

## THE GRADIENT OF VASCULAR PERMEABILITY

### IV. THE PERMEABILITY OF THE CUTANEOUS VENULES AND ITS FUNCTIONAL SIGNIFICANCE

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The recognition that permeability increases progressively along the capillaries of voluntary muscle (1) has led us to study the state of affairs in other organs. An exceedingly pronounced gradient is demonstrable in the skin of the frog, and the small cutaneous venules are even more permeable than the adjoining portion of the capillaries (2). Because of the importance of mammalian skin in water storage and regulation, we have proceeded to study this organ. The skin of the albino mouse was chosen for the purpose because the spread of dyes from the blood can be followed with special ease in it.

#### *The Escape of Dyes from the Blood into the Skin of the Body*

In preliminary tests vital dyes of known diffusibility were injected into a tail vein of unanesthetized animals which were decapitated shortly after. The skin was found to color evenly with rapidly diffusible pigments, whereas with poorly diffusible ones, such as are especially suited to disclose local differences in vascular permeability, there was to be seen a deep staining of the tissue through which the small venules coursed, before any general coloration of the corium had taken place. The phenomenon was visible only when a skin flap was studied from its under side and even then ordinarily the subcutaneous fat prevented a clear view. To do away with it mice were kept on a reduced ration until they had become thin. They yielded pictures that were highly informative.

Young mice of 17 to 19 gm. were used. They had free access to water at all times and remained lively throughout the thinning period of 7 to 9 days. When the weight had fallen to 10 to 12 gm., dye was injected. In type experiments Chicago blue 6B was used in a half strength solution isotonic with the blood (a watery 17.0 per cent<sup>1</sup> solution mixed with an equal quantity of Locke's solution), 0.08 to 0.1 cc. being given in the course of a minute. Decapitation was done 6 to 10 minutes later, the animal was pinned on a board at once, and a skin flap everted. The hair was oiled where it was to be cut through, in order to prevent a scattering of ends on the exposed surface. With blunt scissors a first slit was made along one side in the axillary line and this was extended transversely across the upper and the lower abdomen to the other side. The flap was freed from the web-like underlying tissue with the scissors, and everted and brought in contact with a piece of microscope slide, to which its moist surface at once adhered. Special care was taken to prevent distortion or stretching. The preparation could now be inspected under the binocular dissecting microscope by the cooled light used in our previous work (1). The hair provided an underlying ground of white against which the local color differences stood forth brilliantly. Even the gentlest shaving before injection of the dye resulted in much disturbance of the local circulation, with not a few vascular areas closed off, and irregular staining.

Chicago blue 6B is a poorly diffusible dye (1), as evidenced *in vivo* by its tardy escape from the vessels and its slow secondary spread. It colors the mouse much faster than the rabbit, yet a considerable period elapses before the skin becomes evenly stained. In mice killed 6 to 10 minutes after injection the everted flap has a remarkable appearance (Fig. 1). It is everywhere stippled with small blue dots on a ground which is unstained, appearing white because of the hair beneath. The dots are 0.1 to 0.2 mm. across, 1/2 to 1.0 mm. apart, and in the midst of each ramifies a solitary, contorted, venular tree (Figs. 1, 3, and 4). Some of the corresponding arterioles still contain sufficient dye for the fact to be made out that they are interspersed between the venules with notable regularity, and that each passes to the center of an unstained area (Figs. 3 and 4). The colored dots are most widely separated in the neighborhood of the midline, where vessels in general are fewest and the staining least. Here also the muscle of the panniculus carnosus is practically non-existent (Fig. 6), whereas toward the sides it thickens, as does the connective tissue also, and some general staining is superimposed upon the dotting. In the thinned mice fat has practically disappeared from the skin, and, looking at its under side, one can make out the butts of the hairs, in short, irregular ranks of special whiteness (Fig. 3).

In animals allowed to live more than 10 minutes after the dye injection, some general staining of the corium takes place. This does not come about by an enlargement and coalescence of the colored dots. They remain discrete but are

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<sup>1</sup> Much more of the purified material now employed is required to make an isotonic solution than of that used in our previous studies.

gradually lost in an even coloration, the result of an escape of dye from the capillaries everywhere (Fig. 2).

*Conditions Localizing the Escape*

The spread of Chicago blue through the cutaneous tissue after death is so slow that differences in its local distribution can be scrutinized at leisure. Under the microscope one sees on the under surface of the skin, slightly to one side of the midline, a tracery of fine, roughly parallel blue lines,—the dye-containing capillaries supplying the scanty fibres of the panniculus carnosus (Fig. 5). Focussing shows that the perivenular spots of color lie beyond, in the corium. The arterioles supplying the latter pass almost vertically to it, and so also with the corresponding venules. Between the vessels of the two sorts only capillary channels can be made out in the corium, and these but occasionally since most are empty of the dye-stained blood. Cleared preparations from mice injected with India ink gelatin mass (Fig. 5) show the vascular arrangement excellently described by Kreyberg for the skin of the back (3). Separate arterial and venous networks beneath the panniculus connect with the capillaries of this muscle sheet and send off branches that pierce it to join other, finer-meshed, networks just superficial to it. From these in turn small, solitary arterioles and venules pass at almost regular intervals to the capillary network in the corium. The staining with Chicago blue is localized to the tissue in the neighborhood of these final venules. Hence the dotting with color seen in the gross.

The singular localization of the staining suggests the probability that there are special structures for which the dye has an affinity. But microscopic sections stained with eosin and methylene blue (or hematoxylin) have failed to disclose any such. The mammary gland lies beneath the panniculus (4) and can be ruled from account. The hair follicles fail to "take" the vital dyes, their arrangement has no relation to the dotting with color, and except for them the stratum composing the corium appears to have the same general texture everywhere in the region studied (Fig. 6). The subcutaneous fatty layer had practically disappeared in our thin animals; and it does not stain, nor is it so situated as to be responsible for the localization of color. Even in well nourished mice there is almost no fat in the corium itself

of the abdominal region. Furthermore the edges of the colored dots are ill defined, fading off gradually; and all dye can be forced out of the corium by gentle pressure, leaving it unstained,—facts both which indicate that it is dissolved in the tissue fluids. And finally, dotting of the same sort is produced with other dyes fitted by their rate of diffusibility to disclose it, namely, pontamine blue, trypan red, and brom phenol blue. True, the capillaries are so permeable to this last that it must be injected very rapidly and the corium examined practically at once if the dotting is to be perceived amidst an intensifying general coloration; while with trypan red the examination cannot be much deferred. But these differences are obviously dependent, like those in voluntary muscle (1), upon the degrees of diffusibility of the dye stuffs.

Can local vascular differences be responsible for the stippling of the corium with color? One such difference is very evident. The contorted, venular trees offer a far greater surface for escape of dye from the blood than exists elsewhere. But the dye is not narrowly localized next the venular wall nor does it extend therefrom secondarily to form the spots of color. These arise *in situ* and they extend considerably beyond the region in which the venular tree is situated. To explain their relatively large dimensions one must suppose some dye to have got out from the portion of the capillaries adjoining the venules. Injected specimens show that in the neighborhood of the latter the capillary web is not especially abundant, nor are the channels wider there (Fig. 5).

#### *The Escape into the Skin of the Ear*

It is impossible to watch directly the spread of dyes from the blood into the skin of the body of the living mouse. But the ear presents excellent opportunities for this.

The mouse ear offers great advantages for the study of vascular phenomena, yet it has been little utilized. Leonard Hill has made pressure experiments upon it (5); and Lehmann (6) states that he had been able to see trypanosomes in the blood passing through it. Fröhlich and Zak (7) give the only description of the arrangement of the vessels that we have been able to find; but, while adequate for their purposes, it does not provide the information necessary for ours.

Young mice of 14 to 20 gm. have more transparent ears than older ones, while in the latter, furthermore, ecchymoses of stain often develop, traceable to injuries

not easily recognized beforehand. A young animal of 20 gm. injected into a tail vein with 0.08 to 0.1 cc. of half strength Chicago blue solution during the course of a minute, manifests no symptoms, but the ear becomes pale azure, with thread-like dark vessels sharply outlined under the microscope (Fig. 9). They stand forth against a background as of shimmering ground glass (the underlying fat). After 2 to 3 minutes a patchy coloration begins of the tissue about some of the vessels, and within 5 minutes this has become marked (Fig. 10). With the binocular microscope one can see that the dye has passed out into the tissue traversed by the further portion of the capillary web and the smallest collecting venules, while no staining whatever has occurred as yet in the region occupied by the proximal part of the web and the arteriolar ramifications.

Microscopic observations are best made under ether or luminal, with the mouse so arranged on its belly in a shallow trough of plasticine that the ear stands free and vessels leading to it are not pressed upon. The hair need not be removed, but the skin should be oiled with neutral paraffin oil. Under the ear at a distance of 2 or 3 mm. is fixed an ovoid or triangular plate of opaque, white glass on a dais of plasticine, and the light of the arc is directed obliquely to illumine both glass and ear. In the combination of transmitted and reflected light thus provided the spread of dyes from the blood can be readily followed. For photographic purposes the ear is momentarily flattened in oil between the white plate and a coverslip (Figs. 9 and 10). Under such circumstances, the passage of individual red cells along the capillaries is easily discerned. But even the slight pressure of the coverslip alters the rate of staining and should be avoided.

After an injection of Chicago blue the vessels do not dilate, but the stained blood gives them almost the sharpness of a woodcut. The larger ones, veins and arteries together, radiate in a fan on the upper side of the ear, breaking up near its margin into a multitude of fine branches so interlaced that here it is often difficult to determine the type of vessel from which the dye first escapes. Between the sticks of the fan, though, the situation is better defined. One sees solitary arterioles and venules (Figs. 9 and 10) with only capillaries in the tissue between, indistinct faint blue lines which bend and loop. The posterior quadrant of the ear yields the clearest pictures.

The first escape of dye, occurring 2 to 3 minutes after the injection, stains the tissue lying immediately about the least venules; but almost as it does so the adjacent tissue turns blue too, so that an irregular colored strip is formed, with fading margins and the venule at its center. There can be no doubt, however, that extravascular dye is first perceptible in the tissue immediately next the venules; but it undergoes no evident lateral spread. After 15 minutes the strips are much more intensely blue than at first and definitely larger. In the gross the ear appears brilliantly patched with blue. Each patch or

strip can be seen with the microscope to be separated from the nearest dye-containing arteriole by a region of unstained tissue, supplied by capillaries only, which is often nearly 1 mm. across but averages about 1/2 mm. (Fig. 10). Such regions become diffusely stained later, but the strips of intense blue are often discernible for more than an hour amidst the deepening color.

#### *Structure of the Ear*

The precise situation of the dye can be readily determined. Stained cross-sections show that the ear has a central lamella of cartilage with a fatty layer to either side, overlain by corium and epidermis (Figs. 7 and 8). Amidst the fat on the outer surface are irregular aggregates of short voluntary muscle fibres. These form an almost continuous flaring layer near the base of the ear but further out are scattered, and in the region we have studied, near the margin, are wholly lacking. The fan of large vessels lies on the outer side just under the corium. If a patchily stained ear is cut off, seized at its base with forceps and pulled apart, it separates into two layers, the outer, thinner one consisting only of corium and epidermis with some scattered, easily recognizable fat cells and the fan of vessels. The relation of the patchy staining to these last remains unchanged, and one perceives that the coloration is limited to the corium. Or the intact ear can be cut across with a sharp razor and inspected microscopically, edge on, in paraffin oil. One sees then that the fatty tissue and epidermis have not stained, and the cartilage only where it is thickest, and there lightly and evenly. The blue patches lie entirely in the corium. Hair follicles are equally numerous in the patched and unstained regions. They have not colored. Staining is least pronounced near the margin of the ear, because here the corium is thin and the follicles numerous.

The fatty layer on the inner (under) side of the cartilaginous lamella is shallow and incomplete, and the corium appears thick in comparison with it (Figs. 7 and 8). Both are supplied by derivatives from the fan vessels which pierce the cartilage vertically here and there to ramify and form wide meshed networks beneath it. When the ear of the living animal is viewed on its inner side, arteries and veins are seen to emerge together through a brilliantly translucent partition

as of minute glass beads (the cartilage), and to branch like spider angiomas or contorted dendrites. Here again a blue patching can be seen to develop after the injection of Chicago blue, and one observes that it is localized to the tissue in the neighborhood of the venules.

The ears of mice injected with a gelatin mass containing India ink show far more vessels than Chicago blue renders visible (Figs. 11 and 12). Excellent preparations can be obtained when the aorta and vena cava are clamped off above the diaphragm just before the vessels of the head are washed out and injected by way of a cannula passed up into the aorta through the left ventricle. Not only is each large vein in the fan-work accompanied by an artery, but so too with most of the secondary and even tertiary branchings. Between the sticks of the fan, however, are some regions supplied and drained by solitary vessels; but such regions are far fewer and smaller than one would expect from the study of living animals with dye-stained blood. When the ears of these are closely scrutinized, one finds many regions in which there is no blood flow, the red cells being stationary where present at all. The smaller venules from such regions are devoid of dye. Evidently circulation is not taking place through the entire vascular bed but is much restricted, as is normally the case in the ears of the dog, cat, and rabbit (8). If an India ink suspension is rapidly thrown into the blood and its advancing dark columns are followed, one sees that they go, not from the arterioles to the nearest venules, but across gaps about 0.5 mm. wide bridged only by capillaries.

No arteriovenous anastomoses such as exist in some other species (9) have been detected anywhere in mouse ears cleared after injection with gelatin mass containing India ink; nor does dye or ink injection during life disclose the presence of any, even when the distribution of these substances has been slowed by pressure on the vessels at the base of the ear. Such anastomoses, if they exist, play no part in the phenomena with which this paper is concerned.

As already stated, the main vessels of the ear are situated on its outer side. In proportion as they branch they tend to become more superficial, and the smaller ones, which supply the corium with capillaries, lie next this layer or within it (Figs. 7 and 8). Capillaries are given off also to the fat, but with these we are not concerned since the adipose tissue shows no color; and there are deep ones supplying the voluntary muscle. The muscle capillaries are easily recognizable as grids of crowded, minute, parallel vessels (Fig. 11). They are definitely less permeable to dyes than the capillaries of the corium, but are so close set and numerous that the stained blood within them causes darkenings near the middle of the ear (Figs. 13 and 14). The anterior third of the organ is thicker than the rest, more hairy, and possesses in the corium a meshwork of large, very permeable capillaries. This region we have not studied.

In vitally stained mice the arterioles can be traced to their tips because they lie in tissue that for long is unstained. Their lumen is reduced to even less than capil-

lary size before they break up. The open capillaries are from  $3\mu$  to  $10\mu$  in diameter, averaging  $6\mu$ , the first venules 6 to  $18\mu$ , the next larger  $37\mu$  as an average, the branches into which they give 60 to  $75\mu$ , the primary branches 75 to  $150\mu$ , and the large, fan veins 100 to  $175\mu$ . Even the largest veins are notably thin-walled, and those from which staining occurs show only a single layer of endothelial cells, in ordinary cross-sections.

The capillaries of the corium are no more numerous near the venules than elsewhere, and they first undergo enlargement when entering these vessels, as can be well seen where they enter at right angles. Where the venule continues the direction of the capillary, it is impossible to say precisely where one begins and the other leaves off.

As this description brings out, the vascularization of the corium of the ear differs from that of the body only to the extent necessitated by the shape of the organ. The vessels of the ear lie much more nearly in one plane because of the general flattening. The arterioles and venules do not rise almost vertically toward the surface and branch to all sides but ramify in the direction of the margin of the ear. Hence, as vital staining takes place with Chicago blue, one sees strips of color form, not rounded dots such as appear in the skin of the body. But the difference is merely superficial. The localized staining is traceable in both cases to a special escape of dye from the blood in the region of the venules and the further portion of the capillaries. The same holds true with other dyes, pontamine blue, trypan red, trypan blue, brom phenol blue (Fig. 13), and even the very highly diffusible patent blue V (Fig. 14). All were injected in isotonic solution (1). The variety of the dyes, and the ease with which all color can be forced from the stained tissue by general pressure rule out a fixation upon the structures of the ear.

#### *The Gradient of Vascular Permeability*

The venular trees are broader and more contorted in the ear than in the skin of the abdomen, sometimes actually corkscrewed, and their numerous, fine branches are like widened capillaries. They provide a relatively great expanse of wall through which escape can take place from the blood. That the local conditions are especially favorable to this is shown by the course of the staining, as already described. Pontamine blue escapes from only the smallest of the venules, but Chicago blue, which is more diffusible, passes through the



walls of the veins of next larger size as well, and trypan red, brom phenol blue, and patent blue V get out of veins of still greater magnitude.

Can the patching with dye be explained entirely by an escape from the venules, with secondary distribution through the tissue? The differing course of events with dyes of graded diffusibility provides the answer to this question.

Pontamine sky blue, the most indiffusible of the dyes mentioned, passes out of the blood more slowly than Chicago blue, the patching is more closely localized next the venular trees, and the generalized staining in which the patches eventually are lost takes longer to develop. The dye should be injected in an isotonic half strength solution (a 10.8 per cent watery solution mixed with an equal quantity of Locke's solution (10)), 0.1 cc. for a 20 gm. mouse. The relatively diffusible brom phenol blue (1) must be put into circulation quickly if sufficient is to reach the venules for local differences in the staining of the corium to be perceptible. When given gradually so much passes out from the capillaries that no intenser staining can take place further on from the depleted blood. When quickly injected (0.15 cc. of a 4 per cent watery solution for a 20 gm. mouse) some pale, general staining of the ear appears within a minute, and superimposed upon this a deeper patching round about the venular trees (Fig. 13). The patches are broader than with Chicago blue or pontamine blue, and, as in the case of these dyes, they develop *in situ*, not by lateral spread from the venules. In comparison with them the tissue occupied by the proximal capillary meshes and arterioles appears almost unstained. After less than five minutes the coloration has become uniform throughout the ear. When the very highly diffusible patent blue V (0.15 cc. of an 8 per cent solution for 20 gm. of mouse) has been thrown abruptly into the blood the ear rapidly becomes blue everywhere; but patches of intenser color develop with venular trees in their midst, despite the fact that the plasma has lost much of its dye along the capillary way (Fig. 14). After about 2 minutes the patching is lost in a general staining.

The changes are slower in etherized animals. Tests have shown that the differences with the dyes are not referable to differences in dosage.

The patching caused by the highly diffusible dyes cannot be the result of lateral spread from the venules, since it develops *in situ* and very rapidly. It is soon lost in a general staining. The capillaries are evidently permeable to such dyes throughout their length, but most permeable in their further portion.

Several facts speak for a permeability of the venule wall exceeding that anywhere along the capillary, and for an increasing permeability of the wall of the latter as the vein is neared. When pontamine blue has been introduced into the blood the tissue supplied from the proximal part of the capillary network remains unstained for about 15 minutes after a brilliant blue patching has appeared in the venular region. With Chicago blue the period is less; while with the other dyes a general staining develops within 2 or 3 minutes at most, the time varying with their diffusibility. In the case of poorly diffusible dyes (pontamine blue, Chicago blue) the local differences mentioned persist far too long to be explicable on the basis of relative amounts of vessel wall through which dye can escape; and with the highly diffusible patent blue V this factor would seem to be more than counterbalanced by a great loss of the pigment from the blood as it flows along the capillary way, a loss plainly evident in the relatively light hue of the contents of the venules,—from which vessels, nevertheless, escape is most abundant (Fig. 14). The fact has already been mentioned that the capillaries, while exhibiting great individual variation in calibre, do not increase in width on the way to the venule. Nevertheless with a dye of medium diffusibility (brom phenol blue) a graded increase in tissue staining takes place along the greater part of this way. With less diffusible dyes a similar gradation is limited to the distal portion of the meshwork; while with the very highly diffusible patent blue V the rapidly diminishing concentration of the dye as the blood flows along the capillary sufficiently accounts for the lack of a graded staining save near the venules where the opportunity for passage into the tissues is especially good. The even staining with all the dyes, that succeeds upon the patching, is sufficient evidence that there is plenty of stainable corium everywhere.

In the course of work to be reported later with Dr. McMaster and Dr. Hudack the circulation to the mouse ear was temporarily cut off without damage by compressing the large vessels at its base between

a glass plate and a narrow, sausage-shaped, collodion bag. After this had been done, Chicago blue was injected, a minute or two allowed for it to distribute itself in the blood, and then the circulation was permitted to reenter the ear. Vascular relaxation had occurred as the result of anoxemia and the entire network filled at once with dark blue blood. Immediately that this happened all flow was cut off again. And now in the course of a few minutes a light staining took place with the usual patchy distribution. One could see that the plasma in the venules lost color first,—despite the relatively unfavorable ratio of wall area to vessel content,—with decolorization in the adjoining capillary region a little after. The blood in the proximal part of the capillary meshwork and in the arterioles,—which did not contract,—was still dark blue.

It seems certain from these highly various observations that the opportunity for escape from the blood increases considerably along the capillaries but is greatest in the venular region.

Most of the substances carried by the blood are more diffusible than patent blue V, the best in this respect of the dyes used (1); but the serum proteins are considerably less diffusible than pontamine blue, which stands at the other end of our series. Yet serum proteins normally pass from the blood in no inconsiderable quantity, and are found to the amount of 1 to 2 per cent in the lymph from an extremity (11). Dr. Heidelberger has kindly provided us with two colored azo-albumins, a red compound (12) and a green one, for utilization in tests of the permeability of the cutaneous vessels. The green azo compound caused vascular injury, as shown by ecchymotic staining; but the red one produced no symptoms nor evident lesions, even when concentrated to a 9 per cent solution and injected to the amount of 0.2 cc. The ear of the injected animal became gradually and evenly pink in the course of the succeeding half-hour. Since all free dye had not been removed from the preparation, and there had been abundant time for a secondary distribution of the coloring matter, the finding was uncertain in its implications. For this reason Chicago blue 6B was linked with egg albumen in our laboratory, the last trace of free dye removed, and concentration effected in isotonic saline by differential filtration under pressure through a collodion membrane permeable to the dye but not to its combination with protein. The mate-

rial thus procured was far inferior in tinctorial value to Chicago blue and yielded only an equivocal extravascular coloration.

#### DISCUSSION

In voluntary muscle and in frog skin the arrangement of the vessels is so orderly that local differences in the opportunity for dyes to escape along them find almost diagrammatic expression in colored patterns. In mammalian skin the vascularization is far less regular yet the findings prove that the opportunity for dyes to pass into the tissues increases along the further part of the capillaries and is greatest in the region of the venules. Here poorly diffusible substances mostly escape and the passage outwards of rapidly diffusible ones takes place most readily. That intrinsic local differences in the permeability of the vascular endothelium are responsible for the gradient of vascular permeability cannot immediately be concluded, however; for the fabric just outside may conceivably act as a reinforcing wall, local variations in it conditioning exchange. This possibility has been excluded for the ear vessels by work to be reported later.

That exchange between blood and tissues may take place in part through the walls of venules has long been inferred on anatomical grounds. As far back as 1896 (13) Starling wrote that "the production of tissue fluid is limited to the region of the capillaries and small venules." Krogh has pointed out that in human skin there are practically no capillaries except those in the papillae (8), and he infers that exchange with the tissues must take place mainly through the walls of the venules which he aptly terms "giant capillaries." Lewis (14) believes that all of the small vessels are implicated in this activity and Kreyberg (3) has stated a similar view as concerns the vessels of the skin of the mouse. The significance of our venular findings lies in the evidence they afford that the opportunity for substances to escape from the blood into the tissues is not merely as good in the venules as in the capillaries but far better. In man the cutaneous venules are developed at the expense of the capillaries (15), while even in the mouse their shape, broad and contorted, is manifestly unnecessary to the mere collection of blood. The inference seem justified that the venules are differentiated for special purposes; and the known functions of the skin provide a clue to these. They demand not only

an abundant vascularization, with a highly complex controlling mechanism, as Lewis has pointed out (14), but conditions which can be provided only by broad, thin-walled vessels with a slow current.

The skin is a protective organ continually subject to slight traumata and to local infections. Cohnheim (16) noted that leukocytes get out much sooner and easier from venules than from capillaries; and Tannenbergs (17), extending the observation, has reported that when these cells become attached to the capillary wall, as a first step toward emigration, they are buffeted by the swift current and frequently dislodged and whirled away. This does not happen in the venules, which provide ideal conditions for emigration.

Under ordinary conditions, with many capillaries closed down, there is still a current in the venules, and here the opportunity for exchange with the tissues cannot but be best. The view has been advanced that the rapid current of capillaries is especially favorable to gas exchange with the tissues, and the slow stream of the venules to that of less diffusible substances (18). However this may be, there is no doubt that the latter, when circulating transiently like our dyes, will get out in greatest proportion, other things being equal, where the current is slow. Whether circulating antibodies and the blood proteins reaching the lymph pass out preponderantly through the walls of the venules, present evidence does not enable us to say. Doubtless many factors condition their escape besides the immediate permeability of the vascular tube.

In man the skin of the body acts importantly to regulate temperature, as it does not in furred animals. Lewis has pointed out the obvious rôle of the broad, superficial venous plexuses in this relation. And in mammals generally the skin is one of the main depots of reserve water. There are important occasions, notably after hemorrhage, when water must be mobilized rapidly for circulatory purposes. The broad, highly permeable venular channels provide opportunities for this. In a preceding paper we have described vascular arrangements in the mammalian diaphragm and the pectoral muscle of the pigeon which are obviously adapted to the elimination of waste materials from these actively functioning organs (10).

It may be asked, why skin capillaries exist at all if venules do so well? The question has special pertinence in the case of human skin with its

great development of venules. But some pressure-regulating mechanism there must be; and the capillaries are known to share with the arteries in this function. They withstand pressure as the thin-walled venules cannot, because of their wide lumen. Diapedesis under pressure occurs first from the venules (16), and ecchymosis from these vessels is far more frequent than is generally recognized (19). Under circumstances of venous obstruction Tannenberg observed diapedesis taking place from the venules, then from the venous end of the capillaries, and only later from the arterial portion of these latter, despite the fact that through them the pressure was transmitted that was responsible for the lesions (17).

In preceding communications we have shown that in some regions an effective exchange with the tissues takes place through the small arterioles and venules, capillaries being few or absent where these run (1). From the point of view of exchange with the tissues no reason exists for a tripartite classification of the vessels of such regions. But in the skin the venules are not merely enlarged capillaries, functioning like them. Besides serving for exchange with the tissues and for the collection of blood they have other important purposes.

#### SUMMARY

The permeability of the venules of the skin of the mouse greatly exceeds that of the capillaries. A mounting gradient of permeability exists along the further portion of the latter.

The significance of these facts is discussed with relation to conditions in human skin. The cutaneous venules are differentiated for several functions besides those ordinarily attributed to them, and must be considered as specialized organs.

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## EXPLANATION OF PLATES

## PLATE 50

FIG. 1. Flap of the abdominal skin of a thin mouse, everted 6½ minutes after an injection of Chicago blue,—to show the dotting with color. The staining is palest along the midline. The unstained rectangular area near the upper right hand corner is the navel. × 8.

FIG. 2. A similar flap everted 45 minutes after the dye injection,—to show the generalized staining. Some deeper dots of color can still be made out. × 8.

FIG. 3. A part of the preparation used for Fig. 1, more highly magnified. The stain can be seen to lie in the tissue round about the venules. Here and there the arterial network can be perceived (A, A) but the arterioles are invisible. The interrupted white lines are ranks of hair follicles. × 17.

FIG. 4. Under side of the abdominal skin of a mouse killed 7½ minutes after the dye injection. The venules are completely hidden in stained tissue. Enough dye remains in some of the arterioles (indicated by arrows) for their situation in unstained tissue to be made out. The contrast has been emphasized with a color filter. × 17.

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FIG. 5. Skin of the abdomen of a mouse viewed from beneath. Specimen cleared after injection with an ink-gelatin mass. For comparison with Figs. 1, 3, and 4. From the venous and arterial networks (V and A) branches go off at intervals to the corium. Capillaries are not especially abundant in the neighborhood of the venules. × 75.

FIG. 6. Section of the everted skin of a thin mouse, from the abdomen. The end, M, was near the midline. The specimen consists almost entirely of corium. There are no recognizable fat cells, and the panniculus carnosus, which is shallow, thins in the direction of the midline. Several hair follicles have been cut through. Eosin and methylene blue. × 180.

FIG. 7. Section of the ear of a mouse near its margin. The upper side of the organ is distinguishable by the layer of fatty cells overlying the cartilaginous lamella. The gap to either side of the cartilage is an artefact. Hematoxylin and eosin.  $\times 180$ .

FIG. 8. A similar specimen with ink-gelatin mass in the vessels. Hematoxylin and picric acid.  $\times 180$ .

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FIG. 9. Ear of a living mouse photographed in oil 30 seconds after an intravenous injection of Chicago blue. As yet no dye has passed out into the tissues. The arteries, thin and straight, are readily distinguishable. Some of those which course separately are indicated by arrows.  $\times 13$ .

FIG. 10. The same ear photographed 5 minutes later. The solitary arteries can still be readily discerned (arrows). They pass to tissue which is unstained, whereas that in the neighborhood of the venules is already well colored. The dye has not escaped from the larger veins, however.  $\times 13$ .

FIG. 11. A relatively high magnification of part of an ear cleared after injection with an ink-containing mass. Almost every artery is now seen to be accompanied by a vein. Several grids of the fine capillaries which supply muscle fibres can be made out near the lower border of the photograph (arrows).  $\times 32$ .

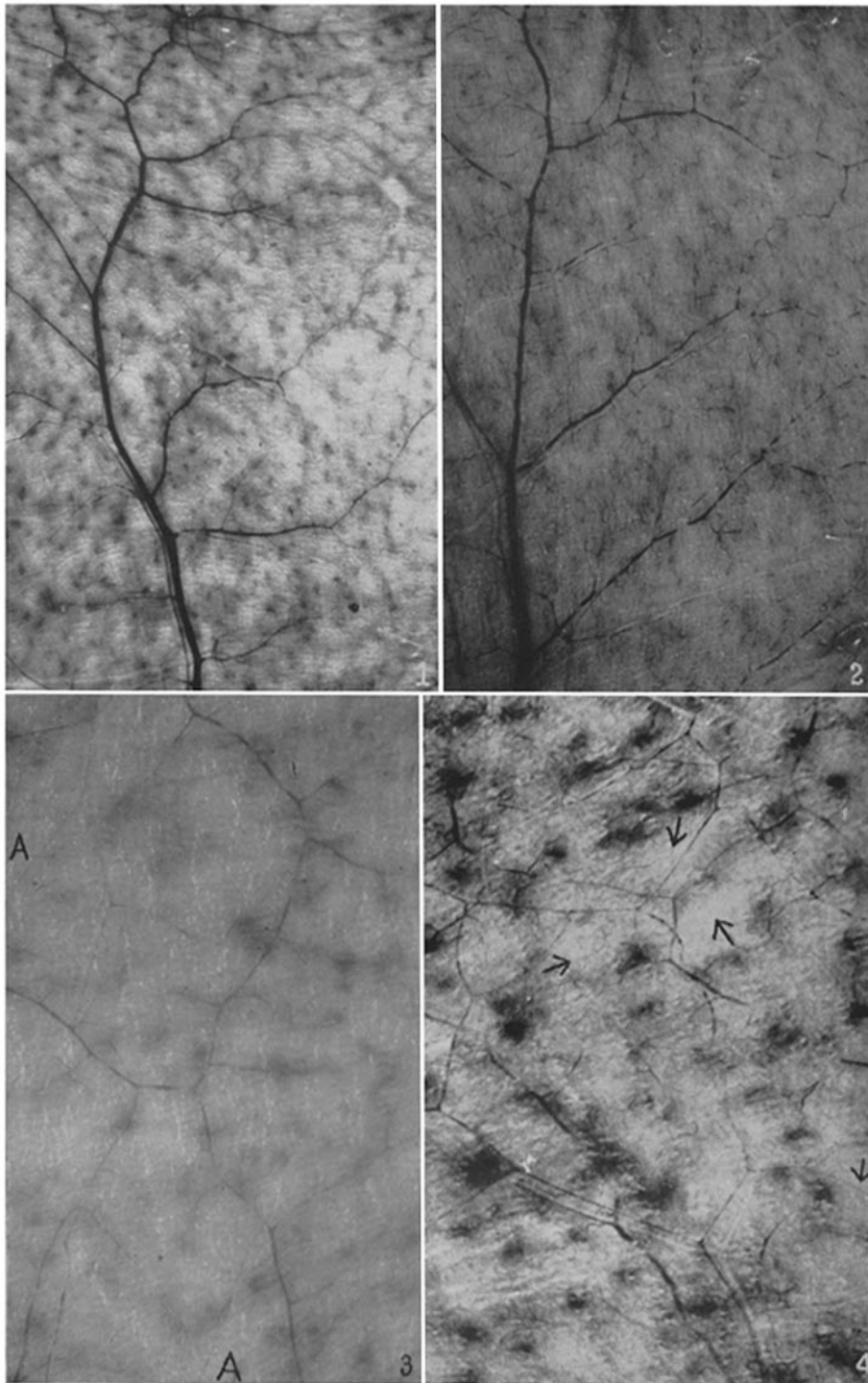
## PLATE 53

FIG. 12. Mouse ear injected with an ink mass and just sufficiently cleared for the vessels of its outer side to be plainly visible. For comparison with the photographs from animals receiving vital dyes. The vascularization is much more abundant than one would suppose from the study of these latter.  $\times 12$ .

FIG. 13. Ear of a living mouse photographed 2 minutes after an injection of brom phenol blue. Some general staining has already occurred. It is least marked in the regions to which the solitary arterioles run (arrows). There are many broad patches of color with venules in their midst.  $\times 13$ .

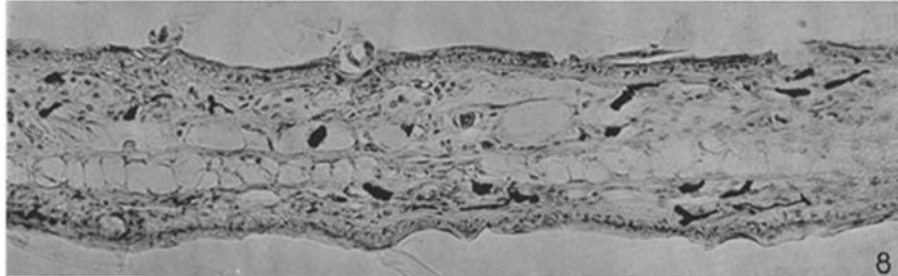
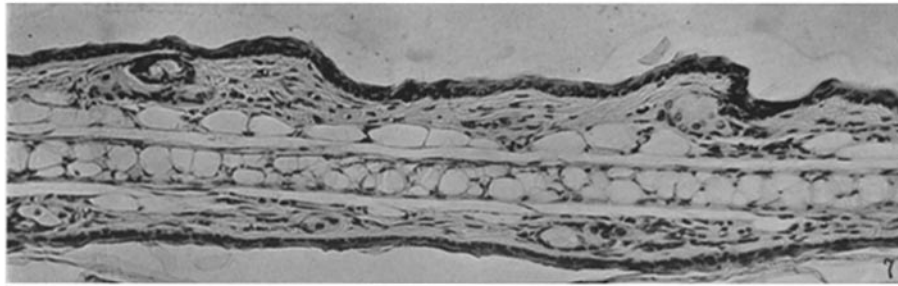
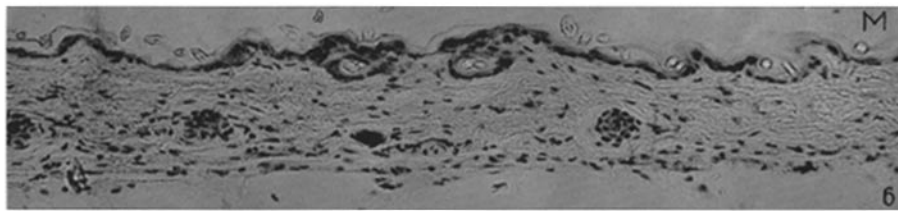
FIG. 14. Ear of a living mouse  $3/4$  minute after injection of patent blue V. Some general staining has already taken place, which is not evident in the photograph. The blood of the venules contains so little dye that their course is difficult to make out. The solitary arteries on the other hand are clearly visible (arrows) by reason of their dye-laden contents; yet in the regions to which they run the staining is at a minimum.  $\times 13$ .





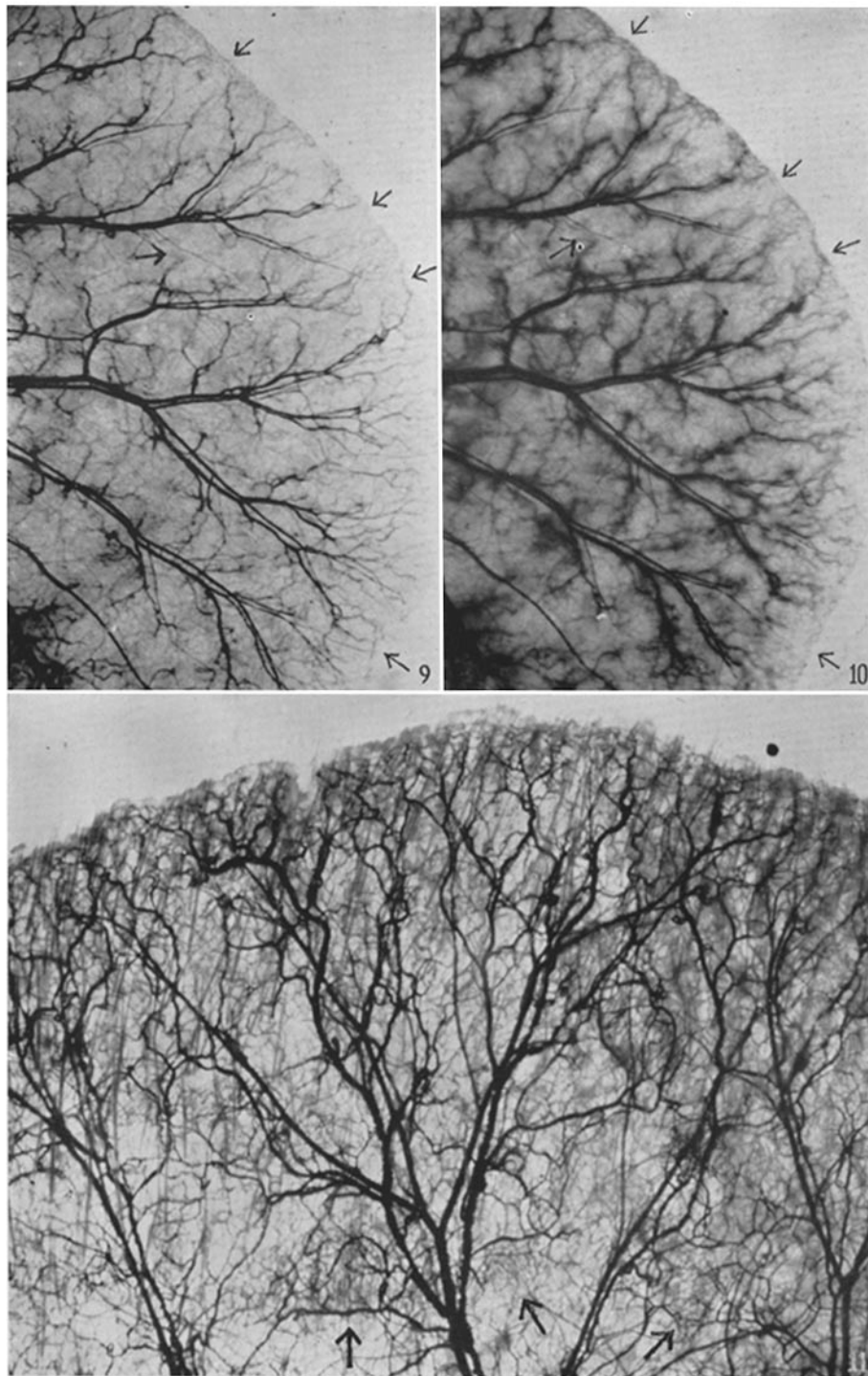
Photographed by Louis Schmidt

(Smith and Rous: Gradient of vascular permeability. IV)



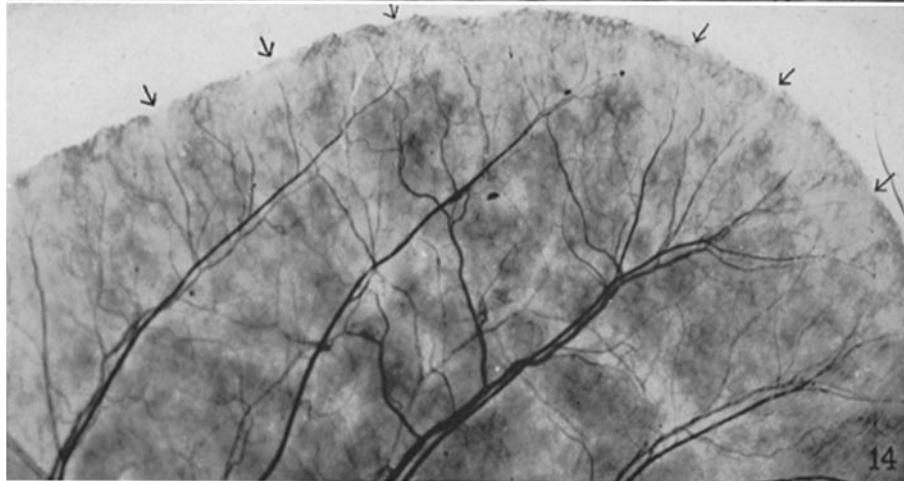
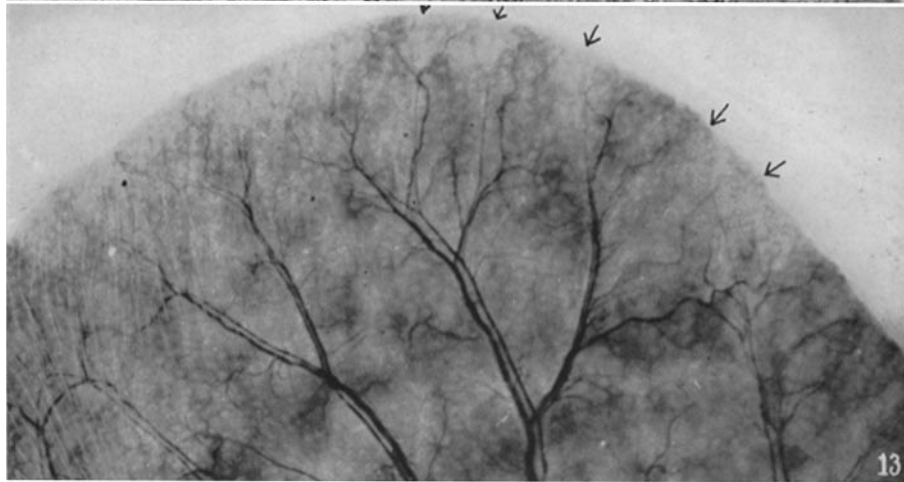
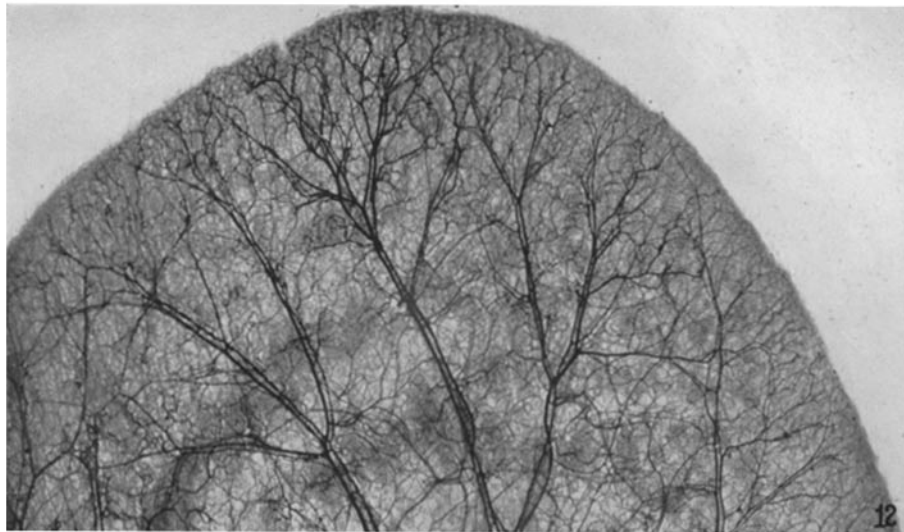
Photographed by Louis Schmidt

(Smith and Rous: Gradient of vascular permeability. IV)



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