THE PARTICIPATION OF SKIN LYMPHATICS IN REPAIR OF THE LESIONS DUE TO INCISIONS AND BURNS

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The lymphatics of human skin participate in the responses to very slight injury. As our previous work has shown (1), poorly diffusible, vital dyes, intradermally injected, enter the superficial lymphatic capillaries of the skin, and ordinarily are retained by them for several minutes before escape occurs into the interstitial tissue. Conspicuous increases in the permeability of the walls of these vessels, as manifested by an almost immediate escape of dye from the channels, follow such stimuli as the application of gentle heat, ultraviolet irradiation, or a firm stroke over the surface, too slight to abrade the skin. Studies of the lymphatic capillaries in the ear of the mouse have disclosed similar changes in the permeability of the walls of these vessels (2, 3). Here too a scratch insufficient to break the skin, a light stroke with a blunt instrument, exposure to ordinary sunlight, or an increase in temperature to but 5° above body heat suffices to induce a great transient increase in the permeability of the lymphatic wall.

The changes described have been effected without causing frank injury. In the present paper we wish to report upon the alterations in the permeability of the walls of lymphatic capillaries of the mouse ear in and about regions subjected to burns or incision. Our purpose has been to examine the responses of lymphatic capillaries under frankly pathological conditions, with special reference to the functioning of these channels during the formation and resolution of sterile inflammatory processes.

Methods

We have found the ear of the mouse an ideal structure in which to observe directly the changes in lymphatic permeability as they occur in living tissue.

As in the preceding work (3), mice of 16 to 18 gm. body weight were best suited to our purpose. In all of the experiments the animals' ears were observed at various intervals after injury had been produced under an anesthetic. When the interval was many hours or several days, ether anesthesia was used when the injury was produced and the final observations were made under complete sodium luminal narcosis. When the times of injury and final observation fell within a period of only a few hours, one anesthetization with sodium luminal sufficed for both. As in the earlier work, 0.125 cc. of a 2 per cent aqueous solution of freshly dissolved sodium luminal was given subcutaneously for each 10 gm. of body weight. All examinations were made under the binocular microscope by a method already described (2).

To render the lymphatic capillaries visible and to test for alterations in the permeability of the lymphatic wall, a highly indiffusible vital dye, pontamine sky blue (2, 3), was injected locally into the skin of the upper surface of the outer edge of the ear. It enters directly the lymphatics draining the region through rents caused by the injecting needle, and extends along the local network rendering it clearly visible (3). In all the experiments save where specific mention has been made, 0.1 cc. of fresh aqueous isotonic pontamine sky blue (21.6 per cent) was added to 2 cc. of a mixture of 1 part mouse serum and 3 parts Tyrode's solution, yielding a final mixture of approximately 1 per cent of dye in an isotonic menstruum of protein content similar to that reported for peripheral lymph (4–7). This was regularly used to obtain comparisons of the rates of dye escape from the lymphatics after different injuries or at varying intervals after a standard injury. It will be referred to as standard pontamine solution. When it entered the lymphatic channels of the normal ear, no perceptible dye escape took place from them for 10 to 15 minutes. In many of the present experiments, on the other hand, so rapid an escape of dye resulted in consequence of the injury to the lymphatics that we were led to employ a suspension of particulate matter to test whether or not there were openings in their walls. For this purpose dialyzed non-waterproof India ink (Higgins' American drawing ink), or else "Hydrokollag," was injected together with the dye solution. The method of preparation of these suspensions has already been described (2, 8). Further to compare changes in the permeability of the blood vessels of the injured ears with the changes occurring in the lymphatics, pontamine sky blue was injected intravenously into many of the mice. The dye was given as described in earlier papers (9–11), employing 0.05 cc. of a 21.6 per cent solution mixed with an equal volume of Tyrode's solution.

To study the physiology of the lymphatics during the progress and restoration of wounds or inflammatory processes, and to render our results comparable from animal to animal, it was necessary to devise methods of traumatization which could be standardized for repetition. This was accomplished in several ways to be described below. Heat was applied in such a manner that mild first, second, or third degree burns were obtained at will, the more severe burns resulting in necrosis.

The Lymphatic Changes Resulting from Skin Incisions

The effects of cutting through the skin were first observed. The incisions were made under the binocular microscope, so that the depth and extent of the wound could be accurately controlled.

The part played by the lymphatics in the process of wound healing is not known. In descriptions of the phenomena of wound healing there is little if any mention of these vessels. How do they behave during inflammation? What is their share in the healing of a wound? Do they become blocked like the blood capillaries when they are cut across? When they reconstitute during the healing process are the lymphatics more permeable than normal or less so? These and many other questions suggest themselves.

In our experiments the lymphatics of the skin of the mouse's ear were rendered visible by local injections of standard pontamine solution, at various times after incising the upper surface of the ear superficially or deeply. In the majority of instances healing took place rapidly and the behavior of the lymphatics could be studied during the healing processes.

Mice were anesthetized with ether and under the binocular microscope cuts about 1 cm. long were made half way between the tip and the base of one or both ears. In some cases the incisions were very superficial, involving only the corium and the subpapillary layer at most, and in these no hemorrhage developed. Probably only the most superficial lymphatics were severed. In other instances incisions were made deeply enough to cut the larger veins and arteries, while in some the upper surface was cut through to the cartilagenous plate of the ear. In still other experiments the cut went completely through the ear. Save in the animals with superficial incisions, more or less hemorrhage ensued, varying from small capillary ecchymoses to frank bleeding of the largest vessels.

A considerable number of mice were operated upon at one time, and at half hour intervals for 7 hours afterwards, groups of three animals each were anesthetized with luminal and local and intravenous injections of dye were made to render the blood vessels and lymphatics visible. On subsequent days other groups of six to ten animals were examined in the same way until all signs of wound reaction had disappeared.

The behavior of the lymphatics after incision differs strikingly from that of the blood vessels in certain respects. Where cut across the lymphatic channels remain open for a variable period of time,

often for 24 to 48 hours or more, instead of constricting at once and closing as do the blood vessels.

Many of the blood corpuscles escaping into the tissue during the hemorrhage after incision pass directly into the lymphatics that are laid open, and continue to do so for many hours. Large numbers of erythrocytes are often found in the lymphatics both distal and proximal to the cut, as late as the day after making the incision. In instances showing this the lymphatics about the cut stand out clearly by reason of the contained blood. Clotting has not occurred and with a micro-spatula the intralymphatic cells can be moved in either direction. In relation to this finding, Clark and Clark (12) in their studies with transparent chambers in the rabbit's ear, report that holes made in lymphatics at the time of operation remain open several days.

When standard dye solution was injected at the ear margin beyond a transverse cut on the same day that the cut was made the channels at the periphery of the ear filled as usual but then emptied their stained contents directly into the incision where they had been cut across. Fig. 1 shows a photograph of the ear of a mouse injected in the usual manner 5 hours after a transverse incision. The wound became filled with blue dye which flowed without hindrance from the severed lymphatics. In a few experiments, instead of injecting the incised ears a tiny crystal of dye was pushed, under the guidance of the microscope, into a minute intradermal puncture wound at the periphery of the ear. The regional lymphatics took up the dissolving dye and within 15 to 20 minutes colored fluid could be seen passing from the severed lymphatic capillaries into the incision. Constriction and spasm of the small blood vessels severed were so immediate and effective, on the other hand, that often no bleeding occurred.

In eight instances within half an hour to 5 hours after a superficial cut had been made, a drop of standard dye solution was placed on the incision. In 3 or 4 minutes colored fluid appeared in the lymphatics on both sides of the wound. Those distal to it lying between the incision and the ear tip filled for the distance of a single pear-shaped segment only, that is to say to the nearest valve; those proximal, extending from the incision to the base of the ear, drained away dye solution toward the head. The finding was not invariable but was frequent enough to prove conclusively that the lymphatics were still open. Whether or not they took up dye seemed to depend upon whether it gained access to them through the fibrin clot in the wound.

The local injections at the ear margin showed not only that the severed lymphatics had failed to close but that the lymphatic capillaries distal to the cut but severed by it were more permeable than usual. Dye began to escape through the walls of these channels within 2 or 3 minutes after it had entered them, and always many localized

dye ecchymoses were seen, as though the channels had received direct local injury, which was not the case (2). In the unharmed ear standard pontamine solution remains within the channels for 10 to 15 minutes before any visible escape takes place (3, 2).

In most instances in which local injections of dye were made at the outer ear margin after a transverse cut had been made, not only were those lymphatic channels filled which drained directly into the cut, but others which skirted it at either end. These intact vessels carried the dye as usual to the base of the ear but they also passed along some of it, by side branches, into the region directly proximal to the injury and even discharged some into the wound itself by collaterals connecting with the open lymphatics in its proximal margin. Like the channels distal to the incision, the proximal collaterals showed an increased permeability to standard pontamine solution, dye beginning to escape from them 2 or 3 minutes after it had entered. Both distally and proximally to the wound, therefore, the lymphatics were more permeable than elsewhere in the ear. Within 5 or 6 minutes after an injection the margins of the cut for a distance of 2 or 3 mm. were stained a bright, diffuse blue.

The results of intravenous injections of dye on the same day the cut was made furnished an enlightening contrast to these findings.

The animal gradually became blue all over save for an area of pallor in and about the incision for a distance of 2 to 3 mm. It was plain that occlusion of the severed blood vessels and spasm of those near-by prevented dye from escaping into the immediate region of injury. Just beyond the area of pallor, the blood vessels allowed more dye to escape than elsewhere in the ear, and within 5 minutes after an injection the pallid incision was surrounded by a ring of diffuse blue stain

Sixteen animals were examined under sodium luminal anesthesia on the day following incision.

These had received superficial cuts in one ear,—extending only through the subpapillary layer of the corium,—and deep cuts in the other ear, reaching to the cartilaginous plate and severing the overlying blood vessels. In all instances both ears were hyperemic and moderately edematous, with the edema most marked distal to the incision, though definite on the proximal side as well. In such regions the ears showed "pitting upon pressure" by a blunt instrument. They were thick, the skin tense, and when punctured by a sharp needle fluid escaped. The wound was ringed by dilated, tortuous capillaries.

After injecting standard pontamine solution intradermally at the ear margin on the day following incision the lymphatics draining the region filled as usual and carried colored fluid to a point about 2 mm. from the incision. In most instances

there was in this region a tendency for the lateral connecting channels to divert the dye to the extremities of the wound, and thence into main trunks leading around the incision toward the ear base. In four animals, though, dye solution also entered the wound showing that some of the severed lymphatic capillaries were still open. As a rule in each animal many dye ecchymoses occurred distal to the wound, but not elsewhere in the ear. Even 20 minutes after injecting the ear but very little dye escape had occurred from the lymphatics in the region proximal to the incision. In a few instances the channels distal to or beyond the cut and several millimeters from its edge were not only highly permeable while those proximal were not, but the former were often dilated and the latter constricted. In those animals in which dye solution from the lymphatics failed to stream directly into the cut, the slightest increase in the pressure of injection served to make it do so. The resistance to the flow of dye into the wound seemed to be located not at the edge of the wound itself, but 2 or 3 mm. away from it; for once dye had been forced through this region it readily entered the incision.

Fig. 2 shows the result of an injection of dye into the margin of an ear which had been incised 24 hours previously. The photograph was taken 5 minutes after the injection. Although some dye entered the incision, by far the greater part was shunted around it as described. The greatly increased permeability of the lymphatics distal to the cut is shown by the abundant escape of dye from these areas. As yet no dye escape had taken place from the channels proximal to the wound.

In four of the sixteen instances examined in this way, dye solution, injected at the ear margin, not only streamed into the wound but passed on directly through the incised region into the channels beyond. A photograph of the phenomenon is reproduced in Fig. 3. It shows the passage of dye in three channels either directly through the incised area or perhaps immediately under it and very close to it, with ecchymosis of colored substance into the incision. The extreme permeability of the channel walls distal to the incision and the lack of proximal dye escape are plainly to be seen.

Can it be that actual reconstitution of these channels had taken place or were they merely injured and not severed by the cut? The question is not to be answered from a single preparation such as the one shown.

To gain light upon it, the ears of six mice were incised transversely and the ear margin injected with dye. In each instance dye entered the incision through three or four channels but was not directly carried beyond, showing that the lymph channels had been severed. Diagrams were drawn to show the distribution of these channels, with the blood vessels as landmarks. The following day, when the ears were re-examined, the colored fluid in the distal portion of the charted lymphatics had completely disappeared. Dye injections were again made at the edge of the ear of three of the animals, inserting the needle through the same puncture wound and expelling dye in the same spot as before. In all, the same channels previously rendered visible by dye solution were again filled, and in

addition, in each ear two or more channels unseen the day before. In two of the three instances one or two of the lymphatics which had filled on first injection and carried dye solution into the incision, now carried dye through this region into a continuation of the channel on the proximal side, allowing it to flow to the base of the ear. In the cut region profuse ecchymoses of dye solution took place immediately, and distal to the wound the lymphatics allowed dye to escape 3 or 4 minutes later, the normal interval between injection and dye escape being about 10 to 15 minutes. Proximally, no dye appeared outside of the lymphatics in 20 to 25 minutes.

Two of the remaining three animals were examined the next day and the last of them on the 3rd day after incision. In these similar results obtained save that with each day more channels were demonstrable traversing the wound area, and delivering dye solution to their continuations beyond. The evidence showed that the severed lymphatic capillaries reunited rapidly.

In four other mice bearing incisions 24 hours old intravenous injections of pontamine sky blue demonstrated an excessive permeability of the dilated tortuous capillaries and venules about the wound. Within 4 or 5 minutes the region was stained a deep blue by profuse dye escape from vessels there, but not elsewhere in the ear. No patent blood vessels entered the wound as yet. Nevertheless there appeared to be a rapid turnover of fluid within the incision; for, in the instances just described, when dye was brought into the incised region from the ear tip by way of the lymphatics, the resulting colored ecchymoses in the neighborhood of the injury disappeared far more rapidly than elsewhere in the ear.

In ten instances the usual routine dye test was postponed for 48 hours after making the wound. The cut ears were still edematous and hyperemic but the edema fluid was transparent.

In all these animals, after local intradermal injections of standard pontamine solution at the ear margin, dye failed to pass into the incised region. In six of the ten instances, no dye reached the margin of the wound, the lymphatic channels filling to a point 2 or 3 mm. distal to the cut and piping the dye through small lateral channels to other larger lymphatics which passed around it. When the pressure of the injection was increased slightly three or four lymphatic capillaries carried dye freely into the cut.

As in our earlier experiment, the resistance to flow of the dye toward the wound seemed located several millimeters distal to it and might be attributed either to pressure from the edema or to the existence of fibrinous plugs which were dislodged by the pressure. In four of the ten instances in which the pressure of injection was not increased some dye solution reached the incisions directly, colored fluid either pouring into the cut from the open ends of the channels or passing by way of a channel that was now intact, but, with profuse ecchymosis of dye into the cut. It was plain that in the wound area itself the severed lym-

phatic channels had either remained open or had reconstituted. They had not been blocked.

In all of the animals examined 2 days after making the skin incisions, the channels distal to the wound appeared much wider than those proximal, and the bulbous swellings were broader and larger. After entering the lymphatics dye began to escape within 4 or 5 minutes while from the proximal channels none appeared in half an hour.

The Minute Lymphatics in Later Stages of the Healing of Incisions

Daily, for 10 days, groups of four or five mice, taken from a number with ears incised at the same time, were anesthetized with luminal and examined for changes in the behavior of the lymphatics during the process of wound healing. The early findings differed but little from those already described, such differences as were noted being attributable to variations in the severity of the wounds rather than to the passage of time. A week after incising the skin of the ear edema was still present both distally and proximally to the wounds. The whole ear still showed moderate hyperemia. Under the binocular microscope many new-formed blood vessels were visible extending into the injured area, more of them on the proximal side. On both sides large numbers of tortuous blood vessels with rapid circulation were present, often with reversed venous flow in some of the larger veins.

The introduction of dye into the lymphatics disclosed a variety of changes. The channels distal to the cut were still far more permeable than normally, profuse dye escape through their walls beginning in 2 to 4 minutes after it had entered them. This was observed even 12 and 14 days after superficial cuts had been made in the ears. At this time the skin appeared healed to the unaided eye. The lymphatics appeared widely dilated either as a whole or in segments. The bulbs adjacent to the valves also appeared greatly elongated and widened, giving the appearance of aneurysmal swellings.

In all these experiments the region immediately next the healed edges of the incision was not entered by the dye, save in each instance by three or four channels which transported colored fluid through the incision into their continuations beyond. In passing the region

of injury profuse ecchymoses of dye occurred, as in the dye experiments done 2 or 3 days after incising the ears. By far the greater amount was passed into lateral channels about 3 mm. distal to the incision, and by them to lymphatics which ran around the wound. Usually at the extreme ends of the incision where healing had just begun several trunks filled with dye and passed it on toward the base of the ear.

New Formation of Minute Lymphatics in Areas of Repair

Occasionally in animals tested on the 6th and 7th days, and characteristically in those examined later from the 8th day on, evidence of new formation of lymphatics was obtained.

When a local injection of standard pontamine solution at the margin of the ear was watched under the microscope one could nearly always observe the column of dye-stained lymph advancing down the lymphatics as usual. But a few seconds later, instead of stopping or being carried around the injury, it spread into a close network of very minute channels, lymphatic sprouts, forming a dense reticulum on the distal edge of the incision itself. Scarcely did dye reach these before it began to escape, long before any had come out from the channels in the uninjured portion of the ear. Within 1½ to 2 minutes the semicircle of the distal circumference of the incision assumed a diffuse blue. Such channels were never found in ears examined less than 6 days after an incision.

Fig. 4*a* shows an ear incised 9 days previously and now injected intradermally at the margin with India ink purified by dialysis. It was amputated at once after injection and photographed while submerged in neutral paraffin oil. At the left end of the incision, one sees several channels which conducted ink through the region of injury. On the right side reconstitution of channels, or a collateral circulation has not yet developed. In the incision itself many very narrow, linear, parallel lymphatics, situated in the new tissue filling the incision are disclosed by the ink. They lie at right angles to the main trunks, having the direction of the original cut. They are better shown in Fig. 4*b*, an enlargement of the same specimen. Unfortunately India ink, owing to its particulate character, fails to fill as many of the very minute lymphatics as a dye solution and it gives no true idea of the richness of the new-formed plexus. When standard pontamine solution was employed in such experiments it escaped so rapidly from the small vessels that good photographs could not be taken though the abundance of new-formed lymphatics was plainly disclosed.

When local injections of dye were made, 1/2 to 3/4 cm. *proximal* to the incision, similar networks of newly formed lymphatic capillaries were found in its proximal lip. Retrograde passage of dye occurred showing that the new capillaries were

without valves. They were exceedingly permeable as shown by the almost immediate escape of dye. The old channels on the proximal side of the wound failed to allow dye to escape in half an hour.

To study the relation of the new-formed lymphatics to regenerating blood vessels, the ears of 20 mice were incised in the usual way and after 10 days the animals were anesthetized and injected intravenously with 0.05 cc. of isotonic aqueous pontamine sky blue solution. Two minutes after the injection was completed, a local injection of 2 per cent vital red in the usual menstruum of 1 part mouse serum and 3 parts Tyrode's solution was injected intradermally at the ear margin. The dense plexus of new-formed lymphatics about the wound filled with red dye and the newly formed blood vessels with blue. The two types of vessels entered the healing area about equally far and both were much more permeable than those elsewhere in the ear. In the center of the healing wounds neither new-formed blood vessels nor lymphatics were visible. In about half the instances the incisions were traversed by two or more larger lymphatics, which passed directly through or just beneath them. No blood vessels appeared in this area.

The Lymphatic Capillaries in and about Burned Regions

To study further the lymphatic capillaries in the periods of formation and recovery from inflammation, advantage was taken of the fact that sharply localized, standard burns of similar intensity could be made in the skin of the ear of the mouse.

Injury by Heat.—Anesthetics were used, as already described. To obtain comparable burns we adopted modifications of methods to apply mild heat stimuli to the ear of the mouse (2). Several thin-walled glass water chambers were blown in various shapes and sizes with a flat surface that could be placed against the animal's ear. Each possessed three openings, one for the insertion of a thermometer and two for circulation of water at the desired temperature. One of these chambers possessed a flat oval surface only 3 mm. in diameter. To produce localized burns, hot water was circulated through the chambers and the ears of the anesthetized mice were gently held against the flat surface. By varying the temperature of the water or the duration of contact of the chamber with the ear, first, second, or third degree burns could be produced at will.

"Stigmatic Burns."—Strictly localized, marked superficial burns, which we may term stigmatic burns, were obtained in another manner. An ordinary bac-

teriological platinum inoculating wire was heated at its mid portion by a microburner until the free tip glowed dull red. The etherized mouse lay with the ear resting on the tip of the index finger and the hot wire was quickly and lightly touched to the central part of the upper surface of the ear. Later at intervals the animals were reanesthetized with sodium luminal in groups of ten, as the experiments required.

Stigmatic burns usually healed rapidly in a few days but occasionally progressed to necrosis, resulting in a neat perforation of the ear, as though it had been punched out.

The burns produced by the small water chambers and the hot platinum wire involved but a small part of the animal's ear, at its middle.

Changes in the permeability of the blood vessels were determined as described earlier in the paper. To test lymph permeability, standard pontamine solution was employed in the usual manner.

The local injections of standard pontamine solution into the tissue at the margin of the ear resulted in its entrance into those lymphatic capillaries which lay in uninjured tissue but which passed through the burned regions and emerged again into normal tissue. Other channels not directly involved in the burn but surrounding it, both near and remote, also took up the dye solution. Control observations were made by injecting the uninjured ears of the same animals. As the processes of repair were often protracted, dye injections were made soon after injury in some instances, in others at half hourly intervals up to 6 hours,—and, finally, at daily intervals up to 10 days or 2 weeks. Always before injecting the standard pontamine solution, the condition of the circulation of the ear was determined under a binocular microscope. The mice lay in plastalene moulds with the ears lying horizontally on porcelain placques and illuminated as previously described (3, 9).

Experiments were done on four series of mice with the water chamber at 46.0°, 53.0–55.0°, 59.0–60.0°, and 67.0°C., respectively. In yet another large series of animals stigmatic burns were produced. It was found that increasing degrees of heat or a longer application simply increased the severity of the reaction. When the burns were severe enough to cause permanent arrest of blood flow the lesions progressed to necrosis within 36 to 48 hours. Such burns almost invariably followed application of the chamber to the ear for 1 minute with water circulating at 59.0–60.0°C., or for as short a period as 20 seconds with the water temperature at 67°C.

When a small spot of skin on the mouse's ear approximately 3 to 5 mm. in diameter was heated at 55°C. for approximately 1 minute moderately severe burns resulted. In the sharply localized region of injury circulation in the smaller vessels was stopped, and often, even in the largest radial veins and arteries, blood flow ceased temporarily. A few seconds afterwards a reactive hyperemia occurred about the heated area, which in 2 or 3 minutes extended over the whole ear. After a few minutes, pronounced edema formed in and about the burn, that is to

say the ear became thicker there, the skin tense, and, to a blunt needle, there was "pitting on pressure." Puncture with a sharp needle led to the escape of fluid, and under the binocular microscope the edematous area assumed a ground glass appearance.

The Immediate Effects of Severe Burns.—When standard pontamine solution was injected into the outer margin of the ear, a few minutes after heating an area midway between the tip and the base, an extraordinary increase in the permeability of the walls of the lymphatics in the burned area was manifest. Dye passing into them from the normal channels nearer the margin at once escaped through their walls into the surrounding tissue.

Fig. 5*a* shows the escape of dye from the lymphatics in a burned area only 2 minutes after dye injection into an ear submitted to the water chamber at 55° for 40 seconds, 8 minutes previously. Such contact usually resulted in a third degree burn with temporary stoppage of the circulation for several hours and severe edema.

The region of dye escape from the channels coincides with the burned area. From the lymphatics in the uninjured portion of the ear there has been no escape of dye. In Fig. 5*b* the same preparation is shown 2 minutes later. Much more colored matter has now escaped from the channels in the burned area and it is distributed more widely.

In ten instances dialyzed India ink was injected together with standard pontamine solution into the margin of ears which had been burned in the same way. Although dye escape in the injured region was prompt and abundant the ink particles failed to pass out and the lymphatic capillaries were clearly defined by it.

Fig. 6 illustrates the immediate findings in a burn of lesser degree when there had been time for the formation of edema. The water chamber at 55°C. was in contact with the ear for 20 seconds. An hour later the standard pontamine solution was injected, the photograph being taken after another 4 minutes. The area of the burn, which corresponds with that of the escape of dye from the lymphatics, was already edematous, yet the channels penetrating it carried much of their contents into the uninjured tissue beyond, where no dye escape from the lymphatics occurred. Ink escaped from none of the lymphatic channels of such burned areas.

When the chamber containing water at 67°C. was applied to the ear of the mouse for 30 seconds or more, or when the ear was touched

with the hot platinum wire, marked punctate burns resulted. As in the experiments in which moderate burns were induced, so in these, stasis in the injured area and reactive hyperemia round about occurred immediately. Edema followed.

When isotonic pontamine sky blue was injected intravenously 10 to 20 minutes after a pronounced contact or stigmatic burn, the blood vessels surrounding the ischemic burnt area were unusually permeable. Within 2 to 4 minutes after the injection, there could be seen about this area a collar of deeply stained tissue, colored by the escape of dye from the blood capillaries and venules close to the burn and long before escape from blood vessels elsewhere in the ear was visible. The tissue within the burnt region itself remained ischemic and therefore no dye entered it.

In contrast with these findings, following a local intradermal injection of dye at the margin of an ear burned in the same way, the lymphatics passed much of the colored fluid into the injured region itself where it escaped from the channels.

In twelve instances, at varying intervals from 3 minutes to 1½ or 2 hours after producing the burn, standard pontamine solution was injected into the skin of the ear, close to its outer margin. In more than half of these, lymph channels could be made out traversing the burned area, but the escape of dye from the portions of these within the burns was so immediate and so great that one might almost suppose that the cellular membrane of the lymphatic wall no longer existed as such. Yet, when the ear was reinjected, in five of these instances with dialyzed India ink or "Hydrokollag," the walls of the lymphatics were demonstrated to be still continuous, for none escaped. In three other experiments standard pontamine solution and India ink combined, injected 2 hours after the production of the burns, was seen to enter the lymphatics as usual; and these carried both dye and India ink through the punctuate, burned area into the lymphatics beyond, though there was some dye loss during the passage.

When the time interval between the production of the burn and the dye injection was increased to 4 or 6 hours, dye often failed to reach the ischemic injured area by way of the lymphatics. The dye was carried to within 2 or 3 mm. of the burn and there immediate and profuse ecchymosis of it occurred. The channels which passed around but close to the burn were extremely permeable so that it was surrounded by a dense cloud of diffuse blue color, while the edematous burn itself remained colorless. In two instances a lymphatic channel carrying dye did penetrate the burn and pass through. As result the patch was stained blue owing to the escape of dye from the lymphatic within it.

When dye escapes from lymphatic capillaries which lie outside an edematous burned area the diffuse blue color it gives to the tissue does not extend into the burn. When, however, dye is carried by a lymphatic into the edematous area, it escapes readily from the containing vessel. The edema fluid would seem to be partly made up of lymph which has escaped from lymphatic capillaries traversing the injured region.

Figs. *7a* and *7b* are photographs of an ear taken 3 and 8 minutes, respectively, after a marginal injection of dye. Three hours prior to this a third degree punctate burn was made (the water chamber for 45 seconds with water at 60°C.). In the first photograph, taken only 3 minutes after injecting the ear, the pale region of the burn had become edged with blue dye owing to escape from the lymphatics at its edge. A single large one carries dye directly through the burn itself and the dye escaping from it has merged with that from the ecchymoses on the right. The second photograph taken 5 minutes later illustrates the progress and extent of dye escape. After burns such as this the entire ear structure remains hyperemic for several days. Capillary dilatation in the region surrounding the burn is pronounced and the edema progresses until the ear may be half a centimeter thick. Under the microscope the blood vessels appear as if seen through ground glass.

Heating the skin to 67°C. for 15 seconds frequently gave rise to blisters. If dye was injected into the ear margin just beforehand, a very pronounced escape of dye took place from the lymph channels. The extravascular movement of dye under these circumstances was almost immediate, and it frequently extended several millimeters from the channels. The observation suggests that ordinary blister fluid is formed not only from vascular transudate but from a lymphatic one as well.

The findings as a whole furnish evidence for the participation of the lymphatics in the formation of and recovery from edema, confirming the observations of an earlier paper (2). Further work on this theme is now in progress.

The Lymphatics in the Early Stages of Restoration and Repair of Superficial Burns

Under ether anesthesia, stigmatic or contact third degree burns were made on one ear of a large number of etherized mice. Each day thereafter groups of eight or ten animals were anesthetized with sodium luminal and a dye injection made intravenously or into the ear margin. As controls the normal ears of the same animals were used.

The findings during recovery from both types of burns were so similar that no attempt will be made to distinguish between them.

Twenty-four hours after the formation of stigmatic burns, each was surrounded by an area of pronounced hyperemia and edema. Under the binocular microscope the whole ear showed a mild reactive hyperemia with dilatation of capillaries and venules about the burn and severe edema at the margin of the latter. Stasis in the blood vessels round about varied in degree, but in all instances the region of actual injury was ischemic.

In these instances and in experiments to be detailed there were many findings similar to those occurring after incision of the ear. After a burn, as after incision, there was a strong tendency for dye injected at the ear margin to be carried around the region of injury by numerous enlarged lymphatic channels. During the first 24 hours after the injury these, when they contained dye, showed a greatly increased permeability, with profuse ecchymotic dye escape wherever a lymphatic approached the injured region itself.

Occasionally, lymphatics transported colored fluid directly into and through the burned areas as through incisions. Though the areas were edematous, dye escaped so rapidly into them that one might doubt the existence of lymphatic walls, were it not that India ink did not escape. However, in most cases the channels surrounding the burn led most of the dye around it. Those immediately next the burns seemed to terminate in blind ends which may have been closed, perhaps by heat coagulation or thrombosis. Only when the injections were made with some pressure was colored fluid forced into the burn itself. Then, if such a burn had been made 2 to 6 days previously, the lymphatics failed to carry dye completely through it into the proximal normal tissue, but instead an ecchymosis of dye resulted within the injured area itself.

A further similarity in the reaction of lymphatics after injury by burns and incision was observed in the fact that 24 to 30 hours after burning the ear the lymphatics proximal to the burn appeared as sharply outlined bands showing no escape of dye into the surrounding edematous tissue, even 20 to 25 minutes after an injection. The color within the channels became rapidly paler as though the contents had been diluted and cleared by an intake of fluid. Instead of the excessive escape of dye from the lymphatics within the burn and distal to it, there was an apparent failure of dye escape in the proximal tissue.

The Lymphatics in the Later Stages of the Healing of Burns

Two days after a burn had been made, the lymphatics which filled most readily, that is to say those which skirted the burn, lay within the hyperemic area surrounding the injury. Little or no apparent escape of dye occurred from them, although they were usually somewhat dilated. The contents were more rapidly carried away than in

normal channels, to judge by the fading of the blue color within them, and without visible dye escape. The few ecchymoses which did occur in the region disappeared in 10 to 15 minutes, but distal to the injury they often persisted for several hours. The observation afforded further evidence of an increased lymphatic turnover going hand in hand with hyperemia and increased vascular permeability.

In five instances this last was easily demonstrated by intravenous injections of dye solution. Dye poured from the dilated capillaries and venules about the burn long before it escaped in quantity elsewhere in the ear. As result a bright blue ring about 4 mm. thick was formed surrounding the colorless area of injury. Fig. 8 shows the result of such an experiment, photographed 4 minutes after the intravenous injection was made.

On the 4th day, local and intravenous injections of dye into such animals yielded findings similar to those just reported.

The blood vessels seemed still to be more permeable than normal, judging by the rapidity of dye escape, but one could not say whether this was due to a more permeable state of the vessels or to an increased supply of stained blood. The lymphatics too were more permeable than normal in the region distal to the burn, and here they seemed wider than those proximal to it. When dyes entered the channels completely surrounding the burned area, profuse escape occurred distally, forming a blue cresent of diffuse staining. The proximal lymph vessels seemed less permeable than normal, for within half an hour after their injection the blue-stained contents had become pale, without showing any visible escape of dye. Whether the failure of dye escape is due to decreased permeability of their walls or to brisk fluid movement into the vessels from without cannot be said. In either case the phenomenon shows drainage of the tissues by the lymphatic capillaries.

The findings in no way differed 5 days after producing the burns but by the 6th and 7th day the inflammation seemed to have subsided. No longer was there evidence of hyperemia in the ears, save for a few dilated capillaries immediately about the injured area. The edema had resorbed, leaving only a small swollen region in and about the burn, which by this time had become indurated. Nevertheless the usual local injections of pontamine solution showed the lymphatics to be still excessively permeable. Between the burned area and the ear margin numerous local ecchymoses of dye occurred, as profusely and rapidly as on the 1st and 2nd day after injury.

New Formation of Lymphatics within Burns

By the 7th or 8th day of healing evidence was obtained of new formation of lymphatic capillaries, as in our experiments with incisions.

When dye injected at the ear margins reached the burned areas, some of it entered a dense, ramifying, twig-like plexus of lymphatic capillaries within the distal edge of the burn itself. Some dye passed through in large channels, the remainder was carried around the injury. Fig. 9 shows such a new-formed network about a burn 9 days old. The photograph was taken 2 minutes after injection of the ear with a suspension of India ink. Only a few of the fine, hair-like projections extending into the new-formed tissue are visible; for ink suspensions did not yield a complete injection of these vessels.

To show the relationship of the new lymphatic capillaries to blood vessels intravenous injections of pontamine blue solution were followed in 1 or 2 minutes by injections of vital red in the ear tissue, as in our earlier work with incisions. The findings were so much alike no separate description will be given.

Similar experiments were made during the later stages of healing. Lymph channels were found completely traversing the burns. The sequence of events when dye was introduced into them has already been described.

DISCUSSION

In our previous papers attention has been called to alterations in the permeability of the lymphatic walls occurring under the conditions of the everyday life of the animal. Even very mild thermal and chemical stimuli result in the escape of substances of large molecule, for example hemoglobin, which are retained within the lymphatics of an unharmed ear. On the other hand true particulate matter, for example India ink, fails to escape, showing that no lacunae exist in the lymphatic wall. In the present paper we have reported upon the changed conditions in the lymphatics following incision and frank injury of other sorts. Profound alterations in the permeability of lymphatic walls, in and about these injured regions, speak for an active participation of the lymph system in the changed processes of fluid exchange. What can be inferred concerning the rôle of the lymphatics during inflammation and repair?

Our experiments make plain the fact that, like the blood vessels, the lymphatics respond to injury first by pouring their contents into the region involved. During the first few minutes or even hours after

an injury the permeability of the lymphatic wall is enormously increased without loss of its anatomical continuity, at least under the conditions of the experiments described. The most indiffusible vital dyes pass out readily but ink particles are retained. The lymphatics are so permeable that it is difficult to suppose that they can still function as channels for the conveyance of fluid. That they fail to do so adequately is indicated by the developing edema. The lymphatics are rendered more permeable for a considerable distance around the immediate region of injury. Inflammatory edema is obviously due to a change in the lymph vessels as well as in the blood vessels. The nature of the various changes is not understood. Soon after a burn the lymphatic capillaries distal to the wound appear dilated and those proximal either constricted or normal,—the former more permeable than the latter. The increased permeability may follow dilatation. Yet obvious changes in permeability have been observed which appear to be functional and not anatomical. Thus, the lymphatic capillaries, skirting a burn or wound and not directly affected by it, neither dilated nor constricted, are usually for the first 48 hours much more permeable than normal.

Our work indicates that the brief isolation of a skin region following injury, an isolation resulting from the excessive permeability of both lymphatics and blood vessels, is first lessened when the lymphatics begin to function again. Their severed ends, unlike those of the blood vessels, remain open and lead away fluids from the wound. The fact that lymphatics remain open and the blood vessels closed in an incision goes far to explain the frequent infection by way of the lymphatics. Soon after an injury in the skin of the ear, the lymphatics lying between the head and the injured region regain their normal permeability and apparently function as draining and conducting channels. Through these and not through the blood vessels resorption from the wound first begins. Through the lymphatics toxins or noxious products of disintegration are first carried away to be sieved or passed through the lymph glands before reaching the body at large. The evidence for this fact, though by no means complete, is sufficient.

In this connection, it should be recalled that we have been dealing with a type of lymph drainage slightly different from that in human skin. The ear of the mouse is very thin and both the superficial and

deep plexuses of lymphatics lie close to the surface. As result the lymphatics usually injected lie in the deeper plexus. It is possible that the current in these channels is more rapid than in the superficial lymphatics of the skin of man, that is to say, the lymphatic capillaries which become filled by intracutaneous injections of dye (1). There results in the mouse ear a lymph current just beneath the epidermis which probably does not exist in the human skin, for there the intradermally injected dye passes, after running 2 or 3 cm. in the superficial channels, into a deeper layer (1). However this may be, the fact should be stressed that the lymphatics injected in these experiments with mice probably correspond in function with the deeper plexus found in the subcutaneous tissues of man, lymphatics which are sufficiently superficial to be involved by any cut causing hemorrhage, or by a third degree burn. In these deeper lymphatics in man there is, of course, a considerable flow (1).

The walls of the lymphatics directly draining a burned or incised area, after having at first been abnormally permeable, within 48 hours after the injury became far less permeable than normally for diffusible substances. At this time intravenous injection of dye shows the blood vessels in the injured region to be still abnormally permeable and, owing to hydrostatic conditions, they must still be adding to the accumulation of tissue fluid. The tissue is edematous and rapid continuous passage of fluid must be taking place through the lymphatics, for dye solution placed within them quickly pales and is swept away. In the later stages of healing, 48 to 72 hours after an ear injury, there is a definite tendency for lymph peripheral to the injured spot to flow around it, instead of through it. Dye injected into the distal channels is carried to a point close to the injury and then is shunted around it. It is as though the lymphatics were obstructed by wound materials or perhaps plugged, as has been suggested by Menkin (13) in his work upon blood vessel changes in large areas of inflammation. Evidence of a participation of the lymphatics in repair is seen in their tendency to rapid reconstitution and a new formation of them not only in the regions where they have merely been severed but where they have been injured or destroyed by burns.

Great numbers of new lymphatics were seen in the regions recovering from burns. When the latter had been severe enough to cause necrosis

of the ear and perforation many new tiny lymph capillaries were found in the granulation tissue of the healing margins obviously taking their place in actively growing tissue. But in the tissue far removed from the healing margins, though still within the burned or incised regions, as shown in Figs. 4*a* and *b* and Fig. 9, many more lymphatic channels became visible after dye injection than in similar areas of normal ear. As the photographs show, some were large and some small. In an earlier paper (3) we have shown that the superficial lymphatic plexus is only partially filled by dye injections, that just as Krogh has shown for blood capillaries, small lymphatics temporarily closed and invisible may be teased open to allow dye to enter. As result one cannot say whether the excessive number of dye-filled lymphatic capillaries in the burned area signified a new formation of channels or a flooding of the entire lymphatic plexus, usually but partially visible after intradermal injection of dye and now entirely filled in a tissue engaged in active fluid exchange. When such burns were examined several days later, the number of dye-containing lymphatics had decreased to the normal number. Whether the channels were cut off or had closed down in a relative period of rest, cannot be said.

The observation that new collateral lymphatics can form is of course not new. It occurs after ligature of the thoracic duct (14, 15) and of large lymphatics of the limbs (16). Regeneration of lymphatics in the ear of the rabbit has also been demonstrated (17) as further their growth into granulation tissue (18). Union of lymphatics has been described by E. R. Clark (19) in the tadpole's tail and by Clark and Clark in transparent chambers in the rabbit's ear (20, 12).

In the past the speed with which a new formation of lymphatics occurs has not been recognized nor has it been appreciated that lymphatic drainage may be instituted from areas of repair before drainage takes place by way of the blood vessels.

SUMMARY

With the aid of solutions of vital dyes the lymphatic capillaries in the ear of the mouse have been studied during the period of immediate reaction to injuries of various sorts and during the period of repair.

The behavior of lymphatics severed by incision differs greatly from that of the small blood vessels. Instead of closing they sometimes

remain open for as long as 48 hours. Materials introduced into the wound pass directly into the lymphatics through their gaping ends, a fact which will explain why infection from incisions is predominantly along the lymphatics.

All around an injury the lymphatics are rendered abnormally permeable. So, too, are the blood vessels, a fact well recognized in the past. Twenty-four to 48 hours later, at a time when the blood vessels in the edematous tissue surrounding the injured area are still much more permeable than normal, the draining lymphatics allow far less to escape than usual. The possible reasons for this have been discussed. The lymphatics participate in the removal of fluid from the edematous tissue.

As repair after injury takes place severed lymphatics may reunite when as yet there are no functioning blood vessels. Later an active hyperplasia of the lymphatic channels occurs, an extraordinarily abundant plexus of minute lymph capillaries budding into the area under repair.

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EXPLANATION OF PLATES

PLATE 28

FIG. 1. Photograph of the ear of a living anesthetized mouse, injected at the ear margin with standard pontamine solution, 5 hours after a transverse incision had been made in the skin of the upper surface. The dye entered the lymphatics of the injected area and gradually extended along them to escape from their severed ends, filling the wound with blue dye. ×6.

FIG. 2. The result of an intradermal injection of standard pontamine solution into the margin of a mouse's ear, which had been incised 24 hours prior to the injection and photographed 5 minutes after it. The blood vessels and lymphatics had been cut through. Some of the dye reached and entered the incision but most of it was shunted around it as described in the text. The lymphatics are markedly permeable distal to the incision, as indicated by the abundant dye escape there, and much less so proximally. ×10.

FIG. 3. Lymphatics in the incised ear of a living mouse photographed 5 minutes after an injection of standard pontamine solution. The incision was made the day before and is easily seen in the photograph. Three lymphatic channels have conducted colored fluid past the incision into the tissue at the base of the ear. In doing so much dye has escaped, either into it or just beneath it. The increased dye escape distal to the incision, and the lack of escape proximal thereto is also well shown. ×10.

FIG. 4*a*. Demonstration with India ink of the lymphatic plexus about a healing wound. Nine days previously the skin had been incised; healing was progressing well. Immediately after a marginal injection of ink the ear was amputated and photographed under neutral paraffin oil. On the left side of the incision several lymphatics are seen carrying ink either directly through the healing incision or just beneath it. In the injured area a few very fine lymphatics can be made out. Linear and parallel, they lie transversely in the healing incision itself. ×10.

Fig. 4*b*. An enlargement of the same specimen. In this figure these newly formed lymphatics are seen to better advantage. ×40.

PLATE 29

FIG. 5*a*. This photograph shows the escape of pontamine solution into a burned region. Eight minutes prior to taking the photograph a chamber containing water at a temperature of 55°C. was brought into contact, for 40 seconds, with an area midway between the tip and base of the ear. Six minutes later the dye injection was made near the tip and 2 minutes later the photograph was taken. The area of dark, fuzzy escape of dye along the lymph channels coincides with the area of the burn. Elsewhere no dye has passed out. ×9.

FIG. 5*b*. The same preparation 2 minutes later, that is to say 4 minutes after the injection. In the unharmed ear standard pontamine solution does not begin to escape from the lymph channels for 10 to 15 minutes. ×9.

FIG. 6. Ear burned in a spot midway between the tip and the base by 20 seconds contact with the water chamber containing water circulating at 55.0°C. One hour later standard pontamine solution was injected at the tip of the ear and 4 minutes afterwards the photograph was taken. The lymphatics, though allowing some escape of dye into the burned region, where it colors the edema fluid, have transported much of it into the proximal, normal tissue. ×20.

PLATE 30

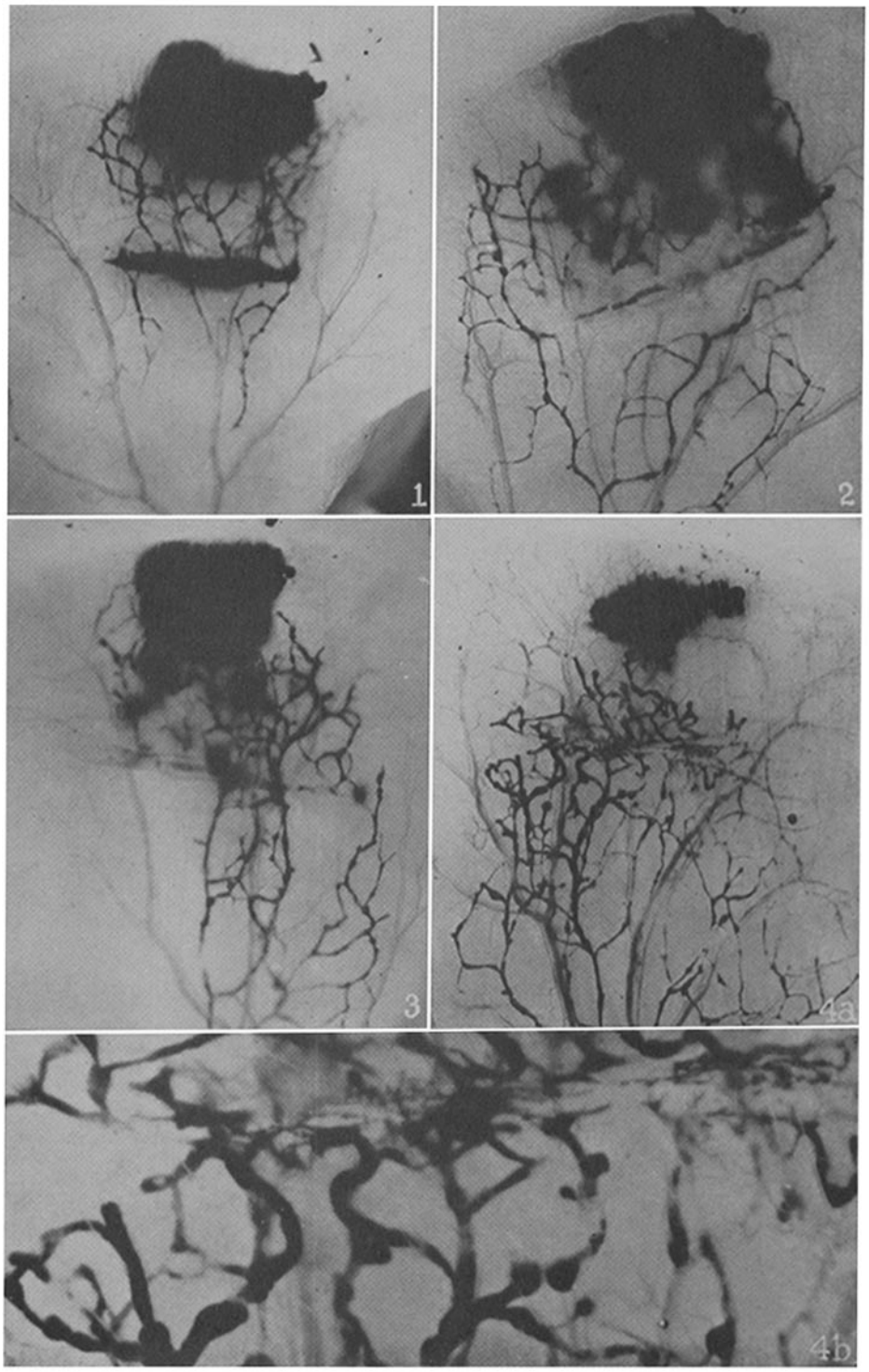
FIGS. 7*a* and 7*b*. Two photographs of the same preparation taken 3 and 8 minutes respectively after a local injection of pontamine solution at the margin of the ear. Three hours prior thereto a severe third degree punctate burn had been made midway between the tip and the base of the ear as described in the text.

The rapid dye escape from the lymphatics close to the burned region is shown and the fact that the latter is almost unstained. A large channel traversed it from which dye ecchymosis occurred after a time. ×6.

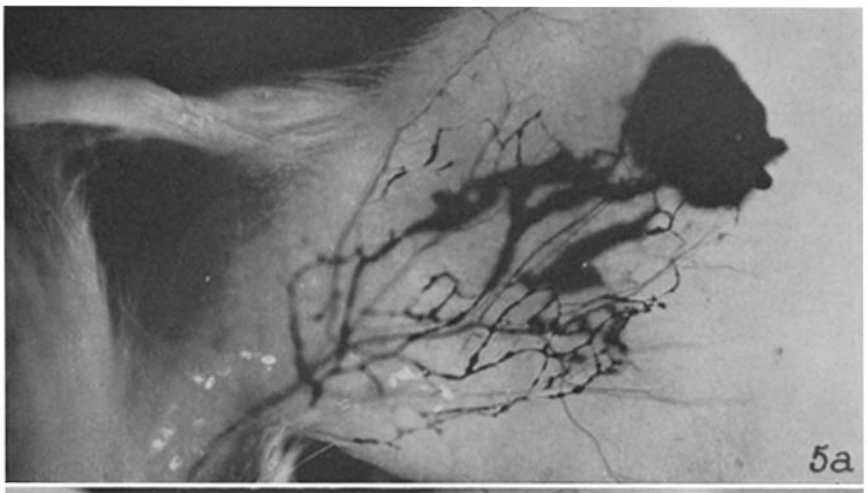
FIG. 8. A photograph of the ear of a living anesthetized mouse 2 days after a stigmatic burn had been induced on its upper surface as described in the text. Four minutes prior to the photographic exposure the animal received intravenously 0.05 cc. of 21.6 per cent aqueous isotonic pontamine sky blue solution.

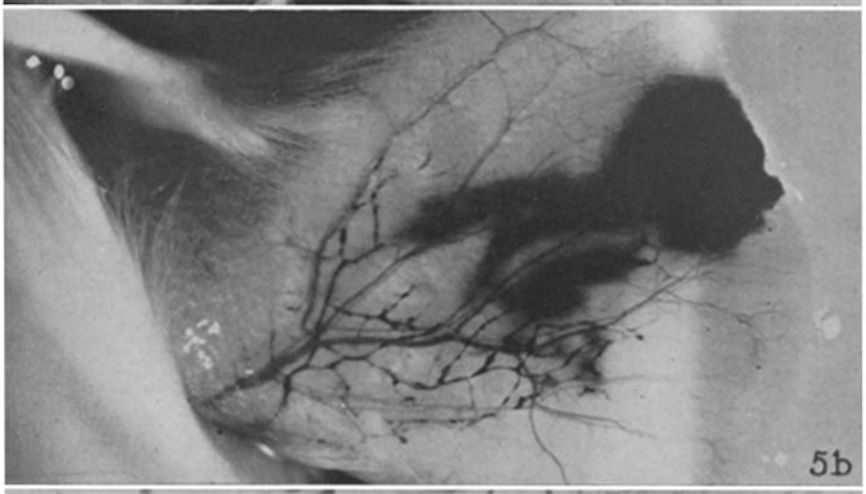
The increased permeability of the smaller blood vessels is evidenced by a ring of intense color about the burn, while elsewhere in the ear very little dye has escaped. At the center of the burned area there is some slight diffuse staining. ×6.

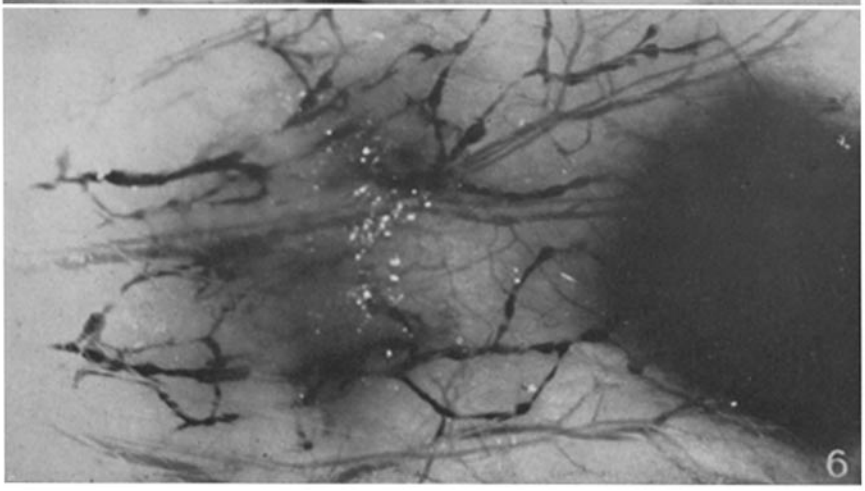
FIG. 9. The ear of an anesthetized mouse injected with a suspension of dialyzed India ink in 5 per cent gelatin solution 9 days after a stigmatic burn. One minute after the injection the ear was severed, placed under neutral paraffin oil, and photographed after another minute. The burn had caused a complete perforation of the ear which at the time of the injection was gradually being closed by granulation tissue. Several very small new-formed lymphatics can be seen in the new-formed tissue and about the healing burn there is an abnormally rich plexus of lymphatics many of which are very small. ×6.



(McMaster and Hudack: Skin lymphatics in repair of lesions)







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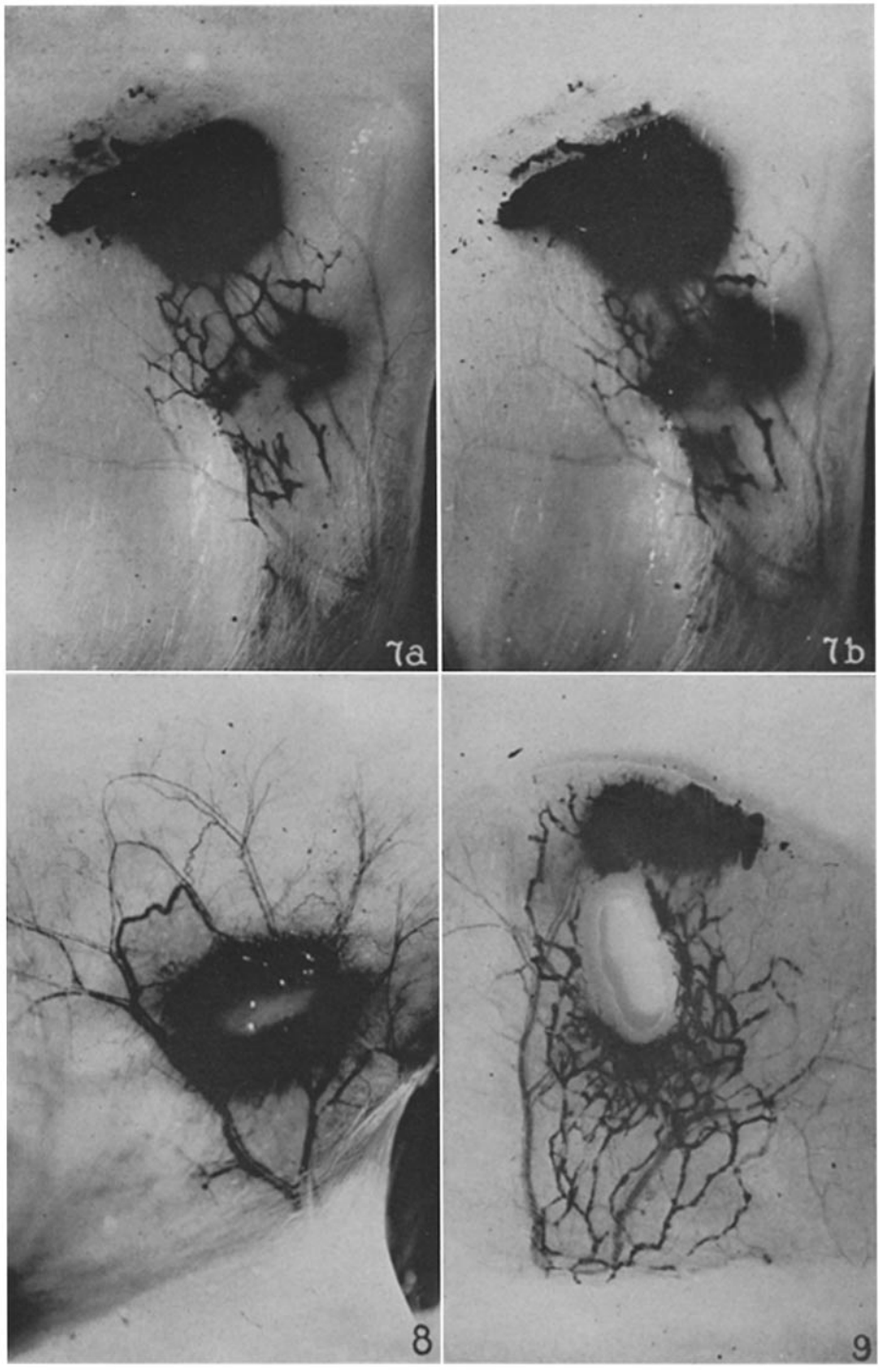

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