with result that the arterioles were contracted almost to invisibility and the stained blood entering the capillaries could be seen barely to creep along them, so slight was the force behind it. None of these changes essentially altered the distribution of the stains. In certain instances the nerves to a hind leg were cut just before the dye injection, to increase the circulation. The gradient disclosed with Chicago blue 6B proved similar to that in the control limb, the mackerel barrings being identical in extent.

In tests previously reported in another connection (4), not only were the nerves cut but the animals were bled prior to injection. Staining proved diffuse in the paralyzed legs whereas barring took place in the normal ones. But highly diffusible dyes were used—patent blue V, brom phenol blue—and the animals were killed so late that diffuse staining was to have been expected wherever vasoconstriction in compensation for the diminished blood bulk had been prevented by nerve severance.

A shock-like state was produced in several etherized cats and rabbits prior to the injection of stain, by traumatizing the muscles of a hind leg, after Cannon's method (8). The eventual weight of the limb as compared with its fellow showed that an enormous extravasation had taken place into it during the period while the blood pressure was falling; and much if not all of the fall must be attributed to reduction in the blood volume. The uninjured muscles where colored at all were characteristically barred (Fig. 12). So too were they in animals depleted with hypertonic solutions given by mouth (9) as also when the muscle circulation was cut down during the period of staining by the slow intravenous injection of epinephrine or pituitrin (10), a procedure which enormously raised the carotid blood pressure.

When large amounts of the dyes are given, as in the experiments thus far detailed, the width of the colored bars varies directly, like the rapidity of the staining, with the diffusibility of the coloring matter em-

ployed. The bars of Chicago blue and of the trypan dyes are slow to form and narrow, those of brom phenol blue appear quickly and are considerably broader, while with patent blue V barring is almost immediate, but so too is diffuse staining and the bars, very broad though they are, can be discerned for but a few minutes. We have ascertained the influence of the quantity of a highly diffusible dye on the gradient of distribution.

Windows were inserted at several places over the external oblique of 2000 gm. rabbits, and from 1/2 to 1/10th the standard amount of patent blue V was thrown into circulation. The largest quantity mentioned gave rise as usual to diffuse staining with a superimposed barring; but the coloration took place slowly and the bars occupied not more than a third of the tissue. With 1/4th the ordinary dose a still more tardy, general staining was produced and upon this, not a barring but a pronounced narrow tracery of color about the transverse venules. With 1/7th the dose the muscle colored to the same slight extent everywhere, and the blood was soon rid of the dye. All these findings were confirmed at post mortem.

Significance of the Gradient

The conclusion seems justified that the gradient responsible for barring with a highly diffusible dye brought in abundance to the tissue leads merely to its equal distribution when little is available. Inequalities develop only when so much dye is carried by the blood, or so indiffusible a dye, that not enough is lost along the capillary channel to counteract the influence of the gradient. Of our materials patent blue V most nearly approaches a normal food stuff in diffusibility. In water it spreads about as fast as dextrose but in gelatin less than one-third as rapidly. There is no reason to believe that a gradient which profoundly influences the distribution of highly various vital stains will fail of effect upon other substances. One may suppose that it acts to offset the progressive loss of normal stuffs along the capillary way, with result that the tissue is everywhere served to the same extent by the blood. But is any such supposition justified? Does there exist the need for an arrangement to equalize opportunity along the capillaries? The structure of the muscle vessels, when considered with the changes undergone by the blood coursing through them, provides an answer to these questions.

The groups of muscle capillaries which connect transverse arterioles with transverse venules vary in length from 0.43 mm. to 1.35 mm. in the adductor magnus, and average 0.69 mm. (1). In the external oblique they are as long. Krogh (11) ascertained that those which are open in resting guinea pig muscle have an average diameter of only 3.5μ. Corpuscles are deformed while passing through them. In injected and fixed rabbit muscle they range between 2.5 and 5.5μ (12). It follows that the capillary length is often several hundred times its breadth. The merest glance at an injected specimen poses the problem of tissue maintenance by such vessels (Fig. 7). Blood coursing through the hairlike channels, running the gauntlet of protoplasm that both takes and gives, is inevitably so different on emerging from what it was on entering that the segment of muscle fibre served from the distal portion of the capillaries would exist in a totally different milieu from that at their beginning were not conditions equalized in some way. The necessity for such equalization lies in the fact that each fibre can be only as strong as the weakest point in its length. One may urge that in working muscle the vessels are distended and tortuous, the current fast, there is a copious lymph flow, and contraction of the fibres, all aiding distribution. Nevertheless the blood when it reaches the veins is largely depleted of food materials, and is loaded with waste. In resting muscle many of the capillaries are closed off (11).

To compensate for the disadvantageous circulatory conditions some mechanism regulatory of exchange must exist along the capillaries, or else graded linear variations in the avidities and habits of the protoplasm of the muscle fibres, variations repeated with each successive relay of little vessels in the series that minister to its great length. The latter conception seems preposterous.

The possibility must be noticed that the stuffs in the blood may maintain the portion of fibre along the first portion of the capillary in a continual state of surfeit, so to speak, with result that this part has a negligible influence to deplete the blood and produce the need for a gradient. Such a condition of affairs occurring, not at the beginning but toward the end of the capillary in animals receiving patent blue V will explain why the barred coloration does not increase with the general deepening in hue, the early presence of dye in the barred region doubtless acting as a deterrent upon later escape there. But granting that under normal conditions distribution to the muscle along the first part of the capillary may perhaps be influenced in this way, there yet remains a further segment to be supplied from a blood that has undergone some depletion and will undergo more the further it travels along the tenuous vessel. The gradient disclosed by our experiments meets the needs of the situation.

Significance of the Vascular Arrangement within the Liver Lobule

The necessity for some arrangement to equalize opportunity where cells of a single sort are ranked along the capillary is clearly shown by the existence of a structural artifice for this purpose in the liver, as also by the untoward changes when the artifice proves inadequate, which frequently happens upon occasions of functional stress.

The cells of the hepatic parenchyma live in an equilibrium easily disturbed by alterations in blood flow, the result being a balanced hyperplasia and atrophy (13). By changes so produced the organ is normally molded from its shape in the embryo to that in the adult. Nevertheless under the ordinary circumstances of life the cells toward the center of the lobule, though served by blood that becomes progressively more venous as this center is approached, thrive in competition with their fellows near the source of supply. They are enabled to do so by the arrangement of the vessels. The little channels carrying the blood which serves the liver cords converge from the periphery of the lobule toward a central vein, and unite with one another, the consequence being that in proportion as this vein is approached more and more blood passes a cell in a given time. Thus, though increasingly impoverished of food stuffs and laden with waste, the blood can still tend the parenchyma adequately.

The distance from the periphery of the lobule to the center is not short. In the rabbit it averages 0.4 mm. in a straight line (14). When the animal is deprived of food an atrophy of the parenchymal cells takes place which is more severe the nearer the center of the lobule these cells are. It matters not that this atrophy has been traced to lessened function (15); for function like food is an opportunity provided by the blood in the form of materials. Not enough of these reach the central cells under the circumstances described for them to maintain themselves in competition with the peripheral elements. The value of the example over others innumerable, of peripheral, central and mid-zone lesions, which could be culled from the literature of pathology, lies in the simplicity of the conditions providing it.

The frequency with which opportunity is unequally distributed within the liver as evidenced by untoward parenchymal changes suggests that the vascular arrangement for distribution is unaided by a gradient of permeability along the capillaries. These let even protein through (16).

Distribution from Capillaries of the Bladder

Where capillaries run in an interlaced felt-work, near to one another and often in opposite directions so that the same cell is served

from different parts of several, there may be no need for a mechanism to equalize exchange. They are thus arranged in the outer layers of the urinary bladder.

The curve of the bladder is too great in the guinea pig, rat and mouse for satisfactory studies. But when the bladder of the rabbit is partially filled with warm milk, exposed under mica, and viewed by cooled, reflected light the more superficial vessels are clearly visible in an illuminated matrix, and one sees that the capillaries widen greatly as they approach the veins and that the blood stream slows concomitantly to such extent that first the flow itself, then the individual cells, become visible. The increased wall surface and the slowing stream would both tend to equalize opportunity along the capillary way. No gradient of vascular permeability has been demonstrable with our dyes, the tissue served by the feltwork of vessels appearing to color everywhere at once. This one would expect under the conditions even in the presence of a gradient.

Relationship of the Vascular Structure to the Gradient

The maintenance of a gradient of distribution in skeletal muscle despite circulatory changes entailing dilatation or great contraction of the vessels indicates that it has a structural basis; and the example of the liver suggests that this may be found merely in local differences in capillary number and size. We have made extensive studies to settle the matter.

Rabbits were etherized, the stomach, intestines and spleen removed through a median incision after ligation of the vessels, and the incision sewed shut. The sternum was split longitudinally, a cannula inserted in the ascending aorta, the right heart opened as a vent, and the animal washed blood-free with warm saline solution containing amyl nitrite. It was next immersed in saline solution at body temperature, a thin gelatin-India ink mass was injected under pressure, then a thick one, and finally enough gelatin-carmine to differentiate the arteries. At intervals the outlet for fluid (through the right ventricle) was obstructed, to aid distension of the capillaries, and finally it was tied off while the injection pressure was maintained. After transfer of the animal to ice-cold 95 per cent alcohol the abdomen was opened by severing the sutures. After fixation for 24 hours *in situ* the external oblique was dissected out and cleared.

In the thinner parts of the oblique of young rabbits the veins and arteries lie in practically a single plane (Figs. 2 and 3). Spalteholz portrays final arterial and venous trunks in regular alternation transverse to the fibres of the adductor magnus and coming off from parent vessels that run side by side. A similar final alternation is achieved in the external oblique but usually in more complicated fashion (Figs. 2 and 3).

Counts of the capillaries are best made in the long muscles. Spalteholz' drawings depict well the minute vascularization. Because of the differing levels upon which it is distributed photographs are unsatisfactory.

At an early period of alcohol fixation, before the injected tissue has become friable, one can tease out portions of individual fasciculi of the long muscles with vascularization intact. One sees after clearing them that relatively few capillaries are present in the region where the arterioles split up (Fig. 6). The latter divide into twigs, some of capillary magnitude, some slightly larger, and the larger ones fork again, often repeatedly, and now into capillaries. The number of these vessels reaches its maximum midway between transverse arteriole and venule, and thence until the venule is neared it does not alter significantly (Fig. 6), the average increase being less than one in fifty, as shown by some forty comparative counts. Cross connections are few. Some of the capillaries enter the venous trunk directly while others may come together just before it is reached, uniting into radicles parallel with the fibres. Even in injected specimens such radicles are almost always shorter and broader than the corresponding arterial twigs, a fact Spalteholz noted. In his precise and comprehensive account no mention is made of any increase in the capillaries as the venule is neared.

None of the several authors measuring injected capillaries mentions any progressive widening along them (12), though a recognition of such widening would have been essential to interpretation of their findings. In our own specimens the capillaries appeared of remarkably even bore.

The vascular arrangement provides a reason for the limitation of the colored bars to the distal half of the region between arterial and venous trunks. The channels from which dye can conceivably pass into the proximal half of the tissue are relatively few, and other things being equal the staining here should be slighter and slower than further on. The barring occurred where the capillaries were relatively numerous and constituted the sole source of supply for the tissues. The graded increase in color as the venules were approached cannot be explained by any increase in the number of capillaries nor, apparently, in the total expanse of vessel wall. But injected and fixed specimens yield only approximate data. In Krogh's report on the capillaries of living guinea pig muscle no mention occurs of any widening toward the venules, and none in v. Hösslin's (12) study of fresh muscle tissue. When in our own work the blood was darkened with India ink, or with dyes that had as yet not passed into the tissue, the muscle should have shown some trace of barring if the capillaries had enlarged toward the venous end, and they should there have been most readily discernible.

Instead it appeared diffusely overcast and under the microscope the capillaries were like threads of even calibre. Poorly diffusible dyes escape only from the distal portion of the capillaries during no inconsiderable time after their injection (Fig. 8), and highly diffusible ones pass out most abundantly there, precisely where the blood is poorest in dye and its pressure lowest. An increase in wall surface vast enough to account for such happenings could not be overlooked. In the absence of it one must ascribe the greater escape of dyes as the capillary end is approached to a graded lessening of the barrier between blood and tissue, that is to say, to an increasing permeability of the capillary wall.

The Literature of Distribution along the Capillaries

The problem presented by the length of the capillaries in its influence upon exchange with the tissues has attracted singularly little attention. One might say that these vessels have been viewed in cross section, seldom in three dimensions.

Schade (17) has invoked the influence of the capillary pressure, that of the blood colloids, and various other factors, in an elaboration of Starling's surmise that the passage of substances into the tissues takes place predominantly through the first portion of the capillary wall with resorption as the major activity further on. Krogh has criticized (18) the hypothesis destructively, but Landis (19) has procured evidence which might be cited in its support. He frequently observed a filtration of dye-stained fluid from the first portion of the mesenteric capillaries of frogs. The vessels had been exposed for more than an hour under Ringer's solution before the observations were begun. In the frog much lymph is continually produced, by filtration through the capillary walls generally. In resting mammalian muscle the amount formed is almost nil (20).

The dyes of our experiments appeared most abundantly in the region where conditions would, at first thought, seem least favorable on Schade's hypothesis. Nevertheless, it is possible to explain certain of the phenomena in terms of his view, if this be taken to imply an active extravascular flow in the direction of the venule. It might be assumed, for example, that the escape of poorly diffusible dyes circulating in great quantity actually takes place from the first part of the capillary but so slowly that no color is visible anywhere until the escaped dye, passing along the outside of the vessel to its further end, fails for some reason to pass into the blood as abundantly as it had emerged, with result that it accumulates in a colored band. But how explain on this basis the fact that the least quantity of poorly diffusible dye that will cause perceptible staining yields characteristic bands of the usual

dimensions at the end of the capillary? Can one suppose that there exists a selective impediment to return just where according to Schade everything should be highly favorable to it?

Highly diffusible dyes stain the tissue next the transverse collecting venules only slightly less than that at the distal end of the capillaries. Is one to suppose that the pigment responsible for this staining escapes primarily in the proximal capillary region and is carried by extravascular ways with the swiftness of the blood itself to reach its eventual situation outside the veins? If the staining is deemed to have come about by direct extension through the vein wall, can one refuse to admit the evidence for an even greater extension from the adjoining portion of the capillaries?

These and the many other respects in which our findings fail to conform with Schade's hypothesis are less impressive as objections to it than is the length and shape of the capillary itself. To assume that the escape of substances takes place preponderantly from the first portion of a hair-like vessel ½ to 1½ mm. long and resorption from its further part is to go afield for a concept which would interpose between the tissue and the blood serving it an obstacle to the equalization of cell opportunity even more considerable than that which would exist were the narrow channel everywhere permeable to the same extent.

The Zone of Effective Vascular Permeability

Physiologists are accustomed to think of the vessels as of a three-part system, arteries to bring, capillaries to exchange, and veins to collect once again. In the main this classification undoubtedly holds. Yet our experiments with patent blue V prove that the wall of the arterioles of muscle will let through a coloring matter somewhat less diffusible than dextrose, while the venule walls are readily penetrated by it. What are the limits of permeability along the vascular system? Obviously they must vary with the substance under consideration, being wide for materials of great penetrative ability, such as CO_2 and urea, and contracted to the vanishing point (at the venous end of the capillary) with substances of very large molecule. Their extent for this or that substance need not be discussed; but it is important to determine the collective outcome of the penetration of the vessel wall by normal stuffs, to learn in other words the limits of effective permeability along the vascular way.

The paucity of vessels in the walls of the larger arteries and their absence from small ones led long ago to the inference that the walls must be nourished partially, or wholly, by direct exchange with the blood. Several authors (21) have described a rapid penetration of highly colloidal dyes into the arterial wall. Veins

are said to stain even more quickly. When a segment of the aorta or renal artery is separated from its surroundings and coated with wax it survives and abnormalities develop only next the wax (22). An effective exchange between the blood and the tissue of the wall evidently takes place; but the high blood pressure and the possible presence of "stomata" may render the instance special.

Where there are *vasa vasorum* in the adventitia the effective influence of the blood in the main lumen cannot extend as far as this layer. Whether it reaches beyond in the case of the arteries and veins with avascular walls has not been directly determined. The small venules of human skin are walled by a single layer of cells, and capillaries are so infrequent that the venules may be supposed to serve in their stead, as Krogh points out (18). Lewis (23) believes that the capillaries, the venules that are simple endothelial tubes, and the "weakly equipped arterioles" all act to nourish the cutaneous tissue; and Kreyberg (24) has stated a like view for the skin of the mouse. But the inference that venules and arterioles must function as capillaries because they are walled only by endothelium is not entirely warranted. Vessel walls cannot be considered to have the same permeability because they are merely one cell thick, as the present work sufficiently attests.

The regularity of the minute vascularization in skeletal muscle enables one to perceive gaps in the capillary spacing. Hence it should be possible to tell whether any of the venules and arterioles serve the fibres effectively, since where they fail to do so capillaries must be present to perform the task.

On teasing out the injected and partially fixed muscles of the rabbit (gracilis, adductor magnus) one sees that the largest vascular trunks running transverse to the muscle fibres lie between the fasciculi and give off branches to these on one or both sides, which branches plunge transversely amid the fibres and ramify. Those to superimposed fasciculi do not quite coincide in position and therefore the colored bars seen in a thick layer of fresh, translucent muscle are broader than is the actual distribution of the stain along the individual capillaries (Fig. 11). Intrafascicular arterial twigs that run parallel with the capillaries are far more frequent than venules (Fig. 6).

In paraffin cross sections the injected capillaries stand forth as dots arranged at the angles of the roughly polygonal muscle fibres, while larger black discs represent the venules and arterioles (Figs. 4 and 5). A light staining with hematoxylin and picric acid greatly aids identification of the structures. One can readily perceive that those vessels larger than capillaries which lie like these latter, within the ultimate muscle fasciculi and next the fibres, are spaced at about the same distance from the nearest capillaries as if they actually were such. Where they exist no other source of nourishment for the tissue can be found. All have walls only one cell thick, but the cell nuclei are nearer together in some of the vessels, doubtless the arterioles. Along the margins of the fasciculi capillaries are relatively

infrequent as would follow from the circumstance that here the demands on the blood are diminished by nearly half. But even here a difference is to be noted between regions adjacent to a small vein or arteriole and those further off. The latter regularly exhibit many more capillaries.

Specimens teased or cut in the direction of the muscle fibres give corroboratory information. The individual fibres pass directly through the forkings of the transverse arterial trunks, and while some few capillaries pass with them, the majority do not, but join the arborization. Where the fibres traverse this latter they must of necessity depend in great part on the blood of its small branches. The venous twigs are more close set than the arterial, and more capillaries pass through the arborization, indicating that it plays a smaller part in maintenance of the tissue.

Interfascicular arterioles equipped with a layer of muscle are not infrequently accompanied by a capillary; but even around these and the venules of corresponding magnitude such vessels are infrequent. But here the determinations are no longer aided by an almost diagrammatic vascularization.

Specimens from cats and guinea pigs yield the same findings.

The conclusion seems justified that in muscle the last arterioles serve the tissue about them at least as well as do the capillaries. Where they course these vessels are dispensed with. The venules share to a minor extent in the task of muscle maintenance.

Contours of the Gradient

Our dye experiments have disclosed a mounting gradient of vascular permeability in muscle, which first becomes effective along the finest arterioles and trends almost vertically upward with the transition to the capillaries. Along these hair vessels the gradient, far from flattening out, mounts again steeply to reach its peak where they join to form the least venules, declining along the veins rather gradually.

The most casual calculation of the area of wall through which exchange can take place from the blood of arterioles, venules and capillaries, respectively, discloses how negligible this is save in the case of the vessels last mentioned, and that of the smallest collecting venules. When the gradient of vascular permeability is considered in the light of this fact one perceives that the arterioles and venules can have but a slight share in serving muscle. The capillaries after all carry out the major task of its maintenance. Yet the function is not so sharply localized to them as general belief would have it. Someone has happily said that in the animal body form fits function like a glove; but when there is more than one function and these not strictly super-

imposed, the glove must bulge a trifle here and pinch there. Thus it is with the blood vessels of muscle, which have the widely disparate tasks of carrying fluid and cells over long distances under pressure and of facilitating exchange with the tissue at one region only along the way. There is no matter for surprise in the realization that through the walls of the finer vascular branches some incidental, beneficial, leakage takes place.

A vast deal has been written on capillary permeability, with the assumption implicit in the term that permeability is the same all along the little vessels. This is certainly not true of those supplying voluntary muscle. Limitations of space forbid discussion of facts in the literature which support the concept that at not a few situations the permeability of the capillary wall increases progressively as the venules are approached and is greatest at the junction with them. In succeeding papers this concept will be expanded.

SUMMARY

The permeability of the capillaries in the skeletal muscles of mammals increases progressively along their course and is greatest where they pass into the least venules. The gradient of permeability is so largely independent of functional states as to give grounds for the view that it is determined by inherent local differences. Through the gradient opportunity is equalized along the capillary. In the liver lobule this object is accomplished by an artifice of arrangement whereby the blood flow past the cells is increased with their distance from the source of supply. In the urinary bladder the interlacing of capillaries, their progressive widening, and a consequent gradual slowing of the blood flow act to achieve the same end. Here a gradient of permeability has not been demonstrable.

Where cells of different sorts are served by a slender capillary, their differing requirements may render unnecessary any provision to equalize their opportunities; but where shortcomings in local maintenance will reduce the efficiency of an entire fabric, as the muscle fibre, and where cells of like character live competitively along the same channel, as in the liver, some arrangement must exist to ensure an even distribution of the services rendered by the blood. In situations of the kind last mentioned the immediate environment of the individual cell,

the "milieu interne" of Bernard, is not only kept as constant as possible but it must be the same, by and large, for all of the cells.

The task of serving voluntary muscle is not strictly limited to the capillaries. The intrafascicular arterioles and venules act so effectively to sustain the tissue about them that where they run no capillaries are supplied.

It is a pleasure to thank the laboratory staffs of du Pont de Nemours and Co., the General Dyestuff Corporation, Hynson, Westcott and Dunning, the National Aniline and Chemical Co., the Sandoz Chemical Works, and the Winthrop Chemical Co. for a most generous provision of dyes and data.

BIBLIOGRAPHY

1. Spalteholz, W., *Abhd. d. sächs. Ges. d. Wiss. M.-Ph. Cl.*, 1888, **14**, 509.
2. Magnus, G., *Arch. klin. Chir.*, 1924, **130**, 237.
3. Colour Index, edited by F. M. Rowe, published by the Society of Dyers and Colourists, 1924, 1st edition, Bradford, England.
4. Rous, P., and Gilding, H. P., *J. Exp. Med.*, 1929, **50**, 189.
5. Goldmann, E., *Proc. Royal Soc. London, Series B*, 1912, **85**, 146.
6. Rous, P., and Gilding, H. P., *J. Exp. Med.*, 1929, **50**, 189; Gilding, H. P., *J. Exp. Med.*, 1929, **50**, 213.
7. Northrop, J. H., and Anson, M. L., *J. Gen. Physiol.*, 1928–1929, **12**, 543.
8. Cannon, W. B., *Arch. Surg.*, 1922, **4**, 1.
9. Rous, P., and Drury, D. R., *J. Exp. Med.*, 1929, **49**, 435.
10. Gilding, H. P., *J. Exp. Med.*, 1929, **50**, 213.
11. Krogh, A., *J. Physiol.*, 1918–19, **52**, 457.
12. v. Hösslin, H., *Deutsch. Arch. klin. Med.*, 1899, **66**, 103; Stoel, G., *Z. wissensch. Biol., Abt. B*, 1925–26, **3**, 91; Duyff, J. W., and Bouman, H. D., *Z. wissensch. Biol., Abt. B*, 1927, **5**, 596.
13. Rous, P., and Larimore, L. D., *J. Exp. Med.*, 1920, **31**, 609.
14. Mall, F. P., *Am. J. Anat.*, 1906, **5**, 227.
15. Rous, P., and McMaster, P. D., *J. Exp. Med.*, 1924, **39**, 425.
16. Starling, E. H., *J. Physiol*, 1896, **16**, 224.
17. Schade, H., *Ergebn. Physiol.*, 1927, **32**, 425.
18. Krogh, A., The Anatomy and Physiology of Capillaries, 1929, New Haven.
19. Landis, E. M., *Am. J. Physiol.*, 1927, **82**, 217.
20. Barcroft, J., and Kato, T., *Phil. Trans. Royal Soc., Series B*, 1916, **207**, 149.
21. Petroff, J. R., *Beitr. path. Anat.*, 1923, **71**, 115; Anitschkow, N., *Klin. Woch.*, 1925, **4**, 2233; Okuneff, N., *Virchow's Arch. path. Anat.*, 1926, **259**, 685.
22. Lange, F., *Virchow's Arch. path. Anat.*, 1924, **248**, 463.
23. Lewis, T., The Blood Vessels of the Human Skin and Their Responses, 1927, London.
24. Kreyberg, L., *Arch. path. Anat. u. Physiol.*, 1929, **273**, 367.

EXPLANATION OF PLATES

PLATE 14

FIG. 1. Edge of the gracilis of a 700 gm. rabbit, photographed by transmitted light after clearing. The vessels had been injected with an India ink-gelatin mass to show the alternation of transverse arterioles and venules. The striae between are the injected capillaries. ×12.

FIG. 2. Thinner portion of the external oblique of a 700 gm. rabbit, prepared in the same way. The entire depth of the muscle is shown. The injected capillaries are represented by groups of parallel striations. The muscle bundles are somewhat separated. ×12.

FIG. 3. Another region of the external oblique, to show arterial and venous trunks that course with the muscle fibres, not across them, to approach each other and spread suddenly into a skein of capillaries. ×12.

FIG 4. Cross section of the semitendinosus of an 800 gm. rabbit injected with ink-gelatin mass. The vessels appear as black dots. The larger of those which represent the intrafascicular arterioles and venules are in general spaced at the same distance from the nearest capillary as if they functioned as such. The capillary next one of them is a branch from it. ×140.

FIG. 5. A similar specimen to show the same fact, from one of the lumbar muscles of a rabbit. ×200.

FIG. 6. Drawing of a final vascular unit in a small group of fibres of the adductor magnus of a rabbit, showing the typical arrangement in unusually pronounced form. The vessels had been injected with ink-gelatin mass and the muscle teased out and cleared. The fibres are not shown. a = arteriole, v = venule. The capillary number reaches its maximum about midway between them. The long, arteriolar branchings are characteristic, as also the stumpy venule in which the capillaries terminate rather abruptly. One of the capillaries in the neighborhood of the arteriole has been broken and bent back. ×70.

FIG. 7. For comparison with Fig. 6. Final vascular unit in the vastus lateralis of a rabbit injected with ink-gelatin mass. a = arteriole, v = venule. Those capillaries only are visible which lie in a single plane. ×130.

PLATE 15

FIG. 8. Fern-like color pattern in the external oblique of a 2700 gm. rabbit killed by cutting the carotids 30 minutes after injection of the standard amount of Chicago blue 6B. The greater part of the muscle is wholly unstained. Photographed between glass plates by a combination of transmitted and reflected light. Natural size. The black spot is an artefact.

FIG. 9. Situation of the dye in such a preparation as shown by the microscope. Each of the venules transverse to the muscle fibres lies in the midst of a cloud of color. The tissue about the arterioles (indicated by arrows) which alternate with the venules is wholly unstained. ×11.

FIG. 10. Similar findings in the external oblique of a rabbit injected with the standard amount of Chicago blue 6B and killed with ether 3½ hours later. The localization of the staining to the distal capillary regions is well shown. The arterioles lie in relatively unstained tissue. The veins contain much dark blood and the transverse venules had been purposely distended with it by pressure. One is entered from a single direction by capillaries and venous radicles, and on this side only has staining taken place. Near the base of the transverse venous trunks where no capillaries enter no staining has occurred. ×17.

FIG. 11. Gracilis of a rabbit killed by cutting the carotids 19 minutes after injection of the standard amount of Chicago blue 6B. The muscle was photographed *in situ* by reflected light, with a glass plate over part of it. Natural size.

FIG. 12. External oblique of a cat brought into shock by Cannon's method, injected with the standard amount of brom phenol blue, and killed 3 minutes later. During the injection the low blood pressure rose transiently. The dye is seen to be less narrowly localized than Chicago blue, but confined like it to the region supplied from the further portion of the capillaries. Photographed between glass plates by a combination of transmitted and reflected light. Natural size.

Schemata to Illustrate the Differing Distribution in Muscle of Dyes of Differing Diffusibility

PLATE 16

Schema I

A. A parallel artery and vein are shown from which trunks arise alternately that run transverse to the muscle fibres and are connected by capillaries (not shown).

B. *Distribution of a Highly Diffusible Dye (Patent Blue V) in a Bled Animal.*

(a) The slow blood stream has as yet carried the dye only to the beginning of the capillaries where it has at once begun to pass out into the tissues. (The tufting is exaggerated, occupying too large an area.)

(b) A bar of dye forms with the distributing arteriole as its axis.

(c) The dye has now progressed to the end of the capillaries, coloring the tissue along them evenly, thus obliterating the bars.

(d) Bars of deeper hue are seen superimposed upon the general coloration, owing to an especially great escape of dye along the further capillary region. Some of the stain has passed into the venous blood. The zone of color along the main collecting vein shows that its wall has been penetrated by the dye, despite the small quantity of it present in the venous blood as compared with the arterial.

(e) All color differences are lost in an intense general staining.

PLATE 17

Schema II

C. *Distribution of a Poorly Diffusible Dye (Trypan Red).*

(a) The dye circulates through all the vessels but is as yet escaping only about the ends of the capillaries. (The tufting is exaggerated, occupying too large an area.)

(b) The dye passing out along the distal portion of the capillaries generally has produced a narrow, colored bar with the collecting venule in its midst.

(c) Some staining has taken place along the entire length of the capillaries, yet that in the distal region is still especially pronounced.

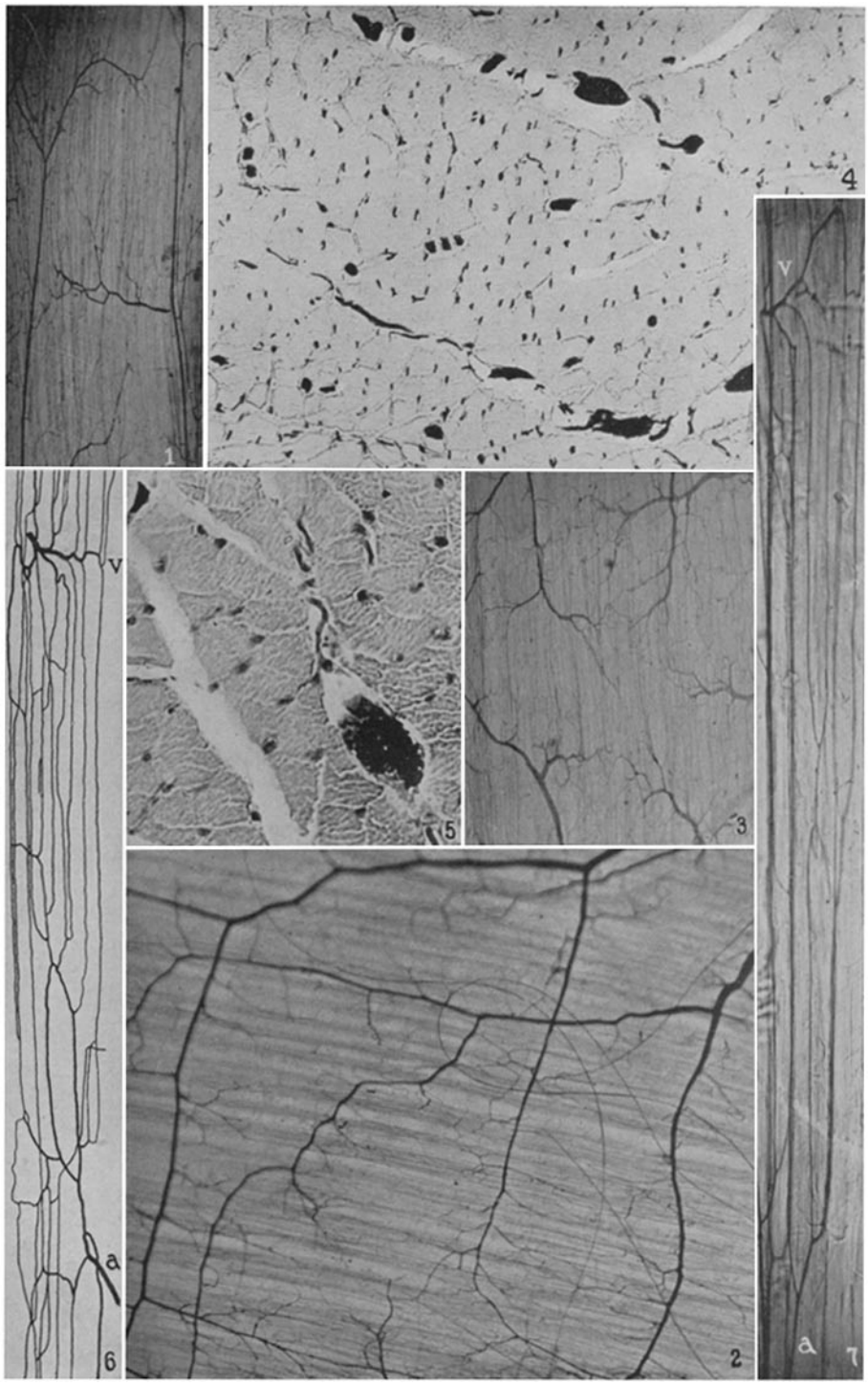
(d) The progressive escape of dye all along the capillaries has obliterated the local differences.

D. *Distribution of a Moderately Diffusible Dye (Brom Phenol Blue).*

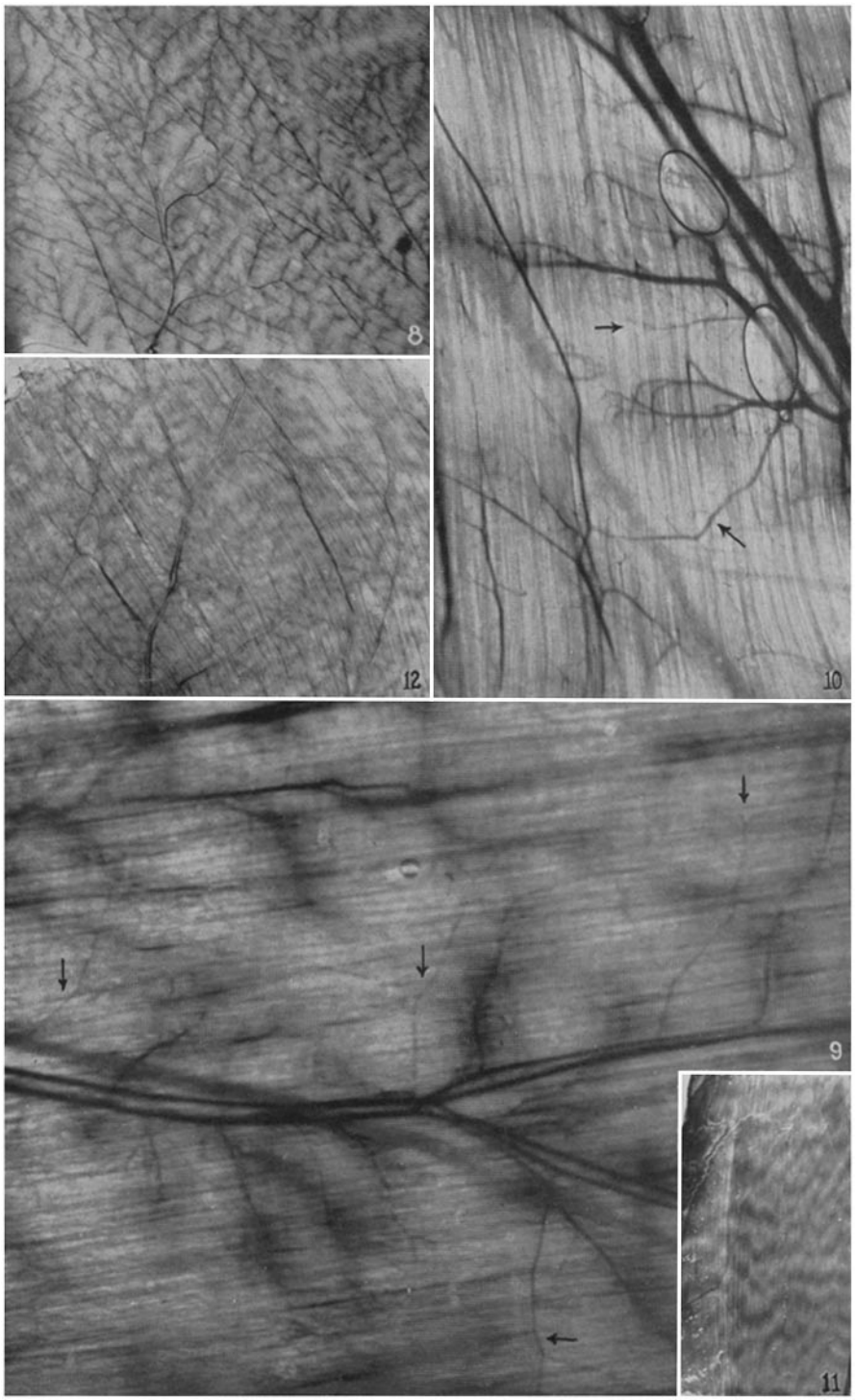
(a) The dye has escaped from the distal portion of the capillaries forming a bar, although the tissue elsewhere is unstained as yet. In consequence of the local escape of dye almost none has reached the venous blood.

(b) The dye has escaped along the entire length of the capillaries, but the staining in the distal region is still especially pronounced, and a zone of color immediately next the relatively large collecting vein shows that it too is permeable to the dye. The venous blood is still poor in dye as compared with the arterial.

Final stage (not depicted). All color differences are lost in an intense general staining.



(Rous *et al.*: Gradient of vascular permeability)



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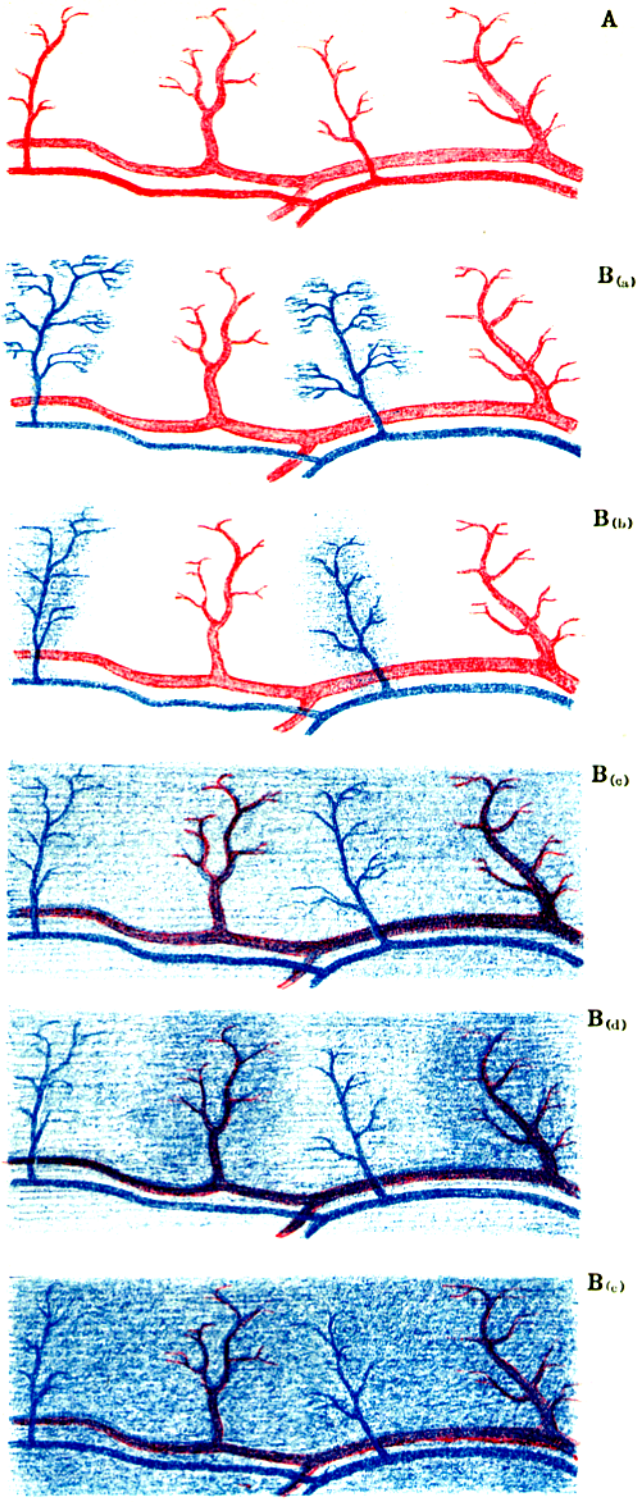
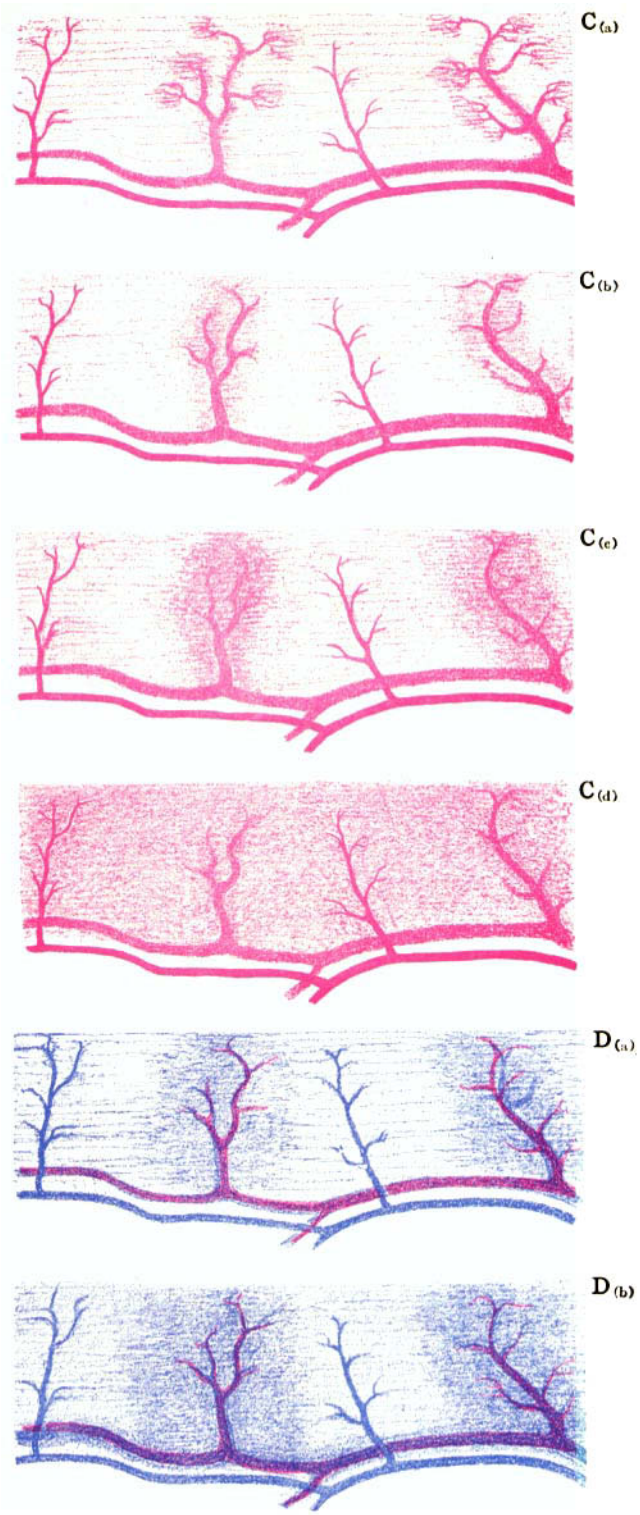


Schema I

(Rous *et al.*: Gradient of vascular permeability)



Schema II

(Rous *et al.*: Gradient of vascular permeability)