I. THE PERMEABILITY OF THE WALL OF THE LYMPHATIC CAPILLARY

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Plates 11 to 13

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Little is known of the permeability of the wall of the minute lymphatics. The neglect of this theme is the more remarkable in view of the great amount of work that has been done on the blood capillaries; but it is to be explained by the technical difficulties of experimentation. Recently in our laboratory a method has been devised whereby the most minute lymphatics can be disclosed and the permeability of their walls subjected to test.

In brief our method has been to render the lymphatics visible by means of well tolerated vital dyes, draining away in them from the point of injection, and to utilize these latter in the study of exchange between the lymph and tissue fluid. Under the circumstances of ordinary vital staining by a distribution of dye from the blood to the tissues the minute lymphatics remain invisible in the midst of the general coloration; but when a local injection of dye is made, the minutest lymphatics deriving from the region become filled with colored fluid and stand out brilliantly from their unstained surroundings. In a tissue accessible to study without disturbance it is possible to test the permeability of the lymphatic wall by the use of dyes of graded diffusibility, to observe the change produced by stimuli of various sorts, and to carry out other investigations. Such a tissue has been found in the ear of the mouse.

The present paper is devoted to orienting observations and to the permeability of the lymphatic wall under normal conditions.

Method

The ear of the living mouse is almost ideal for direct observation of the smaller blood vessels and capillaries (1). The central cart-

ilaginous plate serves as a background against which the overlying blood vessels stand forth sharply under suitable experimental conditions. A small amount of isotonic dye solution or a suspension of finely particulate material is injected into the skin on the outer side of the edge of the ear. The lymphatics, rendered visible by their dye-stained content, are studied. The technique of lighting and inspection has in general been that employed for investigation of the ear capillaries (1).

Young adult white mice were used, which had been kept for a month or more on the same adequate diet. Preference was given to those of long headed type, that is to say, those having large ears so set as to be easily spread upon the platform used in the observations. Anesthesia was induced 1 hour before the experiment by injection beneath the skin of the abdomen of a 2 per cent aqueous solution of sodium luminal, 0.0125 cc. per gm. of body weight. An additional 0.02 cc. per 10 gm. of body weight injected intraperitoneally brought about more rapid narcosis. This dose of freshly dissolved drug produces an even anesthesia lasting 2 to 5 hours. Occasionally, it was necessary to place a broad collar of cardboard about the animal's neck (2) to prevent scratching of the ear before stupor ensued. Throughout the experiment the anesthetized mouse lay on its belly in a frame so moulded of plasteline and wire gauze that the head and body were supported while the ears rested lightly upon small white procelain plaques set upon daises which were in the same horizontal plane, to rule out gravity as a cause for any differences in them. The ears were thinly coated with neutral paraffin oil which served the double purpose of holding them against the procelain without distortion or pressure and of greatly increasing visibility. A beam of light from a Leitz-Wetzlar carbon arc, cooled by passage through one or more filters, 5 cm. thick, of Magnus' fluid, was directed onto the ear by an adjustable mirror. The observations were carried out with a Spencer binocular dissecting microscope.

When dye was to be introduced, the skin was punctured on the outer side of the ear near its edge with a steel dissecting needle which had been ground to an extremely fine point. Through the little wound, either a micro-pipette or a 30 gauge platinum iridium injecting needle was thrust 2 to 3 mm. toward the base of the ear as superficially as possible. Dye solution was then slowly introduced in small quantity, resulting in deeply colored areas 1 to 4 mm. in diameter. Even before the injection was finished the lymphatics deriving from such an area had taken up the dye and colored fluid was seen to stream away toward the base of the ear, the outlines of the containing vessels being sharply demarcated thereby. When it was desired to render single lymphatic radicles visible, a Chambers micro-dissection apparatus and quartz micro-pipettes were utilized for the injection of an extremely minute quantity of dye.

The dyes were in the main those already utilized in this laboratory for studies of

the permeability of blood capillaries. They ranged in diffusibility from the swiftly diffusible Neptune blue (comparable to patent blue V in this respect) to pontamine sky blue which passes not at all through ordinary collodion membranes and only very slowly through the capillary wall. All were injected in isotonic solution, sometimes in sodium chloride solution, again in Tyrode's solution, and yet again in mouse serum diluted thrice with Tyrode's solution—to approximate the composition of the lymph of an extremity (3–5). In many previous experiments on the blood vessels the innocuous character of the dyes has been evidenced (6).

Appearance of the Normal Lymphatics

A general description of the minute structure of the ear has been provided in a previous communication from this laboratory (1); but nothing was said in it of the lymphatics. Those rendered visible by their content of dye-stained fluid appear as irregular channels of highly various size and shape. They form an abundant intercommunicating network. Fig. 1 shows those draining a single colored area resulting from an injection under minimum pressure. Even the smallest lymphatic vessels disclosed by the injection are 15μ in diameter, far larger that is to say than the blood capillaries. Most of them are many times larger. They extend to the very margin of the ear without evident pattern or relation to the blood vessels. The lymph flowing along them toward the base of the ear collects into a few trunks lying as a rule in the tissue between the fan veins of the ear. These trunks are, if anything, narrower than the vessels that they drain. There is none of the gradual increase in size occurring in the veins. At intervals along the course of the lymphatics one sees characteristic bulbous swellings and under high magnification valves can be made out. There are many fine cross-channels without valves or swellings; but few of these are to be seen ordinarily, since the lymph current does not bring the dye into them. They can readily be rendered visible, however, by injecting the large lymphatics under moderate pressure. These can be pierced after they have been rendered visible by a content of colored fluid. Variations in the depth at which dye is placed in the tissue at the edge of the ear have revealed a superficial and a deep plexus of lymphatics, intercommunicating and both possessing valves. The one is in the corium, the other above the fatty tissue immediately overlying the cartilage (1).

No matter where the dye solution is injected, whether at the edge or middle or near the base of the ear, it is always transported toward the head, peripheral flow being prevented. We have repeatedly pierced individual dye-containing lymph channels and have introduced into them dye of another color, with the micro-pipette directed toward the periphery, but seldom have we been successful in forcing colored fluid past more than one or two valves without rupture of the vessel. Also with fine micro-spatulas directed by the Chambers device we have "milked" dye-stained fluid peripherally but only on rupture of the valves. In the extreme ear margin, however, the lymph channels are not guarded by these latter. Here it is possible to obtain a localized retrograde injection from dye proximally placed, and to demonstrate the existence of lymphatic closed loops and culs-de-sac next to the very margin of the ear. Even here the channels are numerous and far larger than the regional blood vessels. At no time has any evidence been obtained of direct communication between the lymphatics and the tissue spaces. Always the lymphatics have appeared to be completely closed.

The study of fixed and sectioned specimens has corroborated these findings. The preparations were obtained by injecting the channels in the usual manner with a gelatin solution warmed to 43.0°C. and stained with pontamine sky blue, after which the whole ear was fixed in Zenker's fluid. Pressure was required to inject the mass.

Lymph Flow

The first demonstration of the lymphatics by means of dyes was accidental and while suggesting the later method, it also indicated the presence of a considerable lymph flow in the ear. In the course of work on the gradient of capillary permeability (6) a dye had been injected intravenously that formed aggregates while in the blood stream and blocked some of the finer arteries of the ear. Almost at once the emboli began to dissolve and as this happened lymphatics draining away colored fluid became visible. Under the circumstances of the later experiments, there was an active flow along the minute lymphatics of the ear, but since this derived from injected regions it cannot be taken as indicative of normal flow; where two lymph channels joined, however, one could often observe that a stream of dye-

containing fluid was displaced, diluted, and swept away by another stream, itself unseen, deriving from tissue remote from the region of injection. This occurred with remarkable rapidity. It could not have been due to the transmission of pressure from the injected area since the tissue of the ear is yielding (7). In certain instances blue and red dye solutions were introduced about 1/2 cm. apart at the margin of the ear. The lymph channels draining the regions coursed to a common trunk, and, carrying as they did different colored fluids, one could see at the point or points of confluence a blue stream joining a red one, or *vice versa*, the two streams flowing unmixed for several millimeters. The findings were not so free from objection as those in which colorless lymph joined that which was colored, because both tributary regions were abnormal.

In twelve experiments a crystal of pontamine blue or a minute droplet of the dye solution was placed in the tissue at the edge of the ear with the aid of a Chambers device and a micro-spatula or injecting pipette. The dye dissolved to form small colored dots, and the margins of these gradually extended in the direction of the base of the ear, and in this direction only, as could be told by measurements with a micrometer scale. In addition to a drainage away of colored fluid by the lymphatic channels described, one saw small streamers of dye, not sharp-edged as with fluid confined in lymphatic channels, but with hazy margins, as if free in the tissue, extending in the direction of the base of the ear. As long as the dot of dye continued to be perceptible as such, its margin continued to advance in this direction, while at the same time the tissue on its outer side which had at first been included in it became colorless. A true migration of the dot had taken place. It gradually became lighter and smaller and within 18 hours after its formation, all traces of it were lost. It is plain from these observations that besides lymphatic drainage and an attendant rapid turn-over of the fluid within the tissue spaces, there is some interstitial flow toward the base of the ear.

The demonstration of the lymphatics by means of the dyes was practically always incomplete. A current does not course through all of the minute lymphatics at the same time, the fluid in some of them being at a standstill. The existence of extremely narrow cross-connections between large lymphatics which had appeared to have a

wall with no openings into it was repeatedly revealed by pressure manipulations. These connections allowed only the finest thread of colored fluid to pass through, and there is every reason to suppose that under ordinary circumstances they were closed off. That they had adequate walls was shown by their sharp outlines and by the fact that their contents even when under slight pressure did not pass into the surrounding tissue. They had no valves or bulbous swellings. The facts suggest that they were accessory lymphatics, not closed off temporarily as are certain of the blood capillaries (8), but channels unutilized under ordinary circumstances.

Continual observation over periods of 3 to 5 hours revealed no movement of the lymphatics; their outlines and relations to one another remained unchanged. The bulbous dilatations appeared rigid, as if their walls were moored in the position of distension. In the region between them, small puckerings, possibly caused by struts of connective tissue, seemed permanently to distort the vessel contour. When India ink or "Hydrokollag," dialyzed against Locke's solution, was injected into lymphatics, these cleared themselves within a few minutes but black particles were left adherent to their walls, outlining them sharply. In such preparations, which can be studied for a much longer period than lymphatics outlined with dye, no contraction of the wall has ever been seen. This is not because the contours of the wall cannot alter. In experiments on the effect of heat and cold, to be detailed in a subsequent paper, marked changes in the size of the channels have been observed. But it must be concluded that the lymphatics of the mouse ear unlike those of the wing of the bat (9) do not actively contract or relax under ordinary circumstances.

Permeability of the Lymphatic Wall

Our first observations showed the walls of the lymphatics to be highly permeable. Despite the fact that fluid was continually entering them from the surrounding tissue, as shown by observations already mentioned, even the most indiffusible of the dyes we utilized passed out. A systematic study of the permeability thus disclosed was undertaken. For the purpose it was important to utilize ears which had not been injured or inflamed in the least. Those of nearly full grown animals of 16 to 20 gm. proved far the best, the ears of younger

mice tending to be too thick for useful observations and of older ones too often marred. Even in ears that appeared wholly normal, an ecchymotic dye escape, testifying to occult injury, was frequently met with. Fig. 2 illustrates this. Several sharply localized dye extravasations here and there along the lymphatics can be seen in the photograph. This irregular type of dye escape can be readily discriminated from that occurring everywhere along an uninjured lymphatic wall in the case of highly diffusible pigments.

The behavior of two groups of dyes was investigated, those of the one group (pontamine sky blue and Chicago blue 6B) being poorly diffusible, as judged by their behavior in the blood stream and by their failure to pass collodion membranes; those of the other (trypan red, brom phenol blue, and Neptune blue), more or less highly diffusible. Isotonic solutions of all these, in various diluents and at various strengths, were introduced into the lymph channels. Several aims were pursued. We sought to learn the effect of differences in the molecular concentration of the dyes upon the rate of passage from the lymphatics. Next we studied the influence upon dye escape through the lymphatic wall of variations in the protein and salt content of the fluid media carrying the dyes. And finally we ascertained whether known differences in diffusibility of the dyes as demonstrated by their passage out of the blood vessels and through gelatin *in vitro* (6) hold good as regards their escape from the lymphatics. A rough comparison of the relative permeabilities of the lymphatic walls and the walls of the blood capillaries, under the conditions of life, was thus carried out.

The molecular weights of the dye specimens, which had been purified of extraneous material, were calculated by the freezing point method with a Beckman apparatus. For the experiments fresh solutions isotonic with 0.9 per cent sodium chloride solution were made in twice distilled water. A 21.6 per cent watery solution of the pontamine sky blue we used was isotonic with blood, 17.1 per cent of Chicago blue 6B, 4 per cent of trypan red, 4 per cent of brom phenol blue, and 5.5 per cent of Neptune blue. Such isotonic solutions were mixed in varying proportions with the following vehicles:—sodium chloride solution 0.9 per cent, Tyrode's solution, mouse amniotic fluid, mouse serum, and a mixture of 3 parts Tyrode's solution with 1 part mouse serum—this fluid having the approximate protein content of lymph from a mammalian extremity, to judge from the literature. In a few instances rabbit serum concentrated fivefold was also employed. The material

injected contained varying percentages of dye, depending upon the purpose of the experiment, but as a rule from 1 to 2 per cent.

In previous experiments (1, 10), and in others to be reported in the following paper, these dyes have been used for vital staining of mice by intravenous injection, and have circulated in the blood in approximately the same concentration as was now used, that is to say 1 to 2 per cent. Variations in the percentage without change in the tonicity of the final mixture were accomplished by using isotonic diluents. Differences in the tinctorial strength of the dyes remained a factor which could not be controlled. Fortunately however, the differences in their diffusibility and those in the influence of the diluents upon their escape from the lymphatics far transcended the observed differences in tinctorial value so that these failed to complicate the interpretation of the results.

The General Phenomena of Dye Escape

The phenomena which enabled us to recognize the passage of dye through the lymphatic wall were very striking. Ecchymotic dye escape has already been considered. The limitation of escape to certain segments of seemingly normal lymphatics sometimes occurs, its cause being as yet obscure. It is encountered only with poorly diffusible dyes. Ordinarily the passage of dye through the lymphatic wall takes place everywhere at approximately the same time and rate. Immediately after the channel has become filled with dye-stained fluid it appears sharply demarcated; but sooner or later, depending on the diffusibility of the dye employed, its outline grows misty. The coloring matter is escaping into the surrounding tissue, and in proportion as this happens the lymphatic becomes surrounded by a colored cloud and obscured.

Invariably in scores of experiments an increase in the concentration of the dye enhanced its escape from the lymphatic channels, irrespective of the character of the fluid in which it was introduced. In this respect the findings resembled those on intravenous injection. Comparison of Figs. 3 and 4 with Figs. 5 and 6 show how greatly a fourfold increase in the concentration of dyes increased diffusion.

Influence of the Fluid Vehicle upon the Escape of Dye

Early in the work the fact was noted that the escape of the dyes was markedly conditioned by the character of the fluid in which they were dissolved. For this reason comparisons of the diffusibility of the dyes were made only when they were introduced in the same vehicle.

Some experiments specifically devised to determine the influence of the vehicle threw light also on the permeability of the lymphatic wall as compared with that of the blood vessels.

The several dyes selected for the work, pontamine sky blue, Chicago blue 6B, brom phenol blue, Neptune blue, trypan red, and vital red, were mixed in equimolecular amount with saline solution, Tyrode's solution, mouse amniotic fluid, mouse serum, a mixture of 1 part mouse serum and 3 parts of Tyrode's solution, and finally with rabbit serum, concentrated fivefold. The proportions used were 0.1 cc. or 0.2 cc. of isotonic watery dye solution to 2 cc. of diluent. Small amounts of two or three of these solutions were injected at different spots along the edge of each ear so that comparisons might be made in the one animal of the rate of dye escape as influenced by the various vehicles. At least fifty injections, and in some cases twice as many, were made with each dye in order to obtain ample data.

It was regularly found that the dye escaped most slowly through the wall of the lymphatics when it had been mixed with a fivefold rabbit serum concentrate. The material was prepared by ultrafiltration through collodion sacs (11). The other vehicles can be arranged in the following order, escape being progressively greater with each one: normal homologous serum, diluted homologous serum (1 part serum to 3 parts Tyrode's solution), homologous amniotic fluid, Tyrode's solution, and saline. The differences observed were very great. When pontamine blue was injected in a fivefold concentrate of rabbit serum, almost none got out through the walls of the lymphatics during the next 2 hours, though these vessels were deep blue with it; and in some instances no escape whatever took place in the hours during which the dye was draining away through them, that is to say before they became decolorized by drainage. A less considerable yet very marked retention resulted when normal mouse serum was used. On the other hand, when the dye had been mixed with amniotic fluid or serum-Tyrode solution mixture, definite escape through the lymphatic wall occurred in 15 minutes or less. In the experiment furnishing the photograph of Fig. 1, pontamine sky blue was injected in a serum-Tyrode solution mixture. With amniotic fluid and the serum-Tyrode solution mixture, dye escape took place at about the same rate.

When present in the lymphatics in Tyrode's solution without serum, pontamine sky blue passed into the surrounding tissue much more easily. Within 6 minutes a moderate perilymphatic staining was usually to be observed, such as that pictured in Fig. 3. Another 10 minutes saw a further spread of color.

By far the most rapid spread of pontamine sky blue occurred when 0.9 per cent saline solution was used as the diluent. Almost immediately after injection this dye, which is notably indiffusible, began to escape from the lymphatics.

The foregoing facts held true of all the dyes with which tests were made. The solutions employed constituted a series with decreasing protein concentration, and in proportion to this decrease the dye escape

increased. Only two protein-free vehicles were used, Tyrode's solution and saline. With the first of these the escape of dye was but little more considerable than when amniotic fluid or a serum-Tyrode solution mixture had been employed. When saline was used on the other hand, the dye escape was pronouncedly greater. It seems more than likely that the saline injured the lymphatic wall, rendering it unusually permeable. The fact is well known that tissues perfused with an unbalanced salt solution rapidly become edematous.

Dye Escape from the Lymphatics Is Conditioned by the Diffusibility of the Dye

When mixtures of the various dyes were brought into the lymphatic channels in equimolecular concentration and in the same fluid, considerable differences in the rate of their escape could be observed, and these accorded with the known differences in diffusibility of the substances *in vitro*. For example, Chicago blue 6B in Tyrode's solution passed through the lymphatic wall with greater difficulty than did an equivalent concentration of brom phenol blue, but with more ease than pontamine sky blue. The differences were found irrespective of whether the dyes were compared in amniotic fluid, serum, saline solution, or another vehicle.

The method of test was as follows:—Solutions of the pontamine blue, Chicago blue, brom phenol blue, and Neptune blue in a single fluid were made as already described, and injected. The moment of first escape of dye was noted with a stopwatch and the general progress and character of the diffusion studied. The behavior of three dyes was compared simultaneously in each of fifty animals by injecting all three into each ear. In fifty more animals only two injections were made into each ear, the behavior of two of the dyes being compared at one time. By shifting the injection sites, local influences were controlled. This precaution, though employed as routine, was unnecessary since the differences in rate and amount of dye escape from the lymph channels were so definite as to transcend the effect of local factors.

In other groups of fifty animals each, the behavior of dyes was compared when mixed with saline solution, mouse serum, and mouse amniotic fluid, and in serum diluted with 3 volumes of Tyrode's solution.

The concentrations by weight of isotonic solutions of pontamine sky blue and Chicago blue are nearly similar, 21.6 per cent and 17.1 per cent. Furthermore, the tinctorial qualities of the mixtures tested were not very dissimilar. As a result, reliable comparisons of the amounts of dye escape from the lymphatics could be

made under the microscope even after twentyfold dilution of the dyes by the various vehicles.

The relatively diffusible blue dyes, brom phenol blue and Neptune blue, were in watery isotonic solution, at about 4 per cent and 5.5 per cent respectively. When these solutions were diluted twenty times with the various vehicles, to yield mixtures comparable to those of the indiffusible dyes previously used, the dye-stained fluid within the lymphatics appeared distinctly weaker in color than pontamine sky blue and Chicago blue. But despite this fact, it was easy to detect the greater diffusibility of the coloring matter because escape from vessels containing it was so early and pronounced.

More effective comparisons of the rate of passage of the two groups of dyes from the lymphatics were obtained when the concentration of the dyes was increased, 0.2 cc. or 0.3 cc. of the isotonic dye solution being added to 2 cc. of diluent. The resulting solutions yielded decisive evidence of the differences in diffusibility already described.

The results of these experiments can be summarized briefly. Pontamine sky blue escaped from the lymphatics with more difficulty than any other of the blue dyes, and Chicago blue 6B was but slightly more diffusible. Brom phenol blue, trypan red, and Neptune blue passed out from the lymphatics with far greater ease, the last very swiftly.

The fact is well attested that the rate of vital staining with the acid dyes varies with their diffusibility as determined *in vitro* (12). So too does the rate of their passage outwards through the capillary wall (6). The present observations prove that the same holds true of their escape through the lymphatic wall. Furthermore dyes which escape from the blood capillaries with difficulty escape from the lymphatics with difficulty too, the ultimate limit of permeability being much the same for the two vascular membranes, despite the greatly differing hydrostatic and osmotic conditions under which they function.

DISCUSSION

Previous knowledge of the permeability of the minute lymphatics has been inferential in the main, being based upon comparisons of the blood and of the lymph obtained from ducts of sufficient size for cannulation. By our method the permeability of the wall of the smallest lymphatics can be directly tested. But the fact should be stressed that this permeability is tested in the direction opposite to that of normal flow,—a flow which, in our material, the ear of the mouse, is by no means negligible. It will be well, before discussing

the findings by our method, to sum up the conditions under which they were obtained. In the majority of our experiments the dyes were injected into the tissue of the ear in approximately the same concentration in which they circulate in the blood when injected intravenously for vital staining. In this concentration they have proved innocuous to the vascular endothelium. The lymphatics draining the injected area promptly filled with the colored fluid,—under negligible pressure in the circumstances of the case. It was the secondary escape of the dyes from these lymphatics that was studied. Their outlines as well as the character of the escape showed plainly that they are, physiologically speaking, well defined, closed channels, a fact which accords with the prevailing view of anatomists.

When a dye is injected into the ear of the mouse there is some gradual misty extension through the tissue from the region in which it is at first located, over and above the frank lymphatic drainage. This extension is in the direction of the base of the ear and is apparently interstitial in nature, the most careful observation failing to disclose that it takes place along definite channels. The lymphatics demonstrated by the dye are of very considerable size, in general far larger than the blood capillaries, and are as big at the margin of the ear as at the base, and, except for the trunks at the base of the ear, which do not let dyes through so readily, are equally permeable everywhere, irrespective of their size. There is, in other words, no gradient of lymphatic permeability.

It is plain that in general we have dealt, not with collecting lymph ducts but with the lymphatic capillaries or radicles. These take up dye so swiftly from an injected area that to all intents and purposes the latter may be thought of as giving directly into them, despite the evidence afforded by the experiments of an anatomical (and physiological) barrier. It would doubtless be possible with the finest micropipette to inject tissue without damaging the lymphatics; but under the circumstances of our work, and under those of injections through hypodermic needles one must suppose that some of the lymphatics are torn. That they do not close down like injured blood vessels but function at once for drainage is indicated by our findings. More will be said upon this theme in a later paper.

In view of the continuous and considerable passage of fluid into the

lymphatics of the ear in normal situations, as disclosed by the current in the channels, the escape of dyes in the opposite direction, that is to say from the lymph into the tissue, is especially worthy of remark. But the lymphatic plexus has a very large surface area, whence it follows that the fluid trend through the wall at any one point is negligible; and furthermore the wall is exceedingly permeable as our results show. These indicate further that salt solution is injurious to the lymphatic barrier and that the escape of dye through it from other fluids varies directly with the diffusibility of the dye, as manifested *in vitro*, and with the character of the fluid menstruum. The more blood protein the latter contains the less is the escape of dye, as would follow both from osmotic conditions and from the circumstance that dyes become in some part adsorbed upon blood proteins (13). The most indiffusible of the dyes we have employed (pontamine blue) is more diffusible than blood proteins, whence it follows that if it is retained by the lymphatic wall, as was the actual case, proteins will be retained also. That the escape of dyes from the blood stream is markedly conditioned by the amount of plasma proteins will be shown in a succeeding paper from this laboratory.

The fact is well recognized that substances of small molecule pass through the walls of the blood capillaries with such ease as to be to all intents and purposes unaffected in distribution by the direction of fluid flow (8). Any alteration of the blood salts and of sugar causes a practically instantaneous readjustment of their relations in plasma and tissue fluids. The most diffusible of the dyes that we have used is far less so than the substances just mentioned, and it is not surprising that while they appear very swiftly outside lymphatics carrying them, they do not immediately attain the same concentration outside as in. One might be disposed to invoke some special mechanism facilitating the passage of substances from the tissue spaces to the lymphatics, as against passage in the reverse direction, were it not that when the dyes are injected into the blood stream the same lag in their distribution is observed, although in this case under circumstances that would seem to favor escape. The fact that the lymphatics remain invisible in tissue stained by the distribution of dyes from the blood instead of being perceptible by reason of a more lightly stained contents is not surprising since poorly diffusible dyes spread so slowly in the interstitial

spaces that there is ample time for the lymph to color like the interstitial fluid, while to highly diffusible dyes the lymphatic wall is an inconsiderable barrier. Furthermore the general coloration would tend to obscure the lymphatics. All of the evidence that we have obtained supports the view that the permeability of the lymphatic wall resembles the permeability of the capillary wall in its essential features and perhaps in its degree.

Certain observations in the course of the work suggest that some of the existing lymphatic channels are not utilized ordinarily. The channels in question are very narrow and are unprovided with valves, yet are so sharply demarcated from the surrounding tissue that they must be considered as preformed channels.

SUMMARY

A technique has been developed for the demonstration of lymphatic capillaries in the ear of the mouse by means of vital dyes and for tests of their permeability under normal and pathological conditions. The lymphatics become visible as closed channels from which the dyes escape secondarily into the tissue. Some of them, cross-connections, with extremely narrow lumen, would seem ordinarily not to be utilized.

There is active flow along the lymphatics of the mouse ear under ordinary circumstances. The movement of dye was always toward the main collecting system. The valves of the lymphatics as well as fluid flow prevented distal spread. There was in addition slow migration, apparently interstitial in character, but in the same general direction, of dots of color produced by the local injection of dye.

The normal permeability of the lymphatics was studied with dyes of graded diffusibility. Their walls proved readily permeable for those highly diffusible pigments that the blood capillaries let through easily, but retained those that the latter retained. Finely particulate matter (India ink, "Hydrokollag"), they did not let pass. No gradient of permeability was observed to exist along them such as exists along the blood capillaries of certain organs.

The observed phenomena of lymphatic permeability, like those of the permeability of the blood capillaries, can be explained on the assumption that the lymphatic wall behaves like a semipermeable membrane.

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EXPLANATION OF PLATES

PLATE 11

FIG. 1. Ear of a living anesthetized mouse, photographed by reflected light, 16 minutes after the injection of 1 per cent pontamine blue solution in a mixture of mouse serum 1 part and Tyrode's solution 3 parts. The plexus of lymphatics, rendered sharply visible by their stained contents, lies in the corium. Other, deeper lymphatics can be dimly seen. No dye has passed out from the lymphatics though these have been full of heavily stained fluid since the injection was made 16 minutes before. The channels are wider than all save the largest of the blood vessels, which latter are visible in gray. ×10.

FIG. 2. Ear of a living mouse photographed *in situ* 5½ minutes after filling the lymphatics with a 1 per cent pontamine sky blue solution in a mixture of Tyrode's solution 3 parts, and mouse serum 1 part. Several sharply localized ecchymoses of dye can be seen, though the channels are in general impermeable to it as yet. Immediately before photographing the ear a cover slip was placed over it. ×8.

PLATE 12

FIG. 3. Ear of a living, anesthetized mouse with the lymphatics containing 1 per cent pontamine sky blue in Tyrode's solution. 6 minutes after injection of the dye, its escape into the tissues from the lymphatics has just begun. ×10.

FIG. 4. The same ear photographed 5 minutes later, that is to say 11 minutes after injection. The color has extended further from the lymphatics, owing to progressive escape of the dye and its secondary spread in the interstitital spaces.

Note that the dye escape is more rapid and diffuse than in the ear of Fig. 1. In

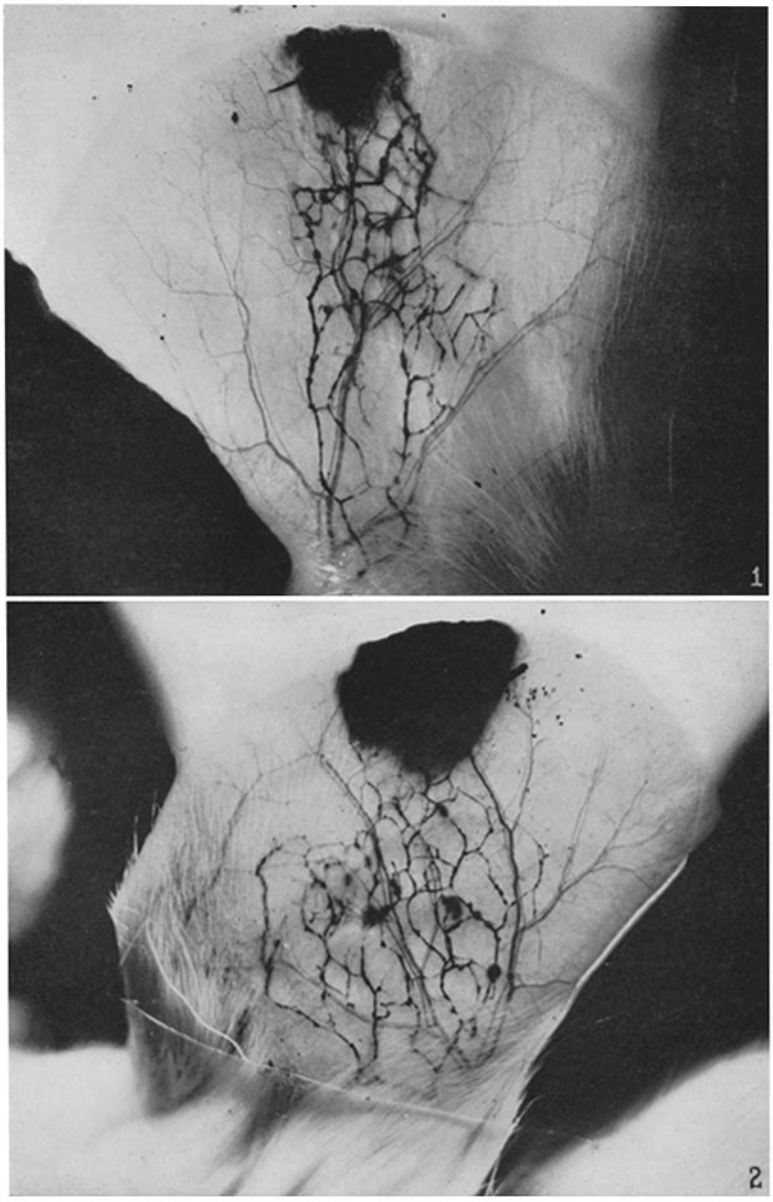
that experiment, a similar concentration of dye was injected but in a protein-containing vehicle. ×10.

PLATE 13

FIGS. 5 and 6. An increase in the concentration of dye within the lymphatics leads to its greater escape. The figures are to be compared with Figs. 3 and 4.

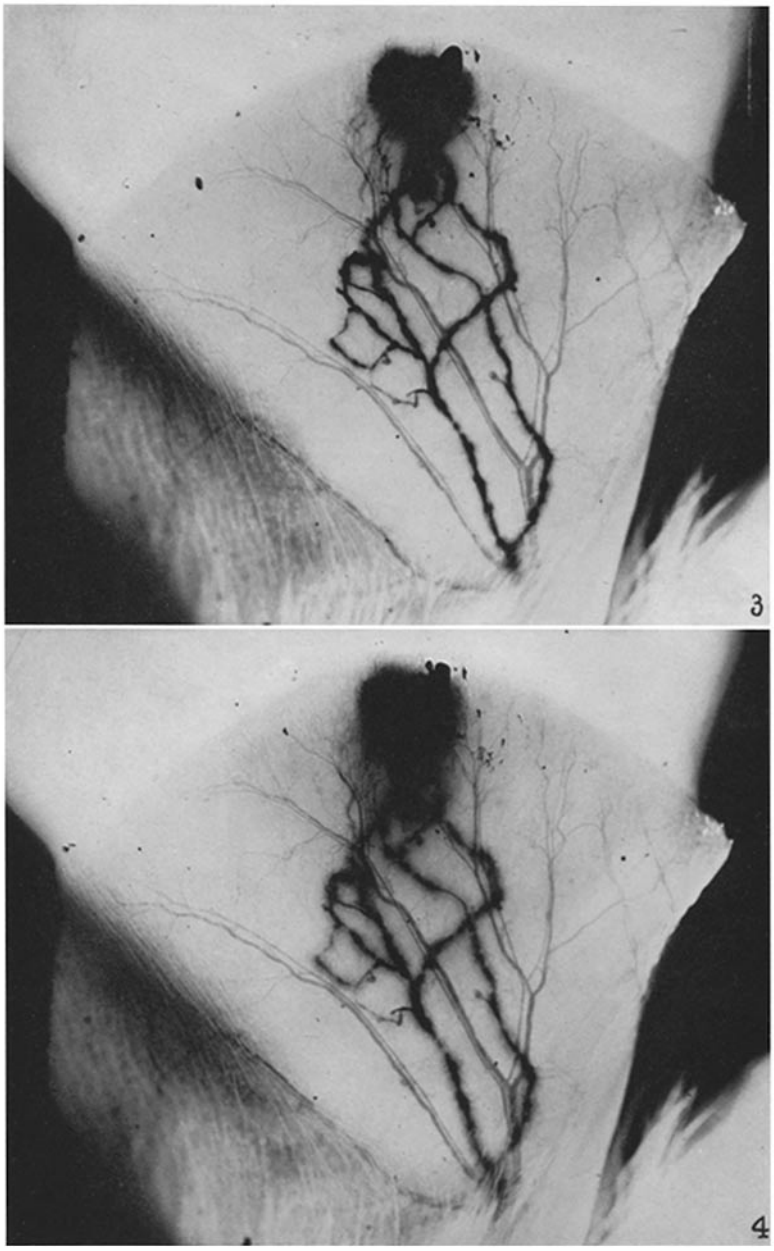
Ear of a living anesthetized mouse, *in situ*, injected with a 4 per cent pontamine blue in Tyrode's solution. The dye concentration was increased fourfold over that employed in the ear photographed in Figs. 3 and 4. The photographs were taken at the same time after injection, 6 minutes and 11 minutes respectively.

There has occurred a profuse escape of dye all along the lymph channels, with a marked secondary distribution through the tissue during the period between the two photographs. ×8.



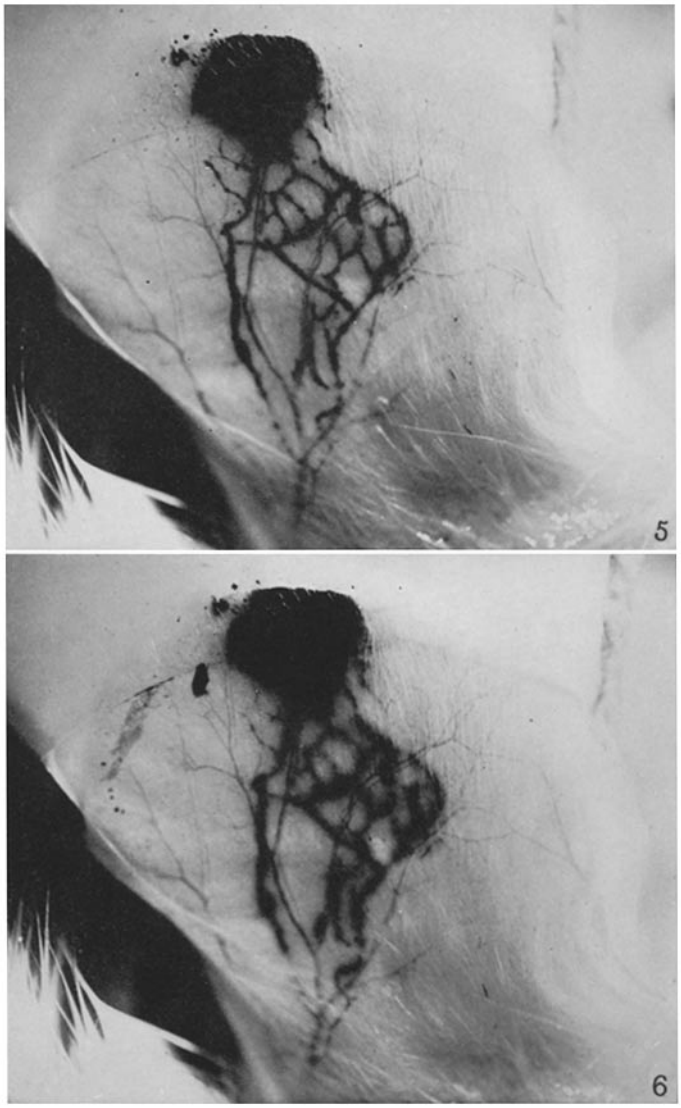
Photographed by Louis Schmidt

(Hudack and McMaster: I. Permeability of lymphatic capillary)



Photographed by Louis Schmidt

(Hudack and McMaster: I. Permeability of lymphatic capillary)



Photographed by Louis Schmidt