

THE RELATION OF HYDROSTATIC PRESSURE TO THE GRADIENT OF CAPILLARY PERMEABILITY

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Despite the recognition in recent years of various physical and chemical factors which may conceivably influence exchange through the walls of the blood vessels, the view prevails that this is governed by diffusion, hydrostatic pressure, and the osmotic pressure of the blood colloids acting along capillaries which are equally permeable throughout their length. The demonstration that the escape of vital dyes from the blood into the tissues increases progressively along the capillaries of certain organs and is greatest precisely where it should be least if the factors just mentioned alone determined the phenomena (1) indicates either that some influence is at work which transcends these factors in importance, or that their rôle has not been wholly comprehended. That diffusibility directly conditions the distribution of dyes from the blood has been recognized since the work of Schulemann (2). The experiments here reported were undertaken to throw light on the influence of hydrostatic pressure. Our method has been, in brief, to modify this pressure markedly, inject a vital dye devoid of complicating affinities, and determine whether the changed conditions have altered the gradient of distribution from the capillaries.

General Technique

The ear of the mouse was used for some tests, but most were made upon the voluntary muscles of rabbits, because the arrangement of muscle capillaries is so regular that the local variations in opportunity for dyes to escape from the blood find expression in an almost diagrammatic color pattern. The dyes had been purified and tested for toxicity and complicating affinities (3). All were injected intrave-

nously in isotonic solution, as a rule in the amounts already found to be optimal for disclosure of the gradient of permeability. The escape from the blood was in some cases watched *in vivo* through mica windows let into the skin, and in others the tissue was examined immediately after the anesthetized animal had been exsanguinated from the carotids. Postmortem spread through the tissues is slow with poorly diffusible dyes like Chicago blue and pontamine sky blue, which were mainly employed. The technique of inspection has been described in previous articles.

Exclusion of the Factor of Specific Affinities

The vital dyes with which the work was done (Chicago blue 6B, pontamine sky blue, and brom phenol blue) are not fixed upon the tissues at once after they escape from the blood,—though later a portion of the two first mentioned is taken up and stored by some of the cells,—but they stain it because present in the interstitial fluid. By forcing this out the tissue can be decolorized. It follows that in determining the influence of hydrostatic pressure upon the escape of the dyes from the blood we are ascertaining how it affects the passage of the pigment from one fluid to another through the capillary membrane, that is to say are really dealing with capillary permeability.

Anesthetized rabbits, guinea pigs, and mice were injected with the dyes, and as soon as staining had become well marked the effect of pressure to drive out the color was tested. The ear of the mouse was frequently used. It was lopped off with scissors at the appropriate time and spread in oil between parallel glass plates, with a flexible sausage-shaped collodion bag about 2 mm. in diameter immediately under it. When the bag was filled with water from a manometer the narrow strip of tissue between it and the upper plate was pressed upon. In other instances the ear was placed between glass slides with a narrow strip of rubber underlying part of it, and the strip was repeatedly pressed against the ear, with some slight shifting, the object being to avoid any collection of dye into pockets in the compromised tissue. Considerable pressures could be exerted without deforming the ear.

The stained muscle (external oblique, gracilis of rabbits and guinea pigs) was excised as a sheet and spread and pressed upon in the same way, but with a bag about 6 mm. in diameter. Only slight pressures could be exerted since considerable ones squeezed the tissue thin, yielding a fallacious appearance of decolorization.

Both in the ear and in muscle the tissue pressed upon lost its color within a few minutes (Figs. 1 and 4). The dye-containing fluid that was forced out often formed

a more or less brilliant border to the decolorized strip. Chicago blue 6B, pontamine sky blue, brom phenol blue, trypan blue, red, and violet all yielded the same results. Since our object was to determine whether fixation upon the tissues acted to complicate the markings indicative of the gradient of vascular permeability, pressure was usually, though not always, brought to bear before these markings were obscured by diffuse coloration. Pressure regularly had the effect of obliterating them.

Effects of Nerve Section

It was necessary in many of the experiments to reduce the hydrostatic pressure greatly. The fact was already known that when this is done by bleeding, the circulation to the viscera is maintained at the expense of that to the superficial tissues and voluntary muscles (4). If the depletion has been great, the blood flows through but a small proportion of the muscle vessels, and after dye injection its advancing, stained columns can be watched as they appear in the arteries, fork in the arterioles, and creep slowly along the capillaries. Despite the low blood pressure, the tissue coloration that gradually takes place has the pattern indicative of an unchanged gradient of vascular permeability, that is to say, the amount of dye escaping from the blood, as usual increases progressively along the capillary way (5). But the possibility suggests itself that this may happen, not because of local differences in permeability but because of a tonic contraction of patent capillaries, greatest in their arterial portion and presumably affecting both the amount of wall through which dye can pass and the local permeability. A less marked contraction of the same sort would account for the gradient of escape that is encountered normally. To test for the presence of such a state of affairs the muscle nerves were cut to relax the vessels, and the distribution of dyes thereafter was ascertained. A leg was used, with its fellow as control.

To effect a wide-spread paralysis of the muscles of the hind leg of the rabbit it is necessary to cut the obturator and femoral nerves within the abdomen, and the sciatic high in the haunch. This was done under ether, with a minimum of trauma. Immediately afterwards a pronounced dilatation of the vessels could be discerned in the muscles studied,—gracilis, tibialis anticus, adductor longus,—a change especially noticeable on comparison with the controls. Sometimes dye was injected into the blood stream at once, and, in other instances, from 4 to 24 hours after the nerve section, with renewed etherization. The legs were placed symmetrically and sometimes mica windows were inserted in the skin over the gracilis muscle. The carotid pressure was followed with a kymograph.

Staining of the paralyzed muscles took place far more rapidly than that of the controls and soon became general. For example, 10 minutes after injecting the ordinary amount of half strength Chicago blue (3 cc. of 8.5 per cent solution per kilo rabbit in 5 minutes), the muscles with cut nerves were deeply and evenly stained, whereas the controls showed the alternation of transverse blue and unstained bars ("mackerel sky" barring) characteristic of the gradient of permeability in long muscles. However, when the dye was injected very quickly and the animal was killed within 2 to 3 minutes the paralyzed muscle also showed these markings; but the blue bars were more intense and much broader than in the control tissue, a brilliant patterning. There was in addition a light general coloration that the control entirely lacked.

In some previous tests by one of us, carried out in the course of work with Dr. Gilding, highly diffusible dyes (patent blue V, brom phenol blue) were injected after nerve section; and a generalized staining of the paralyzed muscles was found at a time when the controls showed only a "mackerel sky" (6). This proved true even when blood bulk and pressure had been greatly reduced by bleeding. A repetition now with the same dyes, injected very quickly with immediate sacrifice of the animal, yielded a characteristic barring of the paralyzed muscles, the control tissue showing almost no color. This evidence of a persisting gradient of permeability had escaped attention before because it had been looked for too late, being already lost in a generalized staining.

The experiments make plain the fact that the gradient of capillary permeability is not the result of local contraction along the capillaries. It is true that when these vessels have been relaxed by nerve section dyes escape from them more rapidly than usual and the effective influence of the gradient of permeability extends over a larger proportion of the capillary; but the distribution is identical with that in normal muscle when a more diffusible dye has been employed, or a greater concentration of the one in present use. Increased blood flow past an increased amount of vascular wall,—which has doubtless undergone some thinning as result of the dilation,—will suffice to explain the findings.

Effects of Nerve Section and Reduced Hydrostatic Pressure

The next experiments were directed to reducing the hydrostatic pressure in paralyzed muscle to the minimum compatible with flow. Something had already been learnt on this point. For the rapid injection into animals with cut nerves of Chicago blue 6B had caused the pressure to fall to about half the normal height, yet the characteristic

banding developed. The tests now to be described were more searching.

Under ether anesthesia the spinal cord of rabbits was cut in the upper lumbar region by a median laparotomy, or else between the third and fourth, or fourth and fifth, thoracic vertebra by way of the back. The operation had the double effect of greatly reducing the systemic blood pressure and of paralyzing many muscles. The best results for our purpose were obtained by a high transection, for then the abdominal muscles,—which are especially suited to observations on the gradient of permeability,—were paralyzed more than half way from the pelvis to the costal margin, a fact rendered evident, with the animal on its back, by a bulging above the pelvis and a relative constriction near the ribs where muscle tonus still existed.

To reduce the flow of blood to a minimum somewhere within the paralyzed abdominal muscle, the aorta was tied about 2 cm. below the left renal vessels. The median incision was closed with special care to prevent traction. Very soon after the ligation the skin of the lower part of the abdomen became pallid and cold, whereas in the region of paralysis further toward the ribs it was hyperemic owing to the vascular dilatation consequent on the cord transection (7).

Chicago blue was now injected, with the animal on its back, and a few minutes later it was bled to death from the carotids. Staining of the skin of the upper abdomen had already developed, extending about midway to the groins. The external oblique muscle proved to be completely unstained in the groin region, that is to say in the lower part of its paralyzed portion; and no circulation could have existed here for the residual blood was not stained in the least. Further toward the ribs the arteries showed dye-stained blood, but not the veins; a little further on both contained it, and there was a definite, if pale, extravascular coloration having the fern pattern referable to the gradient in short muscles (8); while beyond, in the region where the aortic ligation had not interfered with circulation, this pattern was pronounced. Here an unusual amount of blood remained in the small vessels. The zone of transition from unstained to deeply stained tissue was only about 1 cm. wide (Figs. 2 and 3).

After low cord transection and aortic ligation, a feeble circulation sometimes took place through the paralyzed gracilis, this muscle staining very slowly and irregularly. But where it stained at all, no matter how palely, "mackerel sky" bands, attesting to the persistence of the gradient of permeability, could be perceived.

In the animals of these experiments, subjected to cord transection and aortic ligation, dye escaped through the capillary wall into the tissue wherever the blood pressure was sufficient to move the blood along the relaxed vessels of the paralyzed muscle. The staining that developed in such regions showed that the gradient of capillary permeability had undergone no alteration as result of the muscle paralysis

and the minimal blood pressure (Figs. 2 and 3). It cannot immediately be concluded, however, that the gradient of permeability is independent of hydrostatic conditions. The venous system was intact; and pressure in the large veins is known to be almost independent of that in the arteries (9). The gradual increase in staining that took place along the capillaries as the venules were neared, though resembling that due to the ordinary gradient of capillary permeability, might conceivably have resulted from the influence of a venous pressure of normal height in association with an arterial pressure which had been diminished to the limit compatible with flow. In work to be published separately we have found that a slight increase in venous pressure over the normal emphasizes the gradient of permeability along the cutaneous vessels, even more dye than ordinary getting out from the further part of the capillaries and from the venules,—which in the skin have ordinarily a permeability transcending that of the capillaries. Chicago blue does not escape from the venules of muscle under ordinary conditions, nor did it in the experiments just described, as could plainly be seen when a sheet of the external oblique was dissected away and studied under the binocular microscope in the usual combination of transmitted and reflected light (10). But under the circumstances of increased venous pressure an escape does take place, as will be shown further on.

To test the possibility just outlined, high cord transection and aortic ligation were done in some further experiments, and by means of two wire snares the vena cava was occluded below the renal vessels and severed further down, just before a dye was placed in circulation. Since the blood from the portion of the abdominal muscles below the level of the umbilicus reaches the cava by tributaries which enter not more than 1 cm. above the junction of the iliacs, we were able to exclude the systemic venous pressure as a possible factor in the results by opening the cava further up. One experiment will be described in detail as representative of both the procedure and the findings.

A white male rabbit of 1790 gm. was etherized and shaved at 3:40 p.m., about 5 hours after feeding. The spinal cord was transected through an incision in the back between the fourth and fifth thoracic vertebrae, the abdomen was opened in the midline, the renal veins tied, and two snares placed around the vena cava, one just above the right adrenal gland, the other well below the left renal vein. The higher

of the two consisted of a loop of stout thread projecting from the end of a glass tube which had been pinched together in the flame to close it, save for two small openings through which the thread passed. Pulling upon the free ends of the thread obstructed the cava by drawing it against the tube. The other snare consisted of a loop of fine copper wire, instead of thread, and the end of the glass tube had sharp edges. After they had been put in position the snares were left loose about the vein, with the tubes,—which were narrow, and so thin-walled as to be very light,—projecting through the abdominal incision. This was closed around them after ligation of the aorta 2 cm. below the left renal vein. All had been accomplished by 4:30 p.m. At that time the abdomen presented a curious spectacle. Despite a stomach crammed with food its upper two-fifths appeared slender by contrast with a broad pouching of the lower portion, consequent upon a flaccid paralysis of the lower abdominal wall. All of the intestines lay in the pouch, and when pushed toward the diaphragm by manual pressure they slid back into it again. The skin of the upper part of the abdomen showed many abnormally distended venules carrying bright blood. Its pouched lower half and the hind legs were cold and pallid, although the animal, in excellent general condition, lay on a warm electric pad.

At 4:32 p.m. a carotid artery was cannulated and a kymograph tracing started. The pressure proved steady, at 26 mm. Hg.

4:41 p.m. The upper snare was drawn upon to occlude the vena cava, and at once thereafter this vessel was severed by pulling upon the wire of the lower snare. The injection was begun forthwith of 6 cc. of a warmed isotonic solution of half strength Chicago blue through a needle already placed in an ear vein. It was completed in 5 minutes and 5 seconds. At the end of the first 2 minutes the blood coursing in the cutaneous veins of the upper abdomen had become blue; after another half minute staining in this region had begun; and when the carotids were cut $1\frac{1}{2}$ minutes after the injection was finished the skin was blue halfway from ribs to pelvis. Further down its bloodless, pallid appearance was unchanged.

The carotid pressure was 27 mm. Hg at the beginning of the injection; at its end, 24 mm., not varying thereafter. On stripping back the skin immediately after death, fern-like blue markings were to be seen in the muscles of the abdominal wall, but no generalized staining of them. The markings extended somewhat more than halfway toward the pelvis, fading off rather abruptly into unstained tissue further down. The external oblique muscle was dissected away in two large sheets which included the zone of transition from staining to pallor. Under a magnifying glass the fact could be made out that wherever stained blood had got through to the veins in quantity a fern-like blue coloration of the tissue had taken place, such as is the result of the gradient of capillary permeability. At the far edge of the zone of transition from stained to unstained tissue only the arterial blood was deeply colored; and here no perceptible dye had got out into the muscle. Where the staining of this tissue was palest the local differences in it could yet be well seen, much as the pattern of the feathers of a white peacock is seen; and the width of the individual bands of blue appeared to be but little less than where the

coloration was intense. The vessels of the muscle appeared broader and contained more residual blood than usual. There was no diffuse staining whatever. The findings were identical with those when the vena cava was intact.

From the torn cava only 2.5 cc. of blood had been lost, very moderately colored with dye, as would follow from the circumstance that it was derived from a region in which the circulation had been greatly interfered with.

The distribution of dye from the capillaries after the vena cava had been severed did not differ from that when this vessel was intact. It follows that transmission backwards of the systemic venous pressure can be excluded as the cause for the gradient of vascular permeability observed in paralyzed muscle through which blood is coursing at the minimum pressure compatible with flow. One must conclude that this gradient exists independently of local variations in the hydrostatic pressure. This is not to say that it may not be modified by pressure differences. In rabbits with a normal arterial pressure a generalized staining of paralyzed leg muscles was found to take place concomitantly with the more intense local one referable to the gradient. There had manifestly taken place an escape of dye from the first portion of the capillaries as well as further on. Whether this escape was due to the increase in permeable wall surface resulting from dilatation of the capillaries after nerve section, or to transmission of the arterial pressure into their first portion, or to both causes, was not certain from the experiments disclosing it. But observations after cord transection and aortic ligation settled the point. In such instances the quantity of dye carried by the blood was often considerably greater than usual, owing both to a purposeful increase in the amount injected (sometimes double the usual dose) and to a reduction in the volume of blood in which it circulated, consequent upon a locking up of a portion in the parts affected by the ligation; yet no general staining from the dilated capillaries of that part of the paralyzed muscle in which circulation was good accompanied the local one from their further portion which was referable to the gradient. It is plain that capillary dilatation after nerve section does not inevitably result in a generalized escape of dye. The animals in which it was lacking had a greatly reduced systemic blood pressure. That this was the reason for the absence of generalized staining seems the more likely because of experiments now to be

described in which a heightened venous pressure resulted in an increased local escape of dye from the vessels.

Effects of Altering the Venous Pressure

For excellent reasons the results of increasing the hydrostatic pressure were ascertained by raising the venous pressure instead of the arterial. The permeability of the capillaries is greatest at their venous ends, and one would expect alterations in dye distribution as the result of pressure changes to be most pronounced there. Furthermore the venous pressure could readily be quadrupled, whereas the arterial could be raised only fractionally and by a contraction of the arteries themselves.

It was not feasible to make the tests on muscles with cut nerves because of the rapidity with which dye escapes from the dilated vessels when the blood pressure is merely normal. But clear-cut indications were obtained in intact preparations that raising the venous pressure causes an increased escape of dye through the capillary wall.

The saphenous and femoral veins of a hind leg of rabbits under ether or luminal anesthesia were tied, and, as control, the same veins on the other side were dissected out, but without ligation. The legs were then placed symmetrically, with the animal on its back; and the dye was injected into an ear vein as ordinarily. Chicago blue was used for the most part but sometimes brom phenol blue, and in one instance phenol red. After the dye had been in circulation for a few minutes the carotids were cut and the tibialis anticus muscles were compared. It was regularly found that the tissue on the side of venous obstruction showed broader and deeper bars of stain. After phenol red there was a generalized staining also, as would follow from its high diffusibility (11).

To produce a greater degree of obstruction in some cases the common iliac, popliteal, and saphenous veins were tied on one side while on the other they were merely dissected free. The findings did not differ from those just described.

Obstruction of the femoral vein had no effect whatever to cause venous congestion, owing to the abundance of collaterals. Because of these the vessel could be utilized for determinations of the venous pressure in the leg after obstruction of the other veins draining it. In numerous animals the distal end of each femoral was connected with a salt solution manometer after heparin had been injected into the blood stream to prevent clotting. The pressures proved to be approximately the same on both sides, from 5 to 6.5 cm. of salt solution. Now one common iliac vein was tied off through an abdominal incision and the other merely dissected free. This resulted within a few minutes in a rise of the femoral pressure on the side of the ligation to 22 to 26 cm. while on the other side the pressure did not change.

Dye was injected. It appeared promptly in the muscle vessels exposed under a mica window, showing that arterial blood was still entering them despite the accumulation consequent on the iliac tie. Exsanguination was done a few minutes later, both from the carotids and the femoral veins; and several muscles from each lower leg were removed and compared. The staining took the usual banded form, but the bands were broader and deeper colored where there had been increased venous pressure. More dye had escaped from the capillaries on this side and further back along them.

In three animals the superior mammary and the superficial and deep epigastric veins of one side were ligated, and 2 to 6 hours later Chicago blue was placed in circulation. Through mica windows overlying the muscle the fact could be made out that the dye reached the venules of the external oblique more slowly on the side of the ligations. Nevertheless when the animals were killed, after a few minutes, the barring was, in two of three instances, broader and deeper than in the control, and in one case the dye had escaped from the transverse venules as well, forming a blue zone along them.

The vena cava of one rabbit was ligated just above the junction of the common iliac veins and 82 minutes later Chicago blue was injected. The staining of the abdominal muscle proved to be more intense in the region draining into the vena cava below the tie; and in the hind legs the barring was darker and the general staining deeper than in the pectorals and fore legs. In another animal 2 days were allowed to elapse after operation before the dye was given,—during which period no limp or paralysis was observed and a well defined collateral circulation formed by way of the inferior and superior epigastric veins. In the muscle regions affected by the obstruction, the same local intensification of the staining was noted as in the other experiments, and dye escaped from the transverse venules as well as from the capillaries. Like results were obtained 3 days after the vena cava had been tied off above the inferior mesenteric vein. Just prior to the injection of dye into the ear the femoral veins were cannulated and abnormally high pressures found in them—26 to 27 cm. in the distal ends in the case of one rabbit, in another 10 to 13 cm. in the proximal ends.

When only one common iliac had been tied so effective a collateral circulation developed within 3 or 4 days that the pressure in the distal end of the femoral vein on the obstructed side was but slightly higher than on the other. Dye injection yielded a better staining on the free side than on the obstructed.

Raising the venous pressure markedly by obstructing outflow enhanced the escape of dye put in circulation shortly afterwards and increased the proportion of capillary from which it took place. This was clear from the character of the staining. The bands of color were broader and deeper than ordinary and extended further back along the capillaries. When the dye was placed in circulation after obstruction had endured several days the findings varied with whether

collaterals had relieved the abnormal pressure condition or not. When they had done so, as after ligation of a common iliac vein, the staining was not increased on the obstructed side and was often less than on the other. But when the venous pressure was still notably high, as when the vena cava had been tied, not only did more dye escape and the effective permeability extend further back along the capillary but the venules proved permeable to the dye, as happened only exceptionally when the pressure rise had been of short duration.

Flow through the muscles subjected to partial venous obstruction was retarded, as could be told by the rate of passage of the dye-stained blood into the small vessels. There was, of course, an accumulation back of the obstruction and in addition, doubtless, some induced contraction of the artery feeding the part (12). The turnover of blood cannot but have been less than in the control limb. If the substances employed had been so diffusible as to escape readily into the tissue, one might have expected the lessened quantity that was available on the experimental side to be reflected in a lighter staining, despite the heightened venous pressure, since the depth of the staining would be an immediate expression of the amount of dye brought by the blood. This proved to be the actual case in experiments with brom phenol blue. But Chicago blue is so poorly diffusible that only a very small part of the amount passing through the muscle at a given moment escapes into the tissue. Our results show that the rapid replacement of the dye-laden blood with more of the same sort, as when flow is active, has less importance for the distribution of Chicago blue to the tissues than the influence of increased venous pressure. Otherwise the staining could not have been heavier in the leg subject to passive hyperemia than it was in the control

The possibility must be considered that the heightened venous pressure resulted in an increased escape from the capillaries because some of those through which no circulation had been taking place were forced open by it,—not because the permeability of the individual vessels was increased. But this would not suffice to explain the increased permeability of the venules. That even very high pressures fail to open closed muscle capillaries is a truism with those who have attempted an injection of colored mass to demonstrate the vascular arrangement.

The effects of increasing the venous pressure were so marked as to suggest the possibility that the normal blood pressure at the venous end of the capillary may be at least partially responsible for the relatively abundant escape of dyes into the tissue at this situation,—though true it is that the experiments in which the vena cava had been severed after aortic ligation yielded no indications of the sort. To settle the point a procedure was devised whereby the venous pressure could at will be reduced to zero without any loss of blood.

Rabbits of approximately 2000 gm. were closely paired by weight after the withdrawal of food for 24 hours. Cross-agglutination tests had shown their bloods to be compatible. They were anesthetized with a 10 per cent solution of sodium amytal (0.5 cc. per kilo intravenously, with 0.25 cc. half an hour afterwards, and more later if necessary), and laid upon warmed pads, side by side on their backs with the hind legs placed symmetrically. The proximal ends of one common carotid of each animal were connected by a rubber tube having a glass T inserted midway, the stem of which led to a mercury manometer. The tubes had been filled with Ringer's solution prior to insertion. The distal end of one common iliac vein of the rabbit with the lower carotid pressure was then connected with the proximal end of the opposite vein of the other animal, by means of wide-mouthed cannulae and a rubber tube having a larger bore than that of the veins, which connected through a T with a manometer containing Ringer's solution. The uncannulated common iliacs were dissected free as a control to the effects of trauma, and fine threads were put loosely about them so that they could be lifted and cut at will. The abdominal incisions were closed about the tube connecting the veins and $1\frac{1}{2}$ cc. per kilo of a solution containing 10 mg. of heparin per cc. was injected intravenously into each animal to prevent clotting when the connections were opened.

No nerves were severed, since our aim was to determine the effects of abolishing the venous pressure in intact muscles. It was necessary, however, to clamp off the common iliac vein for a few minutes during the cannulation.

The arterial and venous connections were now opened, and at intervals the individual pressures in each animal were taken by temporarily clamping the rubber tube to one side or other of the manometers. Flow from the distal end of the common iliac of the upper animal was not blocked long enough in this way ordinarily for a reading to be obtained of the highest pressure developing on obstruction, but only momentarily so that one could learn from the prompt rise in the manometer column that active flow had been going on. The development of a negative pressure when the salt solution manometer was raised above its previous level constituted another check upon the existence of flow. Now the rabbit receiving venous blood was gradually lowered so that it lay 10 to 20 cm. below the other. After the lapse of a few minutes to allow for circulatory readjustments,—which took place without any change in the mean carotid pressure,—individual

readings were taken again, with the manometers at the levels of the upper and lower animals respectively, and dye was injected simultaneously into one or both of them. They were killed soon after by cutting the carotids and all four common iliac veins.

Six technically adequate experiments were accomplished, three with Chicago blue, three with brom phenol blue. The animals remained in excellent condition throughout, because care had been taken to keep them warm and to minimize trauma. Except during the determinations of pressure, all of the connections were left open, the mean pressure in the connections being followed as indices to the general state of affairs. The mean pressure in the arterial connection, while varying little in the individual experiment, ranged from 70 to 95 mm. Hg for the entire group. The pressure in the proximal end of the common iliac of the lower rabbit varied between 5 and 7 cm. of 0.9 per cent salt solution when flow into this vessel from the other rabbit was cut off, a figure corresponding with that obtained by cannulating the proximal end of a femoral vein under more ordinary circumstances, as in the experiments on venous obstruction already described. The possibility of a positive pressure in the distal portion of the common iliac of the upper rabbit was ruled out by manometer readings which showed the existence of a negative pull when the instrument was raised to the level of this animal. The injection of Chicago blue into the upper animal caused a transient fall in its carotid pressure, which was reflected in some lessening of the mean carotid pressure; and through the walls of the glass T connecting the iliac veins the prompt passage of the dyestained blood to the lower rabbit could be observed, though it never took place in sufficient amount for a noticeable staining of the latter. Sometimes this animal was preserved for a few minutes after sacrifice of the other, to be utilized for a separate test of the effect on the gradient of permeability of ligation of the common iliac vein.

The corresponding muscles of the hind legs of the upper animal were compared both *in situ* and after excision. The tibialis anticus and adductor longus were principally scrutinized.

In these cross-transfusion experiments the lowering of the venous pressure to zero may have brought about local changes by way of the vasomotor system. However this may be, the results in terms of dye distribution were precisely those that would have been expected from the fact that raising the pressure emphasized the gradient of permeability and extend its effective scope. The staining with Chicago blue was slightly but definitely less intense than in the control muscles, and the bars of color were a little narrower. With brom phenol blue no differences could be perceived. As already mentioned, staining with this dye is immediately dependent on the amount of stained blood that circulates through the tissue. The influence of this factor may well have masked that of the differing venous pressures.

The common iliac vein, wherein pressure was to be abolished, was of necessity obstructed for a few minutes during cannulation. This should have caused, if anything, an increased staining with Chicago blue, not the lessened one that was observed.

Total Exclusion of the Pressure Factor

In some final tests the influence of hydrostatic pressure was totally excluded. The ear of the mouse was utilized as providing especially favorable conditions. The gradient of permeability existing in this organ has already been the subject of a paper (13). As in the muscle there exists a mounting gradient of permeability along the capillaries; and the venules into which these empty are more permeable still, instead of less so, as is the case in muscle.

The distribution of dyes from the blood is readily followed in the ear spread between glass plates and covered with paraffin oil (14). In the experiments here under consideration the circulation was cut off by filling with water a sausage-shaped collodion bag, $1\frac{1}{2}$ to 2 mm. in diameter, placed under the organ near its base to press it against the upper plate. To insure the presence of an abundance of dye-stained blood in the vessels the circulation was stopped by pressure in the bag for a few minutes, during which the dye (Chicago blue 6B) was injected into a tail vein. Active hyperemia (Bier's hyperemia) ensued on emptying the bag; and as soon as the stained blood had been distributed to the ear, that is to say before any escape of dye into the tissue had taken place, the bag was again distended, occluding the vessels. Transmitted pressure had of course to be ruled out as a factor in the staining that ensued from the pent blood; and simple tests sufficed for this. Abruptly filling the bag under the maximum occlusive pressure (70 cm. of water) failed to cause a perceptible dilatation of the vessels beyond, and the veins there underwent no sudden collapse when the pressure was relaxed, the blood from them finding its way but slowly into the region overlying the bag.

In the experiments proper a second bag was not infrequently placed across the ear near its tip, and just sufficient pressure was exerted through it to keep the overlying tissue bloodless during the brief period while the dye was circulating. When, immediately thereafter, the circulation through the ear was cut off by means of the proximal bag, the distal bag was allowed to collapse, thus providing an unusual amount of room in the vessels for the blood retained in the ear. The slowness with which this flowed into the empty vessels,—which, as the event showed, were not contracted,—sufficiently proved that it was under no significant pressure. And the results in terms of the distribution of dye were precisely the same as when a single bag had been used.

In man the cutaneous capillaries contract after the circulation has been stopped

for some minutes, forcing the blood from them into the veins (15). This was never observed after flow to the congested ear had been cut off in our mice, nor did the arterioles drive out the thin thread of dark blood within them. Because of the hyperemia that had been induced, many more capillaries were visible than under ordinary conditions; and from their further portions and from the adjoining venules an escape of dye soon took place. So little was available that this escape could be judged more certainly by the decolorization of the vessel contents than by extravascular staining,—which was well defined only around the venules. The blood within the latter soon lost color despite the relatively unfavorable ratio of wall surface to blood volume, and so too with that in the further part of the capillary web, whereas the contents of the proximal portion of the web and of the arterioles remained deeply blue.

DISCUSSION

In a previous paper reasons have been set forth for the utilization of foreign colored substances in direct studies of vascular permeability (16). To avoid the complicating influence of specific affinities tests were made with a variety of dyes. In the present work only a few have been employed, because it was proved that the initial staining with these comes about solely by their escape from blood plasma to tissue fluid, though secondarily they may become fixed upon some of the formed elements or stored within them. It follows that in the experiments here described, involving only the first spread of the dyes from the blood, we have dealt with the effective permeability for these substances of the barrier separating fluid from fluid. This fact, though mentioned already, deserves emphasis.

The gradient of dye distribution from the vessels, studied during the previous work, had appeared incompatible with the view that the capillary is equally permeable everywhere along its course, alterations in the relative influence of hydrostatic pressure, diffusion, and osmosis being responsible for local differences in the character and direction of exchange through the wall of the channel. The present experiments show that when the blood pressure is reduced to the lowest limit compatible with flow the graded distribution of dyes from the capillary persists, and so too when the pressure factor is entirely done away with. From these findings, taken in connection with the fact that dye escape is a measure of the effectiveness of the capillary barrier, it follows that hydrostatic pressure can play no essential rôle in the maintenance of the gradient of vascular permeability.

Pressure importantly conditions the gradient however. Raising the venous pressure extends the effective scope of the gradient and accentuates the local differences in permeability. In whatever way this is accomplished, whether by a stretching and thinning of the vessel, by a filtration process, or by both, accomplished it assuredly is. Landis' direct measurements prove that an increase in venous pressure causes a prompt rise of that within the capillaries of human skin (17). In some of our experiments the positive pressure in the large veins draining the muscles was reduced to zero. Needless to say the pressure in the capillaries,—which are long and very slender,—may not have been profoundly lowered under such circumstances; for it must be largely dependent on the driving force of the blood. All one could hope to produce by the change was a greater pressure fall than ordinary along the capillary way, a fall which might possibly become manifest in an alteration in the rate and character of the distribution of the dyes. Alterations did take place, such as might be taken to indicate that even normal venous pressure is not without a favoring influence on the escape of substances into the tissues from the further capillary region. But induced vasomotor reactions cannot be ruled out as responsible for the changes.

Substances of small molecule leave the blood with such rapidity by diffusion that their spread may be considered as largely independent of any filtration of water (18). Even with dyes of large molecule, diffusion is an important influence in distribution, the rate of vital staining varying directly with the diffusibility of the pigment as ascertained *in vitro* (19). The escape of Chicago blue, after arrest of the circulation and exclusion of the pressure factor, must be laid to diffusion. According to Landis the rate of water filtration, not the intrinsic permeability of the capillaries, is indicated by the spread of dyes (20). Such was doubtless the case with the material, and under the circumstances, of his experiments, which involved long exposure and irrigation of the mesentery of the frog; but a repetition of his tests under controlled conditions has proved their results due to vascular disturbance (21). If the coloration of resting mammalian muscle, an organ from which there is practically no lymph flow (22), were due to a filtration of dye-stained water one would have to suppose a closed circulation of such water in the region supplied by the capillary, occurring pre-

sumably by the influence of the hydrostatic pressure to force water out through the wall of the first portion of the vessel and the osmotic influence of the blood colloids, further on, where pressure had fallen, to bring it in again (23). The mounting gradient of distribution of dyes along the capillary is incompatible with this filtration hypothesis (24), and the persistence of the gradient when the hydrostatic pressure is greatly reduced, or done away with, effectually disposes of it.

The dyes were in watery solution and where water goes they may be supposed to go, unless their molecules are large enough to be held back selectively. When the arterial pressure is markedly lowered, as was the case in many of our experiments, water enters the blood from the tissues; but so swift is the process of readjustment (25) that it was doubtless at an end before our dyes were placed in circulation. Whether a fluid transfer took place in the experiments in which the venous pressure was raised present findings do not enable us to say. In a succeeding paper, however, the influence of water flow, and of the blood colloids, on the gradient of vascular permeability will be dealt with directly.

None of the highly various functional disturbances of the vessels and circulation brought about in the present work and that previously reported has abolished the gradient of vascular permeability. The local changes incidental to plethora, hemorrhage, shock, nerve section, active and passive hyperemia, muscular contraction, circulatory arrest, and anhydremia due to hypertonic salt solution administered by mouth, have alike proved ineffectual in this respect. Consequently it is difficult to suppose that the gradient can be the outcome of functional conditions. Rather should its cause be sought in a structural differentiation along the capillary.

SUMMARY

The gradient of permeability along the capillaries of voluntary muscle and the capillaries and venules of skin exists independently of the hydrostatic conditions, though influenced by them. Its presence cannot be explained by a graded tonic contraction of the capillaries. The evidence,—like that of previous papers,—points to local differences in the barrier offered by the wall of these vessels as responsible for the gradient.

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EXPLANATION OF PLATE 13

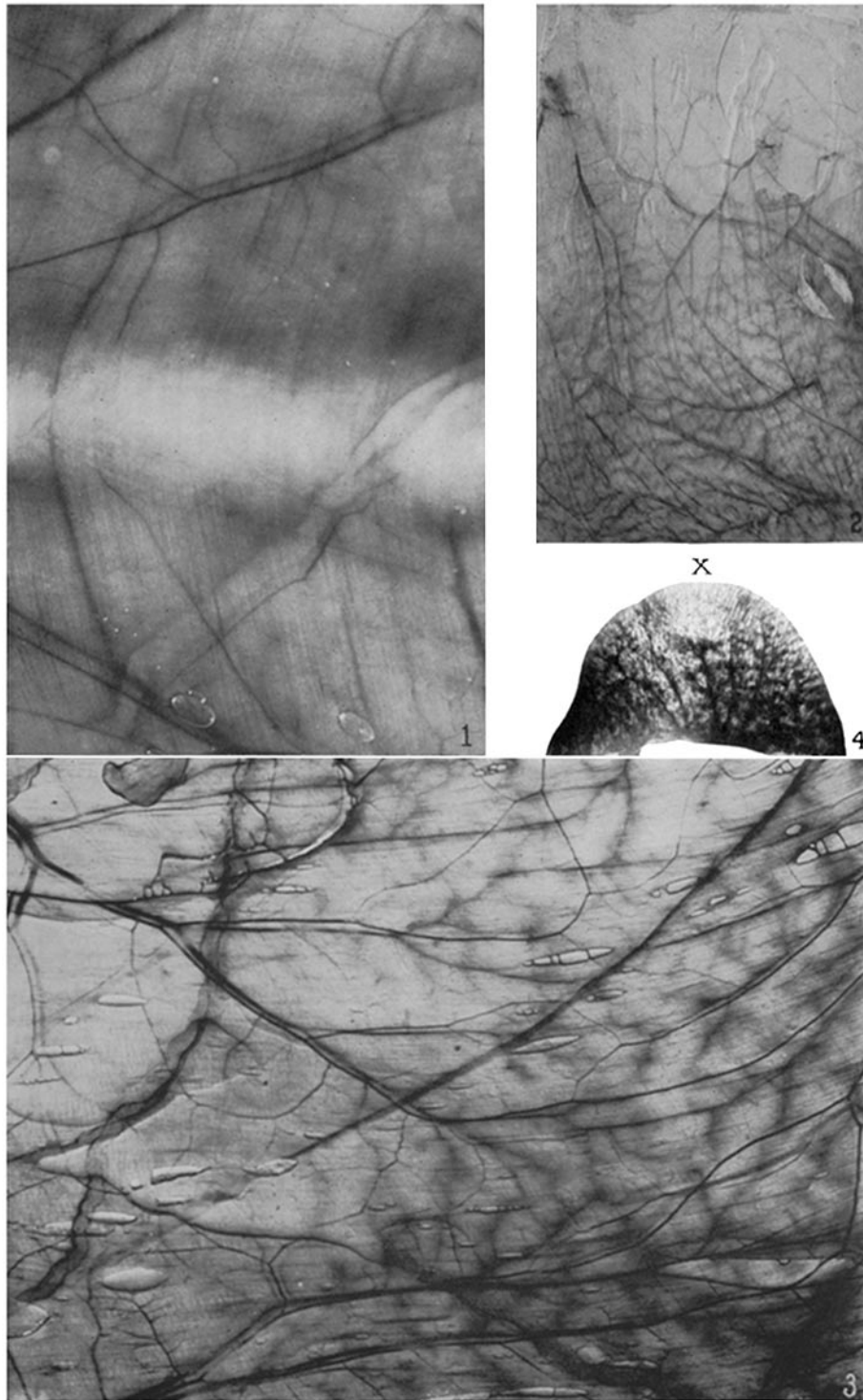
FIG. 1. Decolorization by pressure of diffusely stained tissue. Portion of an external oblique muscle of a rabbit, upon which pressure was brought to bear by means of a bag lying beneath the preparation and separated from it by a sheet of moist, white paper. The dye forced from the tissue has accumulated to either side of the strip pressed upon. In some regions the pressure was insufficient to empty the large vessels entirely. Photographed by reflected light. $\times 8\frac{1}{2}$.

FIG. 2. Part of an external oblique muscle of a rabbit receiving Chicago blue 6B immediately after the spinal cord had been cut between the fourth and fifth thoracic vertebrae, and the aorta tied below the renal vessels. The dye injection

took 5 minutes and the animal was bled to death 2 minutes later. The sheet of muscle was excised immediately and photographed through a red filter to exclude the blood color. The specimen includes the zone of transition from stained to unstained tissue. It will be seen that wherever dye has passed through the vessels in quantity, as shown by the dark contents of the veins, a staining indicative of the ordinary gradient of vascular permeability has developed. Natural size.

FIG. 3. A portion of the transition zone of Fig. 2, enlarged 2 times.

FIG. 4. Decoloration by pressure of tissue showing the markings indicative of the gradient of vascular permeability. The ear of a mouse injected with pontamine blue was lopped off early in the period of dye escape, when the local differences in staining were well defined; and a strip of rubber was pressed upon the organ in the region X, according to the method described in the text. The picture shows that the tissue pressed upon was decolorized. The dye-stained fluid deriving from it forms a dark border on the side toward the base of the ear. $\times 3$.



Photographed by Louis Schmidt

(McMaster *et al.*: Gradient of capillary permeability)