II. INDUCED ALTERATIONS IN THE PERMEABILITY OF THE LYMPHATIC CAPILLARY

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PLATES 14 AND 15

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Lymphatic capillaries can be rendered visible by local injections of vital dyes. In the ear of the mouse, the channels, leading from an injected region, fill with dye and stand forth in deep color, coursing through unstained and undisturbed tissue. The method has been utilized for a study of the normal permeability of the wall of the lymphatic capillaries, as reported upon in an accompanying paper. The present study is concerned with the alterations in permeability that occur under various conditions, some of these within the limits of the normal, others frankly pathological.

General Procedure

Mice, of 16 to 18 gm. body weight, under luminal anesthesia, were laid upon plasteline moulds and the ears were lightly spread upon white procelain placques (see Figs. 1 to 3). Prior to experiment, the ears were carefully examined for gross or microscopic injuries and only those that appeared intact were utilized.

To render the lymphatics visible and to test their permeability as well, pontamine sky blue, a highly indiffusible vital dye, was injected into the tissue at the margin of the ear by a method already described (1). To approximate the probable protein concentration of the lymph, an aqueous solution of the purified dye, 21.6 per cent, which is isotonic with blood, was diluted to approximately 1 per cent with 20 volumes of a mixture consisting of 1 part mouse serum and 3 parts of Tyrode's solution. For brevity we shall call this standard pontamine solution. Under ordinary circumstances, the dye entering the lymphatics of the normal ear does not escape along their course until 12 to 15 minutes have elapsed, and then as a gradually increasing blue haze about the vessels. Changes in the rate of escape were taken to indicate alterations in the effective permeability of the lymphatic wall, though it is true that in some instances, to be commented upon further on, other factors may have been the conditioning ones. It was our practice to utilize one ear of each animal for experiment and the other as control.

Early in the work, the readiness with which the permeability can be altered prompted us to test whether the lighting system employed in the experiments had any such effect. As routine, the light from a Leitz-Wetzlar arc lamp, of 4 to 5 amperes, was filtered through 5 cm. of Magnus' solution (2) while still 60 cm. distant from the ear to be observed. The beam was then reflected to the object by an adjustable plane mirror.

The permeability of the lymphatics of ears exposed to well filtered light of high brilliance for 20 to 30 minutes was compared with that in others exposed to daylight only. No definite difference could be discerned. In the experiments the negligible influence of the filtered light from the arc was exerted upon experimental and control ears alike, since both were equally illuminated.

The Hydrostatic Pressure of Lymph

Evidence of an active flow in the minute lymphatics of the mouse's ear (1) has already been given. It seemed well to investigate the pressures under which this flow takes place and the phenomena consequent upon obstruction. Accordingly, in a series of twelve experiments this was done.

An earlier paper (3) has described the utilization of small collodion bags connected with water manometers for the measurement of blood pressure in the smaller vessels of the mouse's ear. The same apparatus has been utilized for determinations of pressure within the lymphatics. The ear of the anesthetized animal was placed upon a horizontal procelain plaque supported by a glass rod held by a Chambers device. A cylindrical collodion bag 3 cm. long and 2 mm. in diameter lay between the plaque and the ear in such manner that the outer third of the ear extended beyond the bag. The latter, slightly distended with water, was connected with a water manometer of 0.5 mm. bore, which in turn communicated with a reservoir and record syringe by which pressure changes were effected. Above the ear and the bag a transparent glass slide held by a rod in the Chambers device was adjusted parallel to the procelain plaque below. With a fine camel's hair brush a neutral paraffin oil was then run between the ear and the upper platform and immediately the latter was brought down from above until it just touched the ear. With a little practice the adjustments were readily made and the zero point of pressure ascertained, while a second observer watched through the microscope. Slight increases in the distension of the bag led to a narrowing of the lumina of the lymphatics which had previously taken up the dye, and further distension occluded them. Simultaneous readings of the manometer told the pressure required to effect these changes.

Invariably in the twelve experiments the lymphatics were occluded when the bag pressure became equivalent to that exerted by a column of water 2 to 4 cm. in height.

Mechanical Obstruction Increases Permeability

The width of the blood vessels in the ear and the rate of flow therein were watched by one worker while the other raised the pressure just sufficiently to occlude the lymphatics. No narrowing of the veins coursing through the region pressed upon, or interference with the rate of flow through them, occurred as result of the slight pressure; yet when it was maintained there developed within the hour an edema of the ear, not marked but definite. The ear became slightly thicker, the natural folds of the skin appeared less deep and smoother, and with a blunt needle, "pitting on pressure" was demonstrable over the entire outer surface of the ear distal to the bag. Puncturing the skin with a fine sharp needle brought about the escape of a little free fluid. In six out of the twelve experiments standard pontamine solution was injected into the tissue at the edge of the ear after the barrier was placed. In these cases the lymphatics from the colored region filled with colored fluid, but none progressed beyond the barrier. Under the circumstances the lymphatics filled more slowly than usual and the colored lymph flowed off into a greater number of lateral channels than in unobstructed ears. In other experiments, the pressure barrier was placed on one ear after lymphatics of both ears already contained dye. In these instances the secondary escape of pigment through the lymphatic wall was perceptibly greater on the blocked side. The increased permeability was not accompanied by a recognizable dilatation of the obstructed lymphatics.

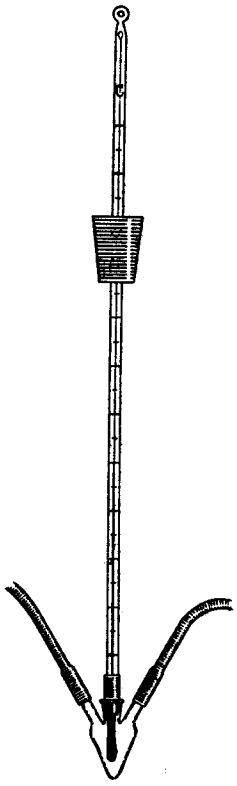
Mechanical Stimulation Increases the Permeability of the Lymphatic Wall

The permeability of normal lymph capillaries can be readily altered. When a light stroke is made with a blunt wire transversely across the surface of the skin on the outer surface of the ear of a mouse, there results an immediate and abundant escape of dye from the underlying lymphatics containing it. This escape is closely limited to the line of stroke (Fig. 1) and results in localized dye ecchymoses along the "tache."

To obtain uniform experimental conditions, the stroking was accomplished with a stout steel wire 5 cm. long and 1 mm. thick, affixed to a wooden handle and ground to a smooth, blunt tip. In all the experiments the strokes were applied by the

same worker holding the instrument at the same slanting angle and exerting as nearly as possible the same pressure. Mouse and plasteline form both rested in the scale pan of a spring balance. With a little practice a pressure during the stroke of about 40 gm. as indicated by the balance was almost regularly exerted. Where the wire pressed upon the small blood vessels during the stroke, constriction occurred, leaving a barely perceptible white line. This rapidly disappeared and only occasionally during the next 2 to 4 hours could a slight narrowing, limited to the veins along the line of the stroke, be perceived with the microscope. Hyperemic flare occurred only with much heavier stroking.

At various intervals after the stroking, standard pontamine solution was injected in the usual way at the edge of the ear to render the lymphatics visible. In all the experiments each animal received but one injection of it.



TEXT-FIG. 1. Water chamber used for heating the ears of anesthetized mice. With the ear lying upon the glass bulb, water at any desired temperature was circulated through the apparatus. Even temperatures were maintained for varying periods with less than 0.5°C. variation.

Twenty-six of the animals were injected as soon as possible after the stroke or within 10 minutes thereafter. Practically at once after the lymphatics filled with colored fluid, that is to say within a minute after the injection, a profuse escape of dye began to take place from them where they crossed the line of stroke. Elsewhere the dye was retained. The photograph of Fig. 1 shows the lymphatic channels 6 minutes after the dye had begun to course along them, and 16 minutes after the stroking.

In another group of twelve animals considerable intervals were permitted to elapse between the stroking and the employment of the dye. Two of the mice were injected with the standard pontamine solution an hour after the stroking, and at half-hourly intervals during the next 5 hours two more were injected. The dye escaped from the lymphatics with the same ease an hour after the "tache" as immediately after it, and the first considerable lessening in escape was noted when the interval had

been $2\frac{1}{2}$ hours. Intravenous injection of 0.05 cc. of isotonic 21.6 per cent pontamine solution, at approximately the same intervals, disclosed, in the area of "tache," an increased permeability of the small blood vessels persisting for 5 hours and easily discernible after the lymphatic reaction had subsided. It is to be noted that the mice used in these experiments had a blood volume of approximately 1 cc. hence the dye circulating in the blood should attain a concentration similar to that of the dye in standard pontamine solution. A localized edema followed the stroke and invariably outlasted the changed state of permeability.

In further experiments of the same kind, dialyzed India ink and "Hydrokollag 300" were employed, either in place of pontamine blue, or combined with it. The graphite and ink particles rapidly adhere to the endothelial wall of the lymphatics of the normal ear, outlining the vessels clearly.

For the purpose Higgins' India ink was dialyzed through parchment paper and against Locke's solution for a week at 2°C. 15 cc. samples of the remaining ink were centrifuged—in 15 cc. centrifuge tubes—for 1/2 hour at approximately 3,000 revolutions per minute. The upper 8 cc. was then aspirated from each tube and portions added to Tyrode's solution for injection into the mouse ears. Usually 1 volume of ink to 3 volumes of Tyrode's solution sufficed to render the lymphatics plainly visible when filled with the mixture. "Hydrokollag 300" was prepared as described by Higgins and Murphy (4). Again, one to four mixtures of the particulate suspension and Tyrode's solution were used.

In twenty animals suspensions of India ink in Tyrode's solution were injected into the skin of the outer margin of the right ear 3 minutes after stroking the organ transversely across its middle. In the left ear, at a similar interval after stroking, suspensions of "Hydrokollag" were injected.

In ten animals both ears were stroked and an injection made into the right one, 3 minutes later, of India ink suspension in thrice its volume of standard pontamine solution. Into the left ears was injected "Hydrokollag" mixed with the same dye solution in the same amounts.

In no instances did the particulate matter escape from the lymph channels in the region of "tache," although the dye readily passed the endothelial barrier. A further attempt to ascertain the nature of the barrier was made by introducing mouse or rabbit hemoglobin as prepared by the method of Sellards and Minot (5), dissolved in Tyrode's solution. Six mice were utilized. In each the lymphatic

barrier let through hemoglobin in the region of "tache," within a minute after it appeared in them, whereas elsewhere they proved completely impermeable to it.

In a previous paper (1) the occurrence of dye ecchymoses from the lymphatics of apparently normal ears was described and pictured. They had the same general appearance as those elicited by stroking and it seemed probable that they might be due to essentially the same cause. To test the point six mice were given sodium luminal as usual, and before complete narcosis developed, the under surface of one ear of each was lightly tickled until the animal responded by scratching the upper surface, though not sufficiently to break the skin. A few minutes later when anesthesia was complete standard pontamine solution was injected into the skin at the margin of both ears. In every case on the scratched side, dye ecchymoses rapidly developed along the lymphatics, whereas none appeared on the control side.

Heat Increases the Permeability of the Lymphatics

An extraordinary increase of lymphatic permeability resulted from the action of heat. It was applied in a variety of ways.

In 20 instances, one ear of the anesthetized mouse was dipped in water warmed to temperatures between 40° and 60°C. for periods varying from a few seconds to 5 minutes. Care was used to submerge only one ear and all other trauma was avoided. At varying intervals, from 2 minutes to 4 hours thereafter, standard pontamine solution was injected into the margin of both ears.

In another series of experiments, to observe the immediate effect of heat, the ear of the anesthetized mouse was placed upon the device shown diagrammatically in Text-fig. 1. A thin-walled glass bulb was moulded in a glass blower's flame to the shape of a flattened blunt spearhead. This bulb had three inlets, and one of its flat surfaces was painted with enamel. Two of the inlets served for the passage of water, while into the third a thermometer was inserted. Water, at any desired temperature, could be circulated through the apparatus while the ear of the mouse lay upon it under the binocular microscope. The white enamel gave the desired background and threw the structures of the ear into sharp relief. Thus, in 22 instances, the influence of temperatures ranging from 43–60°C. was tested on one ear, while the other ear lay upon a similar apparatus at room temperature.

In a few experiments one ear of the anesthetized mouse was subjected to a jet of warmed dry air, according to a procedure already described (6). Compressed air was blown above a gas flame into a funnel; and the ear was exposed to it as it emerged from the 3 mm. opening at the funnel end. A thermometer close to the ear gave approximate readings of the temperature.

The application of heat, even of so little as 43°C., led to a rapid escape of the dye from lymphatics containing standard pontamine solution. A 5 minute exposure at 43.0–43.5°C. sufficed to cause escape in less than a minute after the lymphatics had filled with dye solution injected into the skin 4 minutes after heating the ear. In the unheated control ears no escape occurred for 12 to 15 minutes (1). Greater degrees of disturbance had proportional results. In these experiments, as in those involving mechanical injury, India ink and "Hydrokollag" taken up into the lymphatics failed to escape from them.

A typical experiment furnished the photograph in Fig. 2. Luminal was given to a mouse subcutaneously and after the customary interval of 1 hour the ears were placed upon the water chambers at room temperature. Water between 43.0–43.5°C. was circulated for 5 minutes through the apparatus supporting the right ear. 1/2 minute after the application of the heat, both arteries and veins showed dilatation which continued to increase while the ear lay upon the plaque. At the end of the period of heating, the water chambers were removed and 10 minutes later standard pontamine solution injected into both ears. At this time the left ear exhibited a mild contralateral reflex hyperemia. After another interval of 10 minutes the photograph shown in Fig. 2 was taken. Profuse escape of dye into the tissue had occurred in the heated ear, far more than on the normal side, and by this time, too, moderate edema of it had developed.

In all instances in which temperatures of 43°C. or more were applied, a vascular hyperemia developed almost at once, enduring for a variable period afterwards. The effect of this reaction on the gradient of permeability that exists along the blood capillaries has already been described (6). The reactive hyperemia developed during the same time as the increase in lymphatic permeability, as was found by the study of the animals in which the lymphatic channels had filled with dye solution prior to the heating. In all instances and with all the procedures used, a contralateral reflex hyperemia developed in the unheated control ear. The degree of reaction of the blood vessels on the two sides, though in general unequal, was often similar. The reactive hyperemia in the unheated ear was probably accompanied by a slight increase in the permeability of the lymphatics, judging from a comparison with the findings in mice which had not been subjected to heating.

The Effects of Sunlight

The pronounced changes in the permeability of the lymphatic wall elicited by mild stimuli led us to test the effect of still less considerable ones.

Nine mice were injected with luminal, and an hour later, at high noon, three of them were placed upon a gauze pad on a table standing near an open window in the full sunlight of a clear October day. A sunlit thermometer lying on the pad beside the animals registered 30°C. Three other animals were placed in a black cardboard box, also in the sun, and resting on a warming pad. These mice, though sheltered from the sun's rays, were surrounded by air at a temperature similar to that enveloping the animals directly exposed, that is to say between 29.6° and 30.5°C. Still another three animals, serving as controls, lay on a gauze pad in the middle of the room, out of the direct sunlight and at a temperature of 26°C.

After 15 minutes, standard pontamine solution was injected into the tissue at the margin of both ears of three of these mice, one of each group. The injections were made as rapidly as possible, beginning with the control mouse that had been in the middle of the room, and finishing with the animal exposed to sunlight. During the 3 minutes required for the injections and the subsequent 15 minute observation period, all three mice lay out of the sunlight and at room temperature.

The procedure was repeated at 15 minute intervals, the last mouse of the third batch of three having been exposed to sunlight for 45 minutes.

In all the experiments dye began to escape soonest from the lymphatics of the animals exposed to sunlight, although these were the last of each group to be injected and their lymphatics filled last with dye. In the animal exposed for 45 minutes, profuse escape of dye from the lymphatics occurred within 4 minutes, as compared with 17 minutes and 28 minutes for the control from the box and the one from the middle of the room. Its ears showed a mild hyperemia. That the increased permeability was due to the sunlight and not to temperature was shown by the relatively slow escape of dye in the case of the mice within the box. This was slightly more rapid than in the animals at room temperature, but did not nearly approximate that in the sunlight animals. A further experiment confirmed these findings.

Three further groups, each of three mice, were subjected to the conditions just described, but the ears of one group were injected and observed while the mice lay in the sunlight. Under these circumstances the dye escape from the lymphatics was even greater and more

rapid than before, in the experiments already described, occurring in 2 to 3 minutes. No escape took place from the lymphatics of the control animals for from 10 to 15 minutes.

The Effects of Chemical Irritation

Xylol applied to the ear causes a profuse outpouring of dye from the lymphatics. The results of a few experiments will be briefly given.

The right ears of twenty-six anesthetized mice were painted once with Merck's chemically pure xylol applied by means of a camel's hair brush. From 5 minutes to ½ hour later standard pontamine solution was injected into the tissue at the margins of both ears. Within 1 to 2 minutes afterwards dye began to escape from the lymphatics of the painted ears and after an interval of 5 to 8 minutes the escape had become profuse. Meantime from the control ears little or no escape took place. Fig. 3 displays the difference in the ears of a mouse 23 minutes after a single application of xylol to the right ear and 8 minutes after injection of the dye.

Increased lymphatic permeability was noted irrespective of whether the dye injection was made after the painting with xylol or before. The increased permeability endured for a considerable time. For example, dye escaped within less than 2 minutes from lymphatics of an ear painted with xylol 4 hours previously. Repeated applications of xylol rendered the lymphatics still more permeable.

In 6 instances, about 15 minutes after painting both ears with xylol, India ink was injected into one ear and "Hydrokollag" into the other. The particulate matter taken up by the lymphatics remained within them—despite the increased permeability of the vessels for dye solutions.

The effect of xylol upon the blood vessels in the rabbit's ear (7) is well known. The vascular tree of the mouse's ear reacts in a similar manner, with an intense hyperemia. As in the experiments with heat, so in those with xylol, increased permeability of the lymphatics and reactive hyperemia develop approximately together. The phenomena are followed by edema of the ear. This latter, after a single application of xylol, is relatively slight, but after repeated paintings the ear becomes thick and the blood vessels appear as though seen through ground glass. When standard dye solution is injected subcutaneously, at once or within 2 hours after the last xylol painting, rapid and profuse escape from the lymph channels occurs, an escape far more pro-

nounced than that shown in Fig. 3. The ground glass appearance and thickness of the ear (2 to 6 mm.) prevent good photographic reproduction. The control ears, as in the heat experiments, show a contralateral reflex hyperemia.

The Relation of Increased Lymphatic Permeability to the Development of Edema

In all of the experiments involving the application of heat of 43°C. or over, an edema of the ear appeared. Several minutes elapsed between heating and the development of recognizable edema. During this period, both increased permeability of the lymphatics and an active hyperemia could be perceived. The reflex hyperemia developing in the control ears was frequently as intense as that on the experimental side, yet only occasionally did the control ear show any edema and this always of a very mild degree. The blood vessels of ears subjected to mild heating exhibit a great increase in permeability as we know from previous experiments, while on the control side vascular permeability is but little altered despite the induced hyperemia (6). This has also proved true on painting with xylol. Intravenous injections of 0.05 to 0.1 cc. of an aqueous 21.6 per cent, isotonic, solution of pontamine sky blue were made into a few mice after the study of the lymphatic permeability had been completed. The small blood vessels both of the control and the xylol-painted ears showed increased permeability, but in the latter far more than in the former.

The Lymphatic Condition during Resorption of Edema

What is the state of the lymphatic vessels during the resorption of edema? In the attempt to determine this, a procedure was sought which would give rise in the mouse's ear to a uniform edema lasting but a few hours. It was found that heating the ear for 5 or 6 minutes at 45–49°C. by means of the water chamber already described sufficed for the purpose. The organ was examined under the microscope at half-hourly intervals thereafter. Within 1 hour the ears showed a marked puffiness and swelling which had diminished at the 3rd and 4th hour and had quite disappeared by the next day, without signs of necrosis or vascular injury. The fluid had resorbed during this period. Some quantitative estimates were made of the amount and duration of the edema.

Sixty young, adult mice weighing 16 to 18 gm. were anesthetized with luminal and the left ear of each was carefully amputated and weighed. Twenty individual ears taken previously from animals used for other purposes showed so little variation in the ratio of water content to total mass that the ears of these sixty were weighed at once as one group in a glass-stoppered weighing bottle. The right ears of all sixty animals were heated *in situ* at 46–49°C. for about 5 minutes. 2¾ hours later half were amputated at the base and at once carefully weighed in one group. The heated ears of the remaining animals were amputated 5 hours after exposure and also weighed together. The ears were still edematous but not as thick or hyperemic as at the 2¾ hour intervals after heating. The material provided by the three groups was desiccated to constant weight over phosphorus pentoxide at 56°C. The control ears showed a water content of 64.8 per cent. The heated ears amputated after 2¾ hours, at which time there was a moderate edema, had a water content of 78.8 per cent, and those amputated after 5 hours, 76.8 per cent. The second figure given shows an increase in water content equivalent to 14 per cent of the total mass of the ear. In the following 2¼ hours this had decreased by one-seventh. Subsequent observations have shown that the maximum edema occurs about 1 hour after heating. Amputation at this time would have revealed greater differences than those given.

Resorption had evidently begun within 5 hours after the heat stimulus. Lymphatic permeability at this period was now studied.

Five animals were anesthetized lightly with ether and one ear of each was heated on the water chamber at 44.5–45.0°C. for 6 minutes. Reactive hyperemia in the warmed ears and contralateral reflex vasodilatation in the control ears accompanied the procedure. The animals were allowed to recover from the ether. A moderate edema developed in the heated ears within the hour.

2½ hours later the mice were anesthetized with luminal and at varying intervals thereafter standard pontamine solution was injected into both ears. In three of the animals, edema was still present in the experimental ears 3¾ to 4½ hours after heating, but none could be demonstrated on the control side. Dye failed to escape from the lymphatics of the heated ear in 30 minutes, indeed in one instance it was still retained after 50 minutes, while in the control ears it began to emerge in the usual period, 10 to 15 minutes. In the fourth animal, similar differences were found 3 hours after heating the ear. In the remaining animal, injected 4½ hours after heating, no edema was then present in either ear. Dye passed out of the lymphatics of both ears at the same rate as in normal animals.

In a further experiment one ear of each of five mice was heated at 49.5–50.0°C. for 1½ minutes with the animals under light ether anesthesia. 4 hours later the mice were given luminal, and when fully anesthetized, 4½ to 5 hours after the heating, dye was injected into both ears. To control the effect of contralateral reflex vasodilatation in the unheated (control) ears of the experimental animals, a group of normal mice were anesthetized and injected at the same time and in the same manner.

Similar results were obtained in all the experiments. At the time of dye injections, the heated ears still showed hyperemia and marked edema, the latter less than it had been 2 hours previously. The unheated ears showed slight hyperemia with a dubious or mild edema. In the ears of the control animals one could observe neither edema nor hyperemia. From the lymphatics of the latter, dye escaped in the usual time, whereas in the case of the heated ears of the experimental animals it failed to do so in twice or thrice the time. In these instances then, the most ready escape of dye was found in the mildly hyperemic, unheated ears of the experimental animals.

From these findings it can be said, during the resorption of the edema dye tends to remain within the lymphatics. It is conceivable that a brisk fluid movement into these vessels may account for the failure of dye to escape from them. In the edematous ears, during the period of fluid resorption, the lymph capillaries which at first filled with dye soon after became pale and faded from view. This phenomenon can best be interpreted as a washing out of them by fluid passing into them from without. It cannot be said that the experiments throw any light on the state of the lymphatics during fluid resorption but they indicate a participation of these vessels in the removal of fluid.

DISCUSSION

The lymphatics have been disregarded or overlooked in much of the current reasoning upon fluid transport and exchange. In part this is due to recognition of the fact that the lymph flow from not a few resting organs is negligible, and in other part to the unobtrusiveness of the lymphatics which, unless sought for, usually escape observation; but there is yet a third cause, namely that the means to demonstrate the minute lymphatics in the living creature and to study their physiology have not been available. In the present work the permeability of the lymphatic wall has been tested, though in the opposite direction to normal flow, it is true. Save when fluid is passing into the lymphatics in quantity (as during the resorption of edema), this should make little difference if,—as is ordinarily assumed for the wall of the blood capillary, and as the findings of our previous paper indicate,—the wall of the lymphatic behaves like a semipermeable membrane.

The results of our experiments leave no doubt that the barrier presented by the lymphatic wall, a barrier having under the conditions approximately the same permeability as the wall of the blood capillary, as shown by tests with vital dyes of graded diffusibility, is affected by many slight causes,—and more readily affected than the wall of the blood capillaries. Influences which come within the realm of the normal,—sunlight, slight warmth, a stroke, scratching which does not break the skin,—these greatly, if transiently, increase lymphatic permeability. It seems not merely probable, but certain, that such changes have a meaning for local conditions. Exchange between the blood and tissues is subject to alteration in a variety of ways—by vasodilatation or contraction, alterations in the systemic blood pressure, and so forth. The lymphatics in the nature of things constitute a more passive system; yet much of their usefulness under this or that condition must depend upon the state of permeability of their walls. It will be important to learn whether the active functioning of organs, with its attendant increase in lymph formation, affects this permeability.

None of the injuries that we have utilized to alter the permeability of the lymphatic wall breaks down the barrier so completely as to permit the escape of particulate matter; yet in so far as the lymphatic is rendered more permeable by this or that influence, it ceases to be a walled off channel. We have shown that slight stimuli render the lymphatic wall so very permeable that even hemoglobin passes it readily. What is true of hemoglobin will doubtless hold for proteins of smaller molecule, notably those of the blood plasma. Students of factitious urticaria and of wheal formation have been accustomed to explain whealing solely in terms of the escape of fluid from the blood vessels. But it is plain that if, as result of the injury causing the wheal, the lymphatics traversing the region implicated cease to be physiologically demarcated from the tissue because the barrier provided by their walls no longer exists, the fluid escaping from the lymphatics may readily contribute to the formation of the wheal. Evidence bearing upon this point will be provided in a subsequent communication.

The functional state of the lymphatics in tissues that are edematous for one or another of various reasons has great interest. Indeed,

until it is determined the mechanism of the edema cannot be wholly comprehended. We have shown that the edema which results from obstruction of the lymphatics is attended by an increased permeability of the wall of these vessels, and that during the formation of an inflammatory edema the wall of the lymphatics of the area involved becomes greatly more permeable. There are indications that the permeability returns to normal before the edema disappears, but for reasons that have already been given, they must be considered as inconclusive.

SUMMARY

A standardized solution of a vital dye which escapes with some difficulty from the lymphatics of the ear of the mouse has been utilized in tests of the permeability of the lymphatic wall under various conditions. It has been found that this permeability is subject to great change. The slight pressure that suffices to prevent lymph flow from the ear,—an organ in which such flow goes on normally,—soon results in increased permeability of the obstructed lymphatics without as yet any perceptible dilatation of these vessels. Mechanical stimulation as for example a stroke with a blunt wire, or scratching so light as not to break the epidermis, results in a practically immediate, great increase in lymphatic permeability, which is sharply localized to the region pressed upon. This increase in permeability, though so great that even hemoglobin is let pass by the lymphatics, endures but a few hours. Warming the ear to 43°C. or exposure to mild sunlight increases permeability considerably. Slight chemical irritation increases it greatly, though not so much that particulate matter is let pass. The edema developing as result of lymphatic obstruction or mechanical, thermal, or chemical stimulation is preceded by and associated with a large increase in lymphatic permeability.

The facts are discussed in relation to their bearing upon fluid accumulation within the tissue. It is plain that influences within the realm of the normal suffice to increase lymphatic permeability and that those which lead to edema cause a very great increase in it. In proportion as this increase occurs the lymphatics cease to be channels demarcated by a semipermeable membrane. It seems certain that the changes must be in some part responsible for the local accumula-

tion of fluid. There exist possibilities, on the other hand, of a correlation between the functionings of the blood and lymph vessels under certain pathological conditions, as during the resorption of edema.

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EXPLANATION OF PLATES

PLATE 14

FIG. 1. Ear of a living anesthetized mouse photographed by reflected light 6 minutes after the entry of standard pontamine solution into the lymphatic capillaries. 10 minutes previously the ear was stroked transversely across the middle with a blunt wire, as described in the text. Immediately before taking the photograph a fragment of cover slip was placed upon the ear, which had been coated with paraffin oil to increase visibility, as in the case of the other specimens photographed.

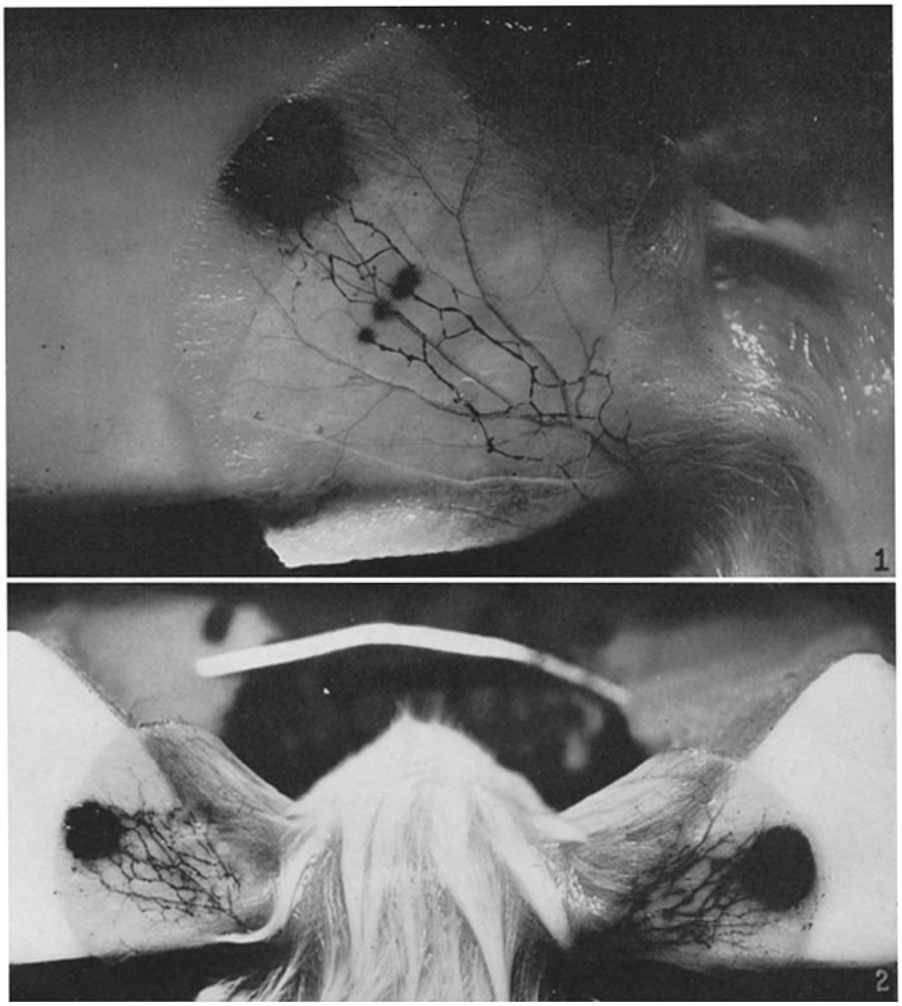
Sharply localized ecchymoses of dye appeared along the line of "tache," although this latter was so weak as not to elicit any reaction of the blood vessels. Under normal conditions no such escape occurs in ½ hour. ×8½.

FIG. 2. The under surface of the right ear was warmed at 43.0–43.5°C. for 5 minutes, as it lay upon the water chamber described in the text. Both ears were then spread on plaques in the usual manner. 10 minutes after the heating, standard pontamine solution was introduced into the skin and taken up by the lymphatics. The photograph was taken after a further interval of 10 minutes. It will be seen that dye has escaped profusely all along the lymphatic channels of the heated (right) ear, while none has occurred in the control (left) ear. ×3½.

PLATE 15

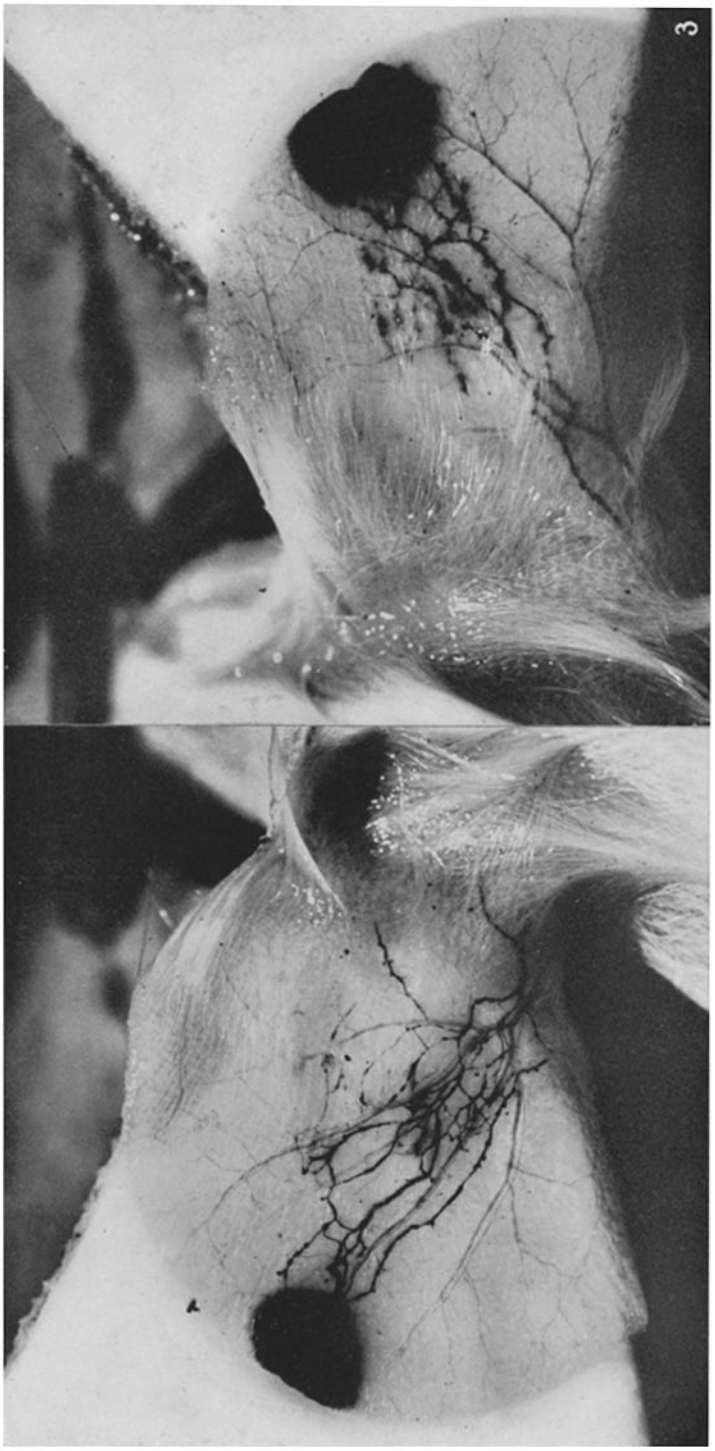
FIG. 3. Ears of a living anesthetized mouse showing the escape of dye from the lymphatics 23 minutes after painting the right ear with xylol, and 8 minutes after the channels had taken up the standard pontamine solution. A marked escape of the dye has taken place from the lymphatics of the ear painted with xylol.

Both ears show a reactive hyperemia, that on the right the more severe. The lymphatics of the control (left) ear show slight dye escape the significance of which is discussed in the text. ×8½.



Photographed by Louis Schmidt

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



Photographed by Louis Schmidt

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