THE LYMPHATIC PARTICIPATION IN HUMAN CUTANEOUS PHENOMENA

A Study of the Minute Lymphatics of the Living Skin

By STEPHEN S. HUDACK, M.D., and PHILIP D. McMASTER, M.D.

(From the Laboratories of The Rockefeller Institute for Medical Research)

Plates 46 to 49

(Received for publication, February 4, 1933)

It is common knowledge that human skin is supplied with a close network of minute lymphatics; yet the physiology of these channels remains obscure, little being known of what takes place in them under normal conditions, and still less of what goes on during inflammation and under other circumstances of injury. The lymphatics of the skin are so constituted that obviously they must be concerned in fluid exchange and fluid transport. Yet because little is known of their functionings they have been generally ignored in appraising skin phenomena.

To determine the share of the lymphatics in skin phenomena it is essential to develop a method whereby these vessels can be studied in the living tissue. Recently we have found (1–5) that the injection of vital dyes into the superficial layer of the skin of mice, rats and rabbits renders the lymph channels plainly visible in regions accessible to study, for example the ear. It has been noted that the walls of the vessels respond promptly to various stimuli, often with marked changes in their permeability. The method has now been adapted to human skin, disclosing facts that form the subject of the present paper.

Procedures

For the greater part of the work the authors served as experimental material, submitting themselves to a series of intradermal and subcutaneous injections with vital dyes. The injections were made at intervals over a period of several months, care being taken in the later tests not to utilize skin areas which might conceivably have undergone changes as result of earlier ones. Supplementary experiments were carried out on six other volunteers. The injections with isotonic dye solu-

tions of high tinctorial value resulted in an immediate entrance of the coloring matter into the minute lymphatics of the locality rendering them brilliantly visible. The anatomy of the lymphatics and the secondary distribution of the dyes could be readily followed. The permeability of the wall of the lymphatic capillaries could be gauged by observing under the microscope the rate of escape of dye from the intact channels into which it had passed.

Several dyes were used, in special the highly diffusible patent blue V and Neptune blue, and the very poorly diffusible pontamine sky blue and Chicago blue (1, 6–8). Because of the tendency of these latter to leave a long-enduring mark, owing doubtless to storage in the local macrophages, most of the experiments were done with readily diffusible dyes, which are only temporarily stored if at all (9, 10). Save in a few exceptional instances noted as such, the dyes were used in aqueous solution isotonic with 0.9 per cent sodium chloride, as determined by freezing point determinations with a Beckman apparatus. Of the materials employed, pontamine sky blue[1] is isotonic with blood in a 21.6 per cent watery solution, Chicago blue[2] in 17.1 per cent, patent blue V[2] in 11 per cent and Neptune blue[2] in 5.5 per cent (1, 3). The amount of dye present in such percentages was far greater than was necessary for the work. Consequently the autoclaved solutions were diluted with Tyrode's solution sterilized by filtration at the time when the experiment was to be done. Sterile homologous serum obtained by vein puncture was occasionally used as a vehicle for the dye.

For the injection, a uniform technic was employed. The freshly prepared solution of dye was taken into a syringe graduated to 0.01 cc. both barrel and plunger, and prepared by dry sterilization. The skin of the volar surface of the forearm, or unfrequently of the inner surface of the lower thigh was gently cleansed with tincture of green soap and sponged off with 60 per cent alcohol, with avoidance of rubbing. The region for injection was then covered with a dry, sterile sponge, the syringe was capped with a 29 gauge hypodermic needle or a special 30 gauge platinum iridium needle; with the free hand the region to be injected was firmly grasped from beneath and the skin was gently drawn taut. The injecting needle was now thrust superficially into the epidermis at an angle of 45° and with the bevel down, was brought as nearly parallel with the skin surface as possible and inserted further. Rocking the bevel of the needle a trifle, and at the same time expressing a minute quantity of dye, caused the torn lymphatic capillaries to take up the colored material immediately. Even the gentlest pressure frequently caused some of the dye to flow backwards about the shaft of the needle and escape to the skin surface, obscuring the injection field and marring the test.

Most of the injections were made under the dissecting microscope. To increase visibility sterile neutral paraffin oil was flooded on the skin either before or after insertion of the needle.

---

[1] Du Pont Dyestuffs Corporation.

[2] General Dyestuffs Corporation.

Demonstration of the Superficial Lymphatic Plexus

The sequence of events which follows an injection has been studied under the binocular microscope and photographed by a microcinema camera. Immediately upon introduction the dye solution passes into and along the lymphatic capillaries, rendering visible an exceedingly rich plexus of them which is all the more remarkable because it is wholly undisclosed by the ordinary means of examination of the skin (Figs. 1 to 6). As the injection continues, dye extends along the lymphatics (Figs. 1 and 2) often for a distance of 5 to 8 cm. from the needle point. This is best seen when slow, gentle pressure is exerted upon the injecting syringe, for under these circumstances the vessels take up most of the injected solution, only a little passing interstitially. When rapid injections are made, the lymphatic capillary plexus often becomes obscured by a localized intense coloration, only a few channels that drain from the margin of the colored area being visible (Fig. 7).

The photographs of Figs. 1 and 2 reproduce the findings at a magnification of approximately 3/2. For purposes of detail Figs. 3, 4 and 5 give some enlargements of Figs. 1*a*, 1*d*, 1*e* respectively. In the test with the individual H (Fig. 1), 0.2 cc. of 11 per cent aqueous, isotonic solution of patent blue V was injected into the skin of the forearm. The dye was expressed from the syringe in about 80 to 85 seconds, only a little more slowly than in the ordinary clinical intradermal injection. The pictures selected represent the state of affairs at 15 or 30 second intervals, save for the last pictures of each figure which were taken 30 seconds after the termination of the injection. The pictures show the course of events during the injection and immediately after it.

Immediately that dye enters the tissue it passes directly into and along the lymphatic capillaries, even though the least possible pressure is put upon the syringe plunger. In the experiment of Fig. 1, within 30 seconds after beginning the injection the plexus of lymphatics showed dye over an area 14 to 15 mm. in diameter (Fig. 1*b*), and in 45 seconds the colored plexus was 2.5 cm. (Fig. 1*c*) wide. Within 1 minute and 15 seconds the outlines of the vessels first entered became blurred by dye that had escaped secondarily from them into the interstitial spaces through the lymphatic wall (Figs. 1*d* and 4). Thus the channels nearest the needle became completely obscured by a cloud of color. After the needle was withdrawn (Figs. 1*e* and 5), dye continued to extend along the channels at the periphery of the injected region. This extension after the injection is completed, and the abundant escape of dye from the channels in which it has been present longest is well seen in Fig. 6, an enlargement from a photograph taken about 5 minutes after

the last one shown in Fig. 1. Escape took place later from the other dye-containing channels.

It is plain that the lymphatic capillary wall is highly permeable for the very diffusible patent blue V. So rapid and abundant was the escape in such an experiment as that just described, that within a few minutes the definition of the individual capillaries was lost save at the margin of the stained region. By this time, too, the tissue into which the needle had been thrust had become distinctly edematous. Within an hour, the edema had spread and with it the diffuse interstitial coloration as well. For the next few hours, secondary dispersion of the dye continued while the color intensity of the stained region faded proportionally. After 8 to 10 hours it became possible once again to see the minute blood vessels around the site of puncture, and edema and hyperemia were but barely perceptible. There was no pain or tenderness. By the next day, the coloration of the skin had disappeared, leaving only faint traces which remained for 48 or even 72 hours. The site of the needle puncture remained visible sometimes as a colored point for several days, but the regions that dye had reached only by secondary escape from injected lymphatics were always practically decolorized within 24 hours. The urine shows traces of the dye as early as 1 hour after the injection and occasionally for as long as 18 hours.

When 0.1 cc. of the dye was very rapidly injected there resulted a localized, sharply demarcated swelling elevated 1 to 3 mm., which spread to a diameter of only 1 to 3 cm., instead of the greater distance observed when the injection had been slow. The removal of dye was more rapid than in experiments such as that just described.

The Permeability of the Lymphatic Capillaries in Human Skin

In earlier work from this laboratory the rate of the escape of vital dyes of known diffusibility from lymphatic capillaries of the mouse ear has served as a means of studying the permeability of their walls and the factors influencing this permeability. Our present experiments have shown that pontamine sky blue escapes more slowly from the intact lymphatic capillaries of normal human skin than does Chicago blue in equimolecular solution, while patent blue V, and also Neptune blue, escape more readily than either of the pigments first mentioned, the rate of escape of all four dyes being roughly proportional to their diffusibility through collodion membranes. This is true also of the entrance of these dyes through the intact lymphatic wall, as will be brought out further on in the paper. Increasing the concentration of any one of the dyes quickens its rate of escape while a decrease retards it. The presence of human blood proteins (serum admixture)

in the injected solution acts to retard dye escape. The technic and the results of these experiments have been similar to those in our work upon the mouse (1) and hence need not be detailed here. But the existence of individual differences in the permeability of the skin lymphatics merits remark.

In repeated observations upon one of the two authors, isotonic patent blue V, 11 per cent in Tyrode's solution, passed through the lymphatic capillary walls in perceptible quantity within 1 to 2 minutes, whereas in the case of the other, 2 to 3 minutes was required. The difference could not be laid to variations in skin transparency since the lymphatics were so superficial that this could not enter as a factor. In five volunteers on whom we made a few tests each, the time of the first visible dye escape also differed, two showing some in 1 to 2 minutes and the other three in 2 to 5 minutes. The findings were consistent in repeated injections of any one subject. One cannot conclude, however, that individual differences exist in the intrinsic permeability of the wall of the lymph capillaries since many other factors can be invoked which will suffice to explain these differences.

Anatomy of the Cutaneous Lymphatics, with Reference to Individual Differences

Dye can be introduced into the lymphatic capillaries of some individuals with great ease, whereas into those of others only with difficulty, the injection material being almost at once led off into the deeper draining trunks. Figs. 1 and 2 serve excellently as examples of the major differences consistently found in experiments on individuals H and M as well as in five other volunteers.

To determine the cause for the individual differences, 24 skin specimens were injected and studied. The material, taken either at a prompt postmortem examination or at surgical procedure included specimens from the new-born and still-born, from infants, children, adults and old people.[3] The postmortem material came from the midline above or below the umbilicus, that of the surgical clinic from amputations of the breast. Normal and pathological instances were available, including the skin of senile hyperkeratosis, and the atrophy of inanition. All specimens were warmed to 44°C., and were then injected intradermally with a mixture of dialyzed India ink and 5 per cent gelatin at 44°C. When concentrations of gelatin higher than 5 per cent were used, the finest superficial lymphatic

[3] For the supply of material, we are indebted to the Departments of Pathology and of Surgical Pathology of the Cornell University Medical School and New York Hospital.

capillaries frequently failed to be entered by it. Many injections were made in each specimen, using for some the same technic which was employed to fill the lymphatics with dye during life. In other instances, to prepare a way for the injection, a finely ground dissecting needle was thrust into the corium and then pushed parallel to the skin surface for 2 to 4 cm. The procedure carried out under the binocular microscope, ruptured many of the superficial lymphatic capillaries, and when a 30 gauge platinum hypodermic needle was forced into the puncture track and injecting fluid introduced through it, many more lymphatics of the superficial plexus filled, at times over a larger area, than in the ordinary test in the living subject. Both methods of injection of the skin specimens gave similar findings, save that the one just described yielded more completely injected preparations.

At once after injection all specimens were chilled in normal saline solution and fixed in acid-formalin or alcohol. For examination some of the injected regions of each specimen were sectioned in the usual manner, others were cleared and preserved in oil of wintergreen for study, and still others, which had been cleared, were stained in solid slices about 1 mm. thick with dilute hematoxylin and eosin, yielding most beautiful preparations which showed clearly the position and relationships of the injected superficial lymphatic capillaries.

All specimens regardless of the age or sex of the individual, yielded pictures of the lymphatics having similarities either to those of Figs. 1 or 2, every gradation between the two being found. In certain specimens a rich superficial plexus of lymphatics was readily disclosed while in other specimens fewer of the superficial lymphatics and more of the deeper draining trunks took up the injection fluid. But even in the latter specimens, it was possible to show the presence of the rich superficial plexus—if several injections were made with great care under the microscope. Because of individual peculiarities of the skin the needle tended, in some specimens, to pass too deeply or else not deeply enough to inject the superficial plexus well. To this simple cause the pronounced difference in results seemed wholly attributable. More will be said of the results in a subsequent paper.

Cleared and stained, injected specimens and sections thereof show the lymphatics described by others, namely superficial lymphatic capillaries lying in the papillary stratum of the corium with blind ends extending into the papillae—and a deeper capillary plexus in the lower layer of the corium and the tela subcutanea. The existence of these plexuses has long been known and recently their relationships have been well shown by diagrams such, for example, as that of Neumann (10) reproduced from von Brunn (11) in Bartels' monograph (12). In

the past von Recklinghausen (13), Gerota (14), MacCallum (15) and others have noted the extreme superficiality of the lymphatic vessels of human skin; but it seems to us that the richness of this plexus of superficial lymphatics has not been sufficiently emphasized. This has resulted from the failure of many workers to inject the channels completely. When thick dye solutions, heavy India ink suspensions and injecting masses were utilized by us to obtain permanent preparations only incomplete injections of the lymphatic capillaries resulted.

That we have been dealing with actual vessels and not artifacts was proven by the characteristic appearance of the vessels when filled with colored fluid, by the speedy drainage into lymphatic vessels high up in the arm when injections were made into living skin and by the finding of endothelial lining cells about the ink-filled channels in cut and stained sections from amputated skin. Frequently, to be sure, in making permanent preparations, the injected fluid broke out into the interstitial spaces, an occurrence readily recognizable in the gross and in cut sections, as MacCallum has already shown (15). Occasionally, too, in making an injection in skin removed from the body the needle point became lodged too superficially, among the horny cells of the corium. In these instances the injecting fluid entered the epidermis itself and spread out yielding an intercellular injection which in the gross has a rough likeness to a lymphatic injection in that the dye solution was distributed like the strands of a net. When such injections were examined under the binocular microscope, and later, when sections were examined, they could readily be recognized as spurious.

The fact that a single injection of isotonic dye solution into living skin fills the superficial lymphatic capillaries over an area 3 to 5 cm. in diameter, or more, deserves special remark for it shows as have the fixed preparations, the existence of innumerable anastomoses among those lymphatic vessels lying in the papillary stratum of the corium, vessels which escape recognition in ordinarily stained sections.

Recently Handley (16) discussing the rôle of skin lymphatics in the genesis of cancer, has stressed the anatomical relationships of these vessels as shown after injection. This author pictures small groups of six or eight blind papillary lymphatic capillaries, each group a unit as it were, meeting to form a common vessel which passes through the tela subcutanea to form with other similar vessels the subcutaneous lymphatic plexus. He holds that in the subpapillary layer of the corium only a few of these vessels anastomose and as evidence for this view (16) cites a specimen injected by Hyrtl but never

described although the latter author had written much upon the theme of skin lymphatics (17). We find anastomoses to be abundant. Doubtless they escaped Handley because of incomplete injection of the vessels.

Many of the lymphatics extend at an inclined angle to the skin surface, communicating between the superficial and deep plexuses, and branching as they run. They do not directly link one capillary plexus with the other but form an almost continuous network. When a lymphatic injection of dye solution or ink suspension is successfully made the superficial plexus conducts the material for a distance of 3 to 5 cm. from the point of injection. If another successful injection is made 8 cm. from the first, the lymphatic capillaries carry material from the latter directly into some of the channels first injected, showing how direct are the anastomoses of these vessels. When four or five injections are spaced in the form of a circle, at a distance of about 5 cm. from each other, the superficial lymphatics of most of the enclosed area may become injected, once again showing the rich anastomosis.

The Lymphatic Involvement in Skin Injury

Invariably in our experience, colored substances injected intradermally on the surface of the forearm have entered the lymphatics directly. This fact prompted us to a further study to determine how considerable an injury is required for direct entrance into the skin lymphatics.

On the surface of the forearm, cleansed with soap and alcohol, linear scarifications about 1/2 cm. long and 2 cm. apart were made, single scratches with a sharp, sterile needle manipulated under the binocular dissecting microscope. Only the superficial epithelium was removed, no skin venules or capillary tufts were visibly torn, and there was no bleeding. To facilitate consecutive observations after known periods of time the scarifications were made at 2 minute intervals. Equimolecular isotonic solutions of pontamine sky blue, Chicago blue and patent blue V were dropped upon the scarifications and immediately covered with sterile neutral paraffin oil to prevent drying.

As one watched, the dye appeared in numerous lymph capillaries leading away from the lesions, these becoming visible under the microscope in 1 to 6 minutes because of their colored contents. In later experiments, we found it best to allow an interval of 6 to 8 minutes between the scarification and the dropping on of the dye, else some irregularity in the findings would occur as the result of wheal formation at the margins of scratch. Work to be reported below has shown that during the formation of histamine wheals, the lymph vessels undergo compression by the edema of the wheal. After the interval mentioned, patent blue V applied to the

scratches became visible in the local lymphatics in 1 to 3 minutes, Chicago blue appeared in them more slowly—3 to 5 minutes,—and pontamine sky blue more slowly still, in 4 to 6 minutes.

It was plain from the differences in the time required for the dye to appear in the lymphatics, this varying inversely with the diffusibility of the pigment, that the lymph channels had not been torn open by the superficial scratches. That patent blue V passed most quickly into the lymphatic stream was of course to be expected, as it was the most diffusible of the dyes used; but the fact deserves stress in the present relation since the permeability of the lymphatic capillary wall was here being tested in the direction which interstitial fluid naturally takes to enter the lymph stream, not in the reverse direction as in the previous experiments. The appearance time of Chicago blue was intermediate between that of patent blue V and pontamine sky blue, and with the aid of color filters the fact could be made out that it entered the lymph channels somewhat less slowly than the latter.

In other experiments, crystals of dye were allowed to dissolve in the fluid which exuded from the scratches. The coloring matter was soon seen in the lymph capillaries draining the lesion. Similar experiments in which crystals of dye were placed in minute punctures of the skin of the mouse ear have yielded similar results. In both the mouse and man, patent blue V invariably appeared in the venules draining the region where the crystal was dissolving. Chicago blue on the other hand appeared in the blood stream but seldom, and pontamine sky blue only when venules or capillaries had been obviously ruptured. The use of crystalline dye was not free from objection because of the marked osmotic influence of the dissolving dye. Nevertheless, it was significant that only the highly diffusible patent blue V passed into the blood stream in visible quantity. Pontamine sky blue, the most indiffusible dye that yielded positive results in the tests, the one nearest to the albumins in molecular magnitude, was drained away chiefly by the lymphatics. In human skin because of poorer visibility, the phenomenon of its entrance into the lymph stream, is not so distinctly seen as in the mouse.

When a sterile, sharp needle is dipped into an isotonic solution of pontamine sky blue and the skin punctured with it, the dye almost invariably appears at once in the lymphatics close to the puncture, obviously as a result of tears in their walls.

Many such experiments, as well as those involving scarification or intradermal injection, have led us to conclude that all slight breaks in the physical barrier of the skin yield opportunity for the passage of dissolved foreign matter into the skin lymphatics, and that the slight-

est penetration beneath the epithelium opens these vessels to the entrance of particulate matter.

The Rate of Lymph Transport

When as little as 0.05 to 0.10 cc. of isotonic dye solution is slowly injected into normal skin the phenomena resemble those already described. Dye spreads within the lymphatic plexus over an area 2 to 3 cm. in diameter, and within 3 to 4 minutes some of the dye escapes secondarily into the interstitial tissue through the walls of the capillaries containing it. The remainder is seen extending somewhat diluted into lymph channels not filled originally, notably into the larger lymphatics draining the region. 5 minutes after the injection dye can usually be seen 15 cm. away from the injection spot in the draining lymphatic trunks, rendering visible those which lie in the subcutaneous fat. In two instances dye has been perceptible in the draining trunks of the axillary portion of the arm 8 minutes after an intradermal injection of 0.10 cc. of patent blue V into the volar surface of the forearm.

The arm in these experiments had throughout rested upon a table, with the elbow slightly flexed, and the shoulder dropped so that the entire limb lay at the heart level. In ten other instances, after similar injections, the dye appeared in the axillary trunks within 10 to 18 minutes. During the period of observation these small injections of isotonic dye solution gave rise to local swelling which required 5 to 10 minutes to form. In estimating from these experiments the rate of lymph transport in untouched skin the factor of pressure at the injection site cannot be ruled out. However, the fact that the swelling required several minutes to form and that before this happened, dye had passed in draining trunks several centimeters distant from it, suggests that the escape of fluid from the blood as result of the puncture injury had only a slight degree of influence, if any, upon the experimental results.

In Fig. 7 is pictured an instance of rapid lymph drainage. The photograph was taken 6 minutes after two simultaneous injections of 0.02 cc. of patent blue V into the individual M. The black, oblong mark on the skin surface near the site of the upper injection is due to dye that passed back along the needle track. The net-like appearance of the injected lymphatic plexus is not seen because already the secondary escape of dye through the walls of these vessels has obscured it, with result a deep and even color of the originally injected region. Immediately surrounding this latter a few lymph capillaries have been rendered visible by dye content and stand out as twig-like extensions. The draining trunks extending up the arm are seen in a lighter shade of the dye. They could be followed almost to the axilla with the unaided eye, a fact which does not appear in the photograph.

The rapid passage of dye along the draining lymphatics cannot have been due, under the circumstances described, to the amount of fluid injected, 1/50 cc. at each site. Nor can it be accounted for by local hyperemia with transudation from the blood vessels, for neither hyperemia nor edema had developed. One is forced to the conclusion that the lymph flow from the skin of the resting human arm is abundant. Experiments on the mouse ear have also led us to conclude that peripheral lymph movement is rapid in the skin of animals (1, 2).

The State of the Lymph Capillaries in Inflamed Skin

Much has been written upon changes of the permeability of blood vessels of the skin in response to various stimuli but the question of whether the lymphatic wall also undergoes changes seems not to have been raised. Dye injection has enabled us to observe the responses of the lymphatic capillaries under various normal and abnormal conditions.

The Effect of Heat.—In two subjects patches of skin on the forearm were heated for 1 minute by applications of a thin-walled glass chamber (2) through which water circulated at 56°C. 5 to 10 minutes thereafter a minute quantity, 0.01 cc. of a mixture of 5.5 per cent solution of Neptune blue and homologous serum, in equal parts, was injected at two points. The first injection was so placed that the dye solution filled lymphatic capillaries lying outside the heated region but included in the hyperemic flare. The second injection was made directly into the heated skin near its edge with result that dye passed into the lymphatics of the injured area and some extended into the channels outside of it.

Five experiments of the sort described were carried out on each subject. The results were the same in all. The lymphatic capillaries lying in the heated area let dye pass into the interstitial spaces far more rapidly and abundantly than did the vessels in the contiguous unheated region. In four other experiments on each subject, patent blue V, diluted to 5.5 per cent with Tyrode's solution, was used, and the same results were obtained. So, too, in tests with the less diffusible dyes, Chicago blue and pontamine sky blue. These observations, confirming as they do those in experiments on the mouse ear (2), extend the findings to human physiology.

Effect of Ultraviolet Light.—In six experiments, pieces of smooth flexible cardboard having rectangular openings measuring 1½ x 3 cm. were strapped with adhe-

sive tape to the volar surfaces of the forearms of two individuals. The arms,—which had not been exposed to sunlight for several months—were subjected to irradiation for 3 minutes at 18 inches from a mercury vapor quartz tube ultraviolet lamp. The rectangular spots of unprotected skin developed a mild hyperemia in 3 hours, and by the next day, stood out upon a background of normal skin as brightly hyperemic and distinctly edematous inflamed areas. Injections of pontamine sky blue and patent blue V were made, within and just without these areas, as in the case of heated skin.

The lymphatics of the region of injury from ultraviolet light let dye through into the interstitial tissue far more readily than did those of normal skin and were the sooner obscured by it. The increased permeability of the lymphatic wall to patent blue V was so great in the injured region that the dye seemed to pass out at once through it, as if it offered practically no barrier at all. It escaped from the lymph capillaries so rapidly, that little or none passed directly to the draining vessels, but within 15 minutes it was visible in these as result of secondary resorption from the interstitial tissue.

Very striking were the results in three instances in which the dye injections were made at the margin of the irradiated area in such a way that about the same quantity of dye passed into the lymphatics of the inflamed area, and into the channels of the adjacent normal skin. For example, in one experiment, 5 minutes after the injection of 0.05 cc. of isotonic 11 per cent patent blue V, the hyperemic area receiving the dye showed a diffusely colored, deeply greenish blue spot in which no lymphatics whatever could be made out whereas in the adjacent normal skin, they could still be seen with some distinctness.

Effect of Local Injection of Toxins and Bacterins.—Two individuals, M and H, were tested for their response to intradermal inoculations of standard Schick test and Dick test toxins, to a concentrated streptococcus toxin,[4] and to the standard typhoid vaccine of the New York Board of Health. As neither volunteer responded sharply to the Schick test material, it was not used further. The technic for the intradermal injection of toxins and vaccines was the same used for the dyes.

The concentrated streptococcus toxin caused within 20 hours after intradermal injection a development of large erythematous macules, slightly raised, moderately well defined and about 1.5 cm. in diameter. To cause this reaction 0.01 cc. suffices, the equivalent of one skin test dose. Scarlatinal toxin in skin test dose gave similar results, but the area of inflammation was about 2.5 cm. in diameter and the margins were not sharply defined.

Both subjects had received one or more courses of inoculation with typhoid

[4] Kindly supplied to us by Dr. Homer Swift and Dr. Currier McEwen, of the Hospital of The Rockefeller Institute.

vaccine 2 or 3 years previously. In both a severe skin response now followed intradermal injection of 0.05 cc. of this vaccine. Following three intradermal injections of 0.02 to 0.03 cc., given at one time on the volar surface of the forearm, axillary tenderness developed after 2 hours and 1 or 2 hours later the axillary nodes became palpable. In 12 hours inflamed lymphatic trunks could be perceived coursing from the region of injection, with hyperemic zones along them. After 22 hours headache and malaise developed.

To determine the lymph movement after intradermal injection of the several toxins used, some preliminary tests were made with pontamine sky blue or patent blue V, which were added to the injection material, using 1 volume of isotonic watery dye solution to 20 or to 10 parts of toxin respectively. In every instance, the fact could be made out that the material injected entered and coursed along the lymph capillaries like an ordinary dye solution. Pontamine sky blue was mixed with the typhoid vaccine in an attempt to determine whether the bacteria, entering a puncture, would pass into the lymphatics as well. The dye of the mixture passed directly into the lymphatic capillaries. It was impossible to recognize the bacterial bodies themselves with the available apparatus.

In the experiments proper no dye was added to the toxins or vaccines. Within 24 hours after the injection, each of the test materials had produced areas of inflammation and these were used for the experiments. Fig. 9*a*, taken from one of these, shows the erythematous macules arising from four injections of 0.01 cc. of the streptococcus toxin, photographed 18 hours and 45 minutes after inoculation. To show the erythema the photograph was taken with a Cooper-Hewitt light. The arm had been used as ordinarily since the injection. In Fig. 9*b* the same arm appears 40 minutes after an injection of 0.01 cc. of 11 per cent patent blue V into three of the four macules, M_1, M_2, and M_3, Macule M_4 was left untouched as a control. Three areas of normal skin, C_1, C_2, C_3, were given similar injections of dye solution at approximately the same time. Thereafter, in the time interval between the injection of dye and the taking of the next photograph for Fig. 9*c*, the arm was not rested or protected in any way but was used as ordinarily in the course of laboratory work. In the instances, Figs. 9*b* and 9*c*, the Cooper-Hewitt light was not used but the photograph was taken through an orange G Wratten filter to accentuate the blue color. As result the deep red uninjected macule (M_4) is difficult to see. Fig. 9*b* shows the injected hyperemic areas deep in color, and the injected control areas more irregular and much paler. The lymphatics in the region of inflammation proved so permeable that the injected dye passed at once from the lymph channels into the interstitial tissue to yield an even, deep coloration. The same amount of dye injected into the control areas C_1, C_2, C_3, was more widely distributed through the lymph channels, escaped much more slowly into the interstitial spaces and colored the tissue irregularly.

Fig. 9*c* is a photograph of the same arm 3 hours and 10 minutes later. Already the major portion of the coloring matter has disappeared from the inflamed regions into which it was injected whereas the control areas remain heavily colored. These were not entirely free from dye 18 hours after injection by which time the inflamed

spots were completely cleared of blue color. When standard typhoid vaccine or Dick test toxin was used to excite an inflammation, similar results obtained.

In two other experiments, like that just described, four areas of inflammation were caused by intradermal injection of standard typhoid vaccine. The day after it had been introduced the inflamed regions as also two control areas of normal skin were injected with 0.01 cc. of the highly indiffusible 1 per cent pontamine sky blue in Tyrode's solution. The same immediate findings were obtained, and in one of the experiments all of the inflamed areas decolorized before the control areas did, while in the second experiment this was the case with three out of four of the inflamed areas.

In no instance did the inflammation progress; and except for a residuum of brownish pigmentation all the lesions resolved themselves within a week. In one injection following the use of typhoid vaccine and a poorly diffusible dye in which some of this latter passed subcutaneously the color persisted locally for several weeks.

It is plain from these various findings that in skin recently inflamed by heat, ultraviolet light or by bacterial vaccine or toxin, the walls of the lymphatic capillaries become far more permeable to vital dyes, irrespective of the grade of diffusibility of the latter. After intradermal injection into areas inflamed with toxins and bacterins secondary escape from the lymphatics occurs more rapidly, and the dyes are removed in much less than the time required to clear a normal area of skin of a similar injection. The alternative explanation that the dyes are changed to colorless derivatives in the inflamed region, need not be entertained since they are stable compounds. Similar though less sharp findings as concerns the rate of decolorization were obtained in the experiments upon heated skin. In our experience the inflamed skin decolorized in about one-fourth the time necessary in the case of the controls.

The Skin Lymphatics during Wheal Formation

What are the changes which take place in the lymphatic capillaries of skin stroked or otherwise stimulated to wheal formation? To test this point we have employed a subject H who responded to the usual clinical test,—a firm stroke with a blunt instrument,—by the formation of wheals which were perceptible within 2 minutes and well defined in 3. When the normal skin of this subject was stroked, the walls of the lymphatic capillaries in the affected region were found by the dye injection method to be more permeable than normal during the 2 minute latent period before appearance of the wheal.

In six experiments, a solution of equal parts of aqueous isotonic Neptune blue and homologous serum was injected intradermally during the latent period. Wherever the dye-containing lymph channels crossed the area eventually involved in the wheal, there was dye escape from them, whereas from those outside the region of stroke no such escape occurred. These findings with human skin corroborate the results in mice (2) (see Fig. 1 of paper referred to).

Pontamine sky blue in isotonic approximately 1 per cent solution, that is to say, 0.1 cc. of isotonic 21.6 per cent aqueous dye solution mixed in 2.16 cc. of homologous serum, passes out of normal human skin lymphatics very slowly. Only after 15 or 20 minutes is extravascular color seen. This poorly diffusible mixture, injected during the latent interval after stroking, and extending along the lymphatic capillaries within the area which had been stroked, escaped from the channels in the line of stroke.

In the experiments with pontamine blue the fact was noted with the microscope that as the wheal developed it pressed the colored fluid along the channels and out of the region involved. Two intradermal injections of 0.1 cc. of isotonic 11 per cent patent blue V in Tyrode's were made into a normal area of skin on the forearm. When 4 hours had elapsed, and the dye had spread evenly through the interstitial tissue forming two colored patches across which the skin was firmly stroked to evoke a wheal. On both sides of the line of stroke wheal formation became visible 2 minutes later and in 3 minutes was well defined, with sharp margins. At this time the photograph reproduced in Fig. 8 was taken under a pool of oil, held from flowing away by plasticine placed on the skin round about. Although the definition thus obtained was excellent the phenomena were more marked to the naked eye than in the figure which does not render the color contrast in its entirety. Along each side of the relatively pallid wheal was a dark line of dye. The dye in this line lay in the interstitial tissue, not in the lymphatics and must have been pressed out of the wheal region by the edema fluid. Tests of the sort described were repeatedly made on the same subject and the results were always the same. As the wheal edema developed it compressed the lymphatics contiguous to the injury, as could be directly seen, thus narrowing or blocking the channels. This point has been demonstrated by applying patent blue V to a scratch made in the region where whealing has just started, according to the technic already described, or introducing it by means of a needle puncture. The appearance of the dye in the lymphatics in which the test was made immediately after the whealing stroke or after the wheal had begun to regress was not delayed as compared with the event in instances above.

The experiments show that the lymphatics of the skin submitted to a whealing stroke become at once abnormally permeable; that as the wheal develops fluid previously present in the region involved is forced into the surrounding skin, and that the lymphatics in the wheal region lose their effectiveness as draining channels. This last change would seem to be the result of direct pressure on the lymphatics nar-

rowing or blocking their lumina combined with such an increase in the permeability of their walls that they no longer retain and pass on their contents.

The Effects of Histamine

A study was now made of the lymphatics in the wheals produced by histamine.

In some initial experiments histamine solutions containing dye were injected into normal skin, result being that the two substances were distributed together. In five experiments 1 part in 50,000 of histamine was present in an aqueous solution of patent blue V (11 per cent), injections were made simultaneously of the dye solution with and without histamine. The skin of the volar surface of the forearms of the two subjects was utilized. The injections were made 5 to 7 cm. apart, at the same level on the arm. Whealing occurred in 2 to 3 minutes with the histamine-containing dye solution. During the preliminary period and in the following 2 minutes, far more dye escaped from the lymphatics than in the localities injected with control solution. Thereafter the swelling caused by the histamine mixed with dye obscured the findings so that further comparisons were not possible. In four other experiments the test mixture consisted of histamine 1 to 100,00 and patent blue V in 5.5 per cent concentration with Tyrode's solution as the diluent to preserve isotonicity. The control solution, a 5.5 per cent solution of patent blue V in Tyrode's solution and containing no histamine was injected at the same time. Although in these instances the formation of wheals was slower, similar results obtained, showing that histamine in 1 to 100,000 increased the permeability of the lymphatic capillary wall.

In six further experiments, 1 to 50,000 watery histamine solution was pricked into a region of skin in which the lymphatic capillaries had been injected 2 minutes previously with 11 per cent, aqueous, isotonic patent blue V. As usual the procedure caused the development of a wheal, and as the wheal developed the pressure at its extending margin squeezed the colored solution along the vessels into the normal skin beyond, a course of events plainly visible under the microscope. Dye which had already escaped into the interstitial fluid and had colored the latter diffusely was also pressed out of the whealing area. The result was that the wheal became surrounded by a darkly colored ring, itself appearing pale against a dye-stained patch.

In seven experiments 0.005 to 0.01 cc. of a watery 11 per cent isotonic solution of patent blue V was injected into the skin over a freshly and fully formed histamine wheal. The dye solution passed at once into the lymphatics, was retained by them and extended along them just as in ordinary skin. The lymphatics appeared to be less compromised by pressure than in the wheals produced by stroking, and dye-stained fluid passed into and along them. During a period of 6 to 10 minutes dye solution escaped hardly at all into the interstitial fluid, but instead

the injected lymphatic channels became paler and were apparently cleared of dye in 12 to 18 minutes. During the regression of the wheal the dye in the lymphatics appeared to be diluted rapidly.

It would seem from these findings that the abnormal permeability of the lymphatics noted immediately after histamine injection no longer existed in the fully formed wheal. Lewis (18) in his studies of the responses of blood vessels of the skin called attention to the finger-like extensions of histamine wheals as indicative of a spread of the substance through the lymphatics, with its subsequent escape and secondary wheal formation along the course of the vessels. In some of our experiments evidence was obtained in support of this supposition.

In five instances fully formed histamine wheals with finger like projections were elicited by puncturing the skin through a drop of aqueous, histamine solution 1 to 1000 dilution. Subsequent intradermal injections of the dye were made directly into the skin overlying the wheal. In all five instances as the dye solution was carried away in the lymphatic channels vessels of this sort were disclosed passing directly along the axis of the whealed finger-like projections. It seemed clear that the histamine must have escaped from them and given rise to the secondary whealing about them.

The experiments show that lymphatic capillaries affected by histamine allow dye to pass into the interstitial spaces more readily than do those of the normal skin, a state of affairs also found in skin submitted to stroke for whealing purposes. Although histamine clearly increased the permeability of the lymphatic wall in the experiments in which the substance was injected together with dye solution, this did not seem to be the case when dye was injected into fully formed wheals. As whealing developed the fluid previously present in the skin was forced into the adjacent tissue and the lymphatics within the whealed region were compressed.

DISCUSSION

The experiments here presented show that it is possible to study directly the lymphatics of living human skin. They have demonstrated the existence of a rich plexus of minute lymph vessels in the superficial layer of the corium, a fact many times proved by others but not ordinarily taken into account in appraising the factors involved in skin reactions. Some have supposed the capillary lymphatics to be divided into small groups, each group draining into the tela subcutanea and essentially separate from the others because of the absence of superficial anastomoses. We find on the contrary that in

the skin of the arm and thigh, the regions subjected to investigation, the superficial lymphatic network is continuous, material injected at one spot often spreading directly through the channels of a large area. The abundant anastomoses deserve emphasis because of a further finding that every least wound of the corium tears lymphatics open with result that material enters them directly. Scratches which merely abrade the epidermis without entering the corium facilitate absorption through the walls of the intact, underlying lymph channels.

To judge from our experiments the permeability of the lymphatic capillaries in the skin of man and in the ear of the mouse appears to be similar in nature and degree (2), the lymphatic wall behaving like a semipermeable membrane, irrespective of whether dye is passing in or out of the vessel. Before considering the latter conclusion as definitely established it would be desirable to carry out more work upon human skin with highly indiffusible dyes; but unfortunately the use of these leads to prolonged discoloration and hence they cannot be used freely. Since little is known about the physiological effects of the dyes actually used it has seemed best to exercise caution in their employment upon human beings, limiting the experiments so far as possible.

Slight causes suffice to increase greatly the permeability of the minute lymphatics. A stroke on the skin of an individual susceptible to whealing causes practically at once such a change in the lymphatic walls that dyes ordinarily held back by them for some time pass through at once. This is the case also when the skin has been slightly inflamed by the application of heat or ultraviolet light, and during the more considerable inflammation that results from the intradermal injection of a bacterial toxin or of killed pathogenic bacteria. How great may be the effect of very mild heat, or of moderate exposure to sunlight we are unable to say; but judging from the general similarity of the results of our experiments on man to those with the mouse already reported by us, it seems probable that such stimuli, well within the realm of the normal, may much increase lymphatic permeability.

The clinical significance of these findings is not inconsiderable. So much material enters the lymphatics when an intradermal injection is made that every injection of the sort is to a greater or less extent intralymphatic injection. In some individuals material injected in-

tradermally spreads through the superficial lymphatics of a considerable area whereas in others it is more localized, a difference due, as we have found, to differences in skin texture such that in some persons the injecting needle tends to penetrate deeper than in others, with result that material is deposited mainly beneath the lymphatic plexus. To this fact, certain of the differing individual reactions to intradermal test injections that are observed clinically, can be laid. In both types of individuals, however, injected material finds its way promptly into the large draining lymphatics. The rapidity of this occurrence when minute quantities of dye-containing solution are introduced into the skin proves that the flow along the lymphatics draining the skin is rapid even when the limb is at rest, and by corollary that fluid turnover within the skin must be abundant. Dye can often be perceived in the lymphatics at the axilla within 10 minutes after the injection of 1/50 cc. of dye-containing solution into the skin of the forearm. It follows that not only is an intradermal injection an intralymphatic one, often almost entirely so, but it is also to some extent a general injection, not merely a local one—more or less of the material introduced passing quickly along the lymphatics to the blood stream.

The work of previous authors has led them to conclude that there is almost no lymph flow from the resting limb. But they employed anesthetized creatures, animals which had not the greatly specialized skin of the human being with its abundance of blood vessels provided for highly developed functions. The fact should, however, be mentioned as bearing on our findings that a reason for the rapid passage of dye-bearing lymph to the axilla may be found in the circumstance that only a few of the lymphatics draining the forearm enter the supratrochlear or epitrochlear glands, most of the injected dye being carried directly into the lymph channels of the upper arm. This does not alter the fact that lymph flow from the human cutis is remarkably rapid. Earlier work on the lymphatics in the ear of the mouse has led us to believe that peripheral lymph turnover in this organ as well is great (2). Working with dogs, Field and Drinker (19) reported the fact that subcutaneous injections of horse serum appeared in the lymph draining from an injected limb, but did not do so more rapidly when multiple injections were made, evidence that direct injection of the lymphatics did not occur. The point illustrates well the differ-

ence between the lymphatic involvement resulting from subcutaneous as contrasted with intradermal injections. Certainly in man the skin cannot be injected through ordinary hypodermic needles without entering the lymphatics directly.

The participation of the lymphatics in skin lesions must obviously be great. But it will not be susceptible of accurate analysis until more is known of the change taking place in such vessels under normal and pathological conditions. Some inferences appear justified, however. As is well known the blood capillaries in inflammatory foci become so permeable as to let out into the skin much more material than ordinarily; but in this skin the lymphatics have been so changed that they can be entered from the interstitial tissue much more readily than usual. It follows that there should occur in the inflamed area a much greater fluid turnover than usual. Our experiments show this to be the case. Dyes injected into inflamed and normal control areas of skin spread much further to begin with in the lymphatic plexus of the normal area because the walls of the channels into which the dye is introduced through tears made by the needle do not so readily let it escape into the interstitial tissue. The resulting stained patch in the normal area is broader and lighter than in the inflamed one. Nevertheless decolorization is accomplished much sooner in the inflamed area. The relative shares of the blood and lymph vessels in the process have yet to be appraised. The findings as described seem in direct contradiction to those of Menkin (20, 21) who observed that foreign materials circulating in the blood tended to localize and be held within inflamed regions, largely as the result of lymphatic thrombosis, or so he supposed. But Menkin dealt with purulent or necrotic types of inflammation and ourselves with that giving rise to no more than rubor, calor and turgor.

Under certain circumstances of turgor the lymphatics may undergo mechanical compression as we have found. This was the case in fully developed wheals due to histamine or to stroking. Quite possibly the same thing happens in sudden acute swelling due to inflammation the result being that the local accumulation of fluid from the blood persists longer than it might were the channels not pressed upon. During the development of wheals fluid escaping from the blood vessels forces from the interstitial tissue the fluid previously present therein—a fact readily demonstrated by the dye method.

A further word may be said upon the process of wheal formation since the interpretation of this phenomenon well illustrates the current disregard of lymphatic functioning. The fact is well known that the minute blood vessels involved in the wheal become abnormally permeable even to the point of letting out substances which would not otherwise pass into the tissue, as for example Congo red (22). But it has not been realized that concurrently the lymphatics become more permeable and that though this is the case they, like the blood vessels, become so compressed by the wheal fluid that their efficiency as drainage channels is interfered with. Herein lies a reason why wheals become sharply delineated. However, a complete understanding of wheal formation and disappearance necessarily waits upon further knowledge.

SUMMARY

A technic is described for the demonstration of lymphatic capillaries in living skin and for their study. By means of vital dyes injected intradermally these vessels can be rendered plainly visible. They form an extraordinarily abundant anastomotic web. The least scratch, one which does not penetrate through the epidermis, gives rise to such conditions that lymphatic absorption readily takes place from the abraded surface; and so close-meshed is the lymphatic web that an intradermal injection with even the finest hypodermic needle tears some of the constituent vessels open with result that they undergo direct injection. In many individuals much of the fluid introduced at an ordinary intradermal injection, like that made in the clinic, spreads through the superficial lymphatic network, whereas in others it tends to enter the deeper lymphatics at once, the difference being due to merely physical factors determined by skin texture. Normal flow along the skin lymphatics is rapid even when the body is at rest, dye introduced into the skin of the resting forearm reaching the axilla within a few minutes. The observations make plain the fact that every intradermal injection is an intralymphatic one, often preponderantly such, while furthermore every local injection into the skin becomes within a few minutes a general one, so rapidly is the introduced material transported to the blood.

The normal permeability of the skin lymphatics of man is approximately the same as that of the mouse. Tests indicate that in both

instances the lymphatic wall behaves like a semipermeable membrane. The permeability of the human lymphatic wall like that of the mouse is subject to rapid and great changes. A stroke on the skin with a blunt instrument to produce a wheal, causes the lymphatic capillaries to become so permeable temporarily that dyes pass through their walls as if practically no barrier existed, instead of being held back for a greater or less period. Slight inflammation due to heat, ultraviolet light or bacterial products has a similar effect. So, too, has histamine. When fluid pours rapidly into the tissue from the blood, as when a wheal is formed, the lymphatics are compressed and their efficiency as drainage channels is interfered with.

These facts are briefly discussed in their bearing upon skin phenomena in general. The lymphatics cannot be disregarded in considering such phenomena, in which it is plain that they have a large share.

BIBLIOGRAPHY

1. Hudack, S. S., and McMaster, P. D., *J. Exp. Med.*, 1932, **56,** 223.
2. McMaster, P. D., and Hudack, S. S., *J. Exp. Med.*, 1932, **56,** 239.
3. McMaster, P. D., and Hudack, S. S., *Proc. Soc. Exp. Biol. and Med.*, 1931, **28,** 852.
4. Hudack, S. S., and McMaster, P. D., *Proc. Soc. Exp. Biol. and Med.*, 1931, **28,** 853.
5. Hudack, S. S., and McMaster, P. D., *Proc. Soc. Exp. Biol. and Med.*, 1932, **29,** 944.
6. Rous, P., Gilding, H. P., and Smith, F., *J. Exp. Med.*, 1930, **51,** 807.
7. Smith, F., and Rous, P., *J. Exp. Med.*, 1931, **53,** 195.
8. Rous, P., and Smith, F., *J. Exp. Med.*, 1931, **53,** 219.
9. Schulemann, W., *Biochem. Z.*, 1917, **80,** 1.
10. Neumann, J., Zur Kenntnis der Lymphgefässe der Haut des Menschen und der Saugetiere, Wien, W. Braunmiller, 1873.
11. von Brunn, A., Handbuch der Anatomie, Jena, 1897, **5,** pt. 1.
12. Bartels, P., Das Lymphgefässsystem, in von Bardeleben, K., Handbuch der Anatomie des Menschen, Jena, G. Fischer, 1909, 120.
13. von Recklinghausen, F. D., Die Lymphgefässe und ihre Beziehung zur Bindegewebe, Berlin, A. Hirschwald, 1862.
14. Gerota, D., *Anat. Anz.*, 1896, **12,** 35.
15. MacCallum, W. G., *Bull. Johns Hopkins Hosp.*, 1903, **14,** 1.
16. Handley, W. S., The genesis of cancer, London, Kegan, Paul & Co., 1931.
17. Hyrtl, J., *Oesterr. Z. prakt. Heilk.*, 1860, **6,** 293.
18. Lewis, Sir T., The blood vessels of human skin and their reactions, London, Shaw & Sons Ltd., 1927.

19. Field, M. E., and Drinker, C. K., *Am. J. Physiol.*, 1931, **97**, 40.
20. Menkin, V., *Arch. Path.*, 1931, **12**, 802.
21. Menkin, V., *J. Exp. Med.*, 1931, **53**, 171, 179, 647, 919.
22. Ebbecke, *Klin. Woch.*, 1923, **2**, 1725.

EXPLANATION OF PLATES

PLATE 46

FIGS. 1 and 2. Successive stages in the distribution of dye during injection of the skin of two individuals, M and H. Patent blue V in 11 per cent solution was used, 0.1 cc. of it in the subject M, 0.2 cc. in H. The photographs were selected from a moving picture film to show the course of events or further some characteristic individual differences. Magnification × 3/2.

PLATE 47

FIGS. 3, 4 and 5. Enlargements of *a*, *d* and *e* of Fig. 1. These figures show the spread of dye in the rich network of lymphatics lying in the subpapillary layer of the corium. In Fig. 5 the spread has continued after removal of the injecting needle; in the upper left hand corner a pointer marks a region of such spread. Magnification ×5.

FIG. 6. The edge of the injected area shown in Fig. 1, 5 minutes after the last picture was taken. The rapid escape of dye from the lymphatics has obscured their outlines in a dense blue cloud. At the margin many small lymphatics can be seen draining away the dye. Midway between these and the bleb is an area in which dye is escaping from the lymphatics into the interstitial spaces.

In the lower right corner a deep draining lymphatic is visible. It is one of those channels which lie in the subcutaneous fatty layer of the skin.

PLATE 48

FIG. 7. The results of the rapid injection of minute quantities of dye solution into the skin of M. Photograph taken 6 minutes after two simultaneous injections of 0.02 cc. of patent blue V, each injection extending for a period of seconds. At this time—but too faint to show in the photograph—dye could be seen in a draining trunk close to the axilla. Some dye escaped along the needle to the skin surface forming heavy oblong marks.

FIG. 8. The displacement of dye-stained interstitial fluid by the pressure of a wheal. 0.1 cc. of 11 per cent patent blue V in Tyrode's solution was injected intradermally in two places and 4 hours later when it had spread diffusely through the tissue, wheal formation was evoked by a firm stroke across the colored regions and the skin surface was covered with paraffin oil. 3 minutes later, the photograph was taken. The wheal appears as a broad pale band along the horizontal line of stroke. On both sides of it, there can be made out a dark line of dye-stained interstitial fluid displaced by the wheal fluid.

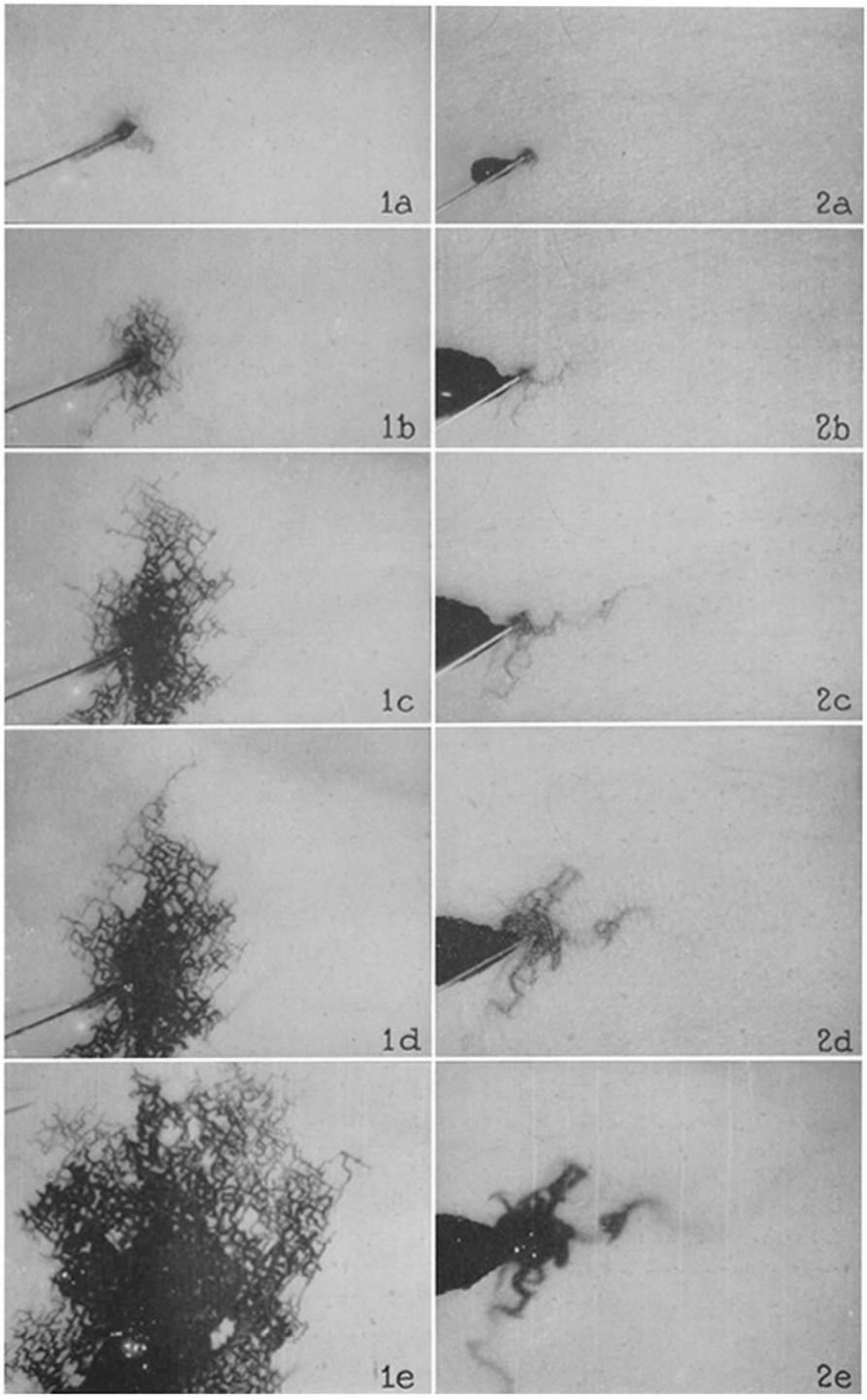
PLATE 49

FIG. 9*a*. Four erythematous macules 18 hours and 45 minutes after intradermal injections of 0.01 cc. of a streptococcus toxin described in the text. To bring out the differences from the normal skin the photograph was taken with a Cooper-Hewitt mercury arc light. An old scar, directly above the antecubital fossa is to be ignored.

FIG. 9*b*. The same arm shown in Fig. 9*a*, 1 hour and 10 minutes later, and 40 minutes after an injection, of 0.01 cc. of an 11 per cent patent blue V in Tyrode's solution, into the macular areas M_1, M_2 and M_3. Three similar control injections were made intradermally into the normal skin, at C_1, C_2, C_3. The arm has for the moment been supinated slightly for photographic purposes so that areas M_1, M_2 and M_3 appear higher than the others. To bring out the blue color of the dye, the photograph was taken through an orange Wratten G filter with result that the uninjected macule M_4 which was actually deep red appears almost invisible.

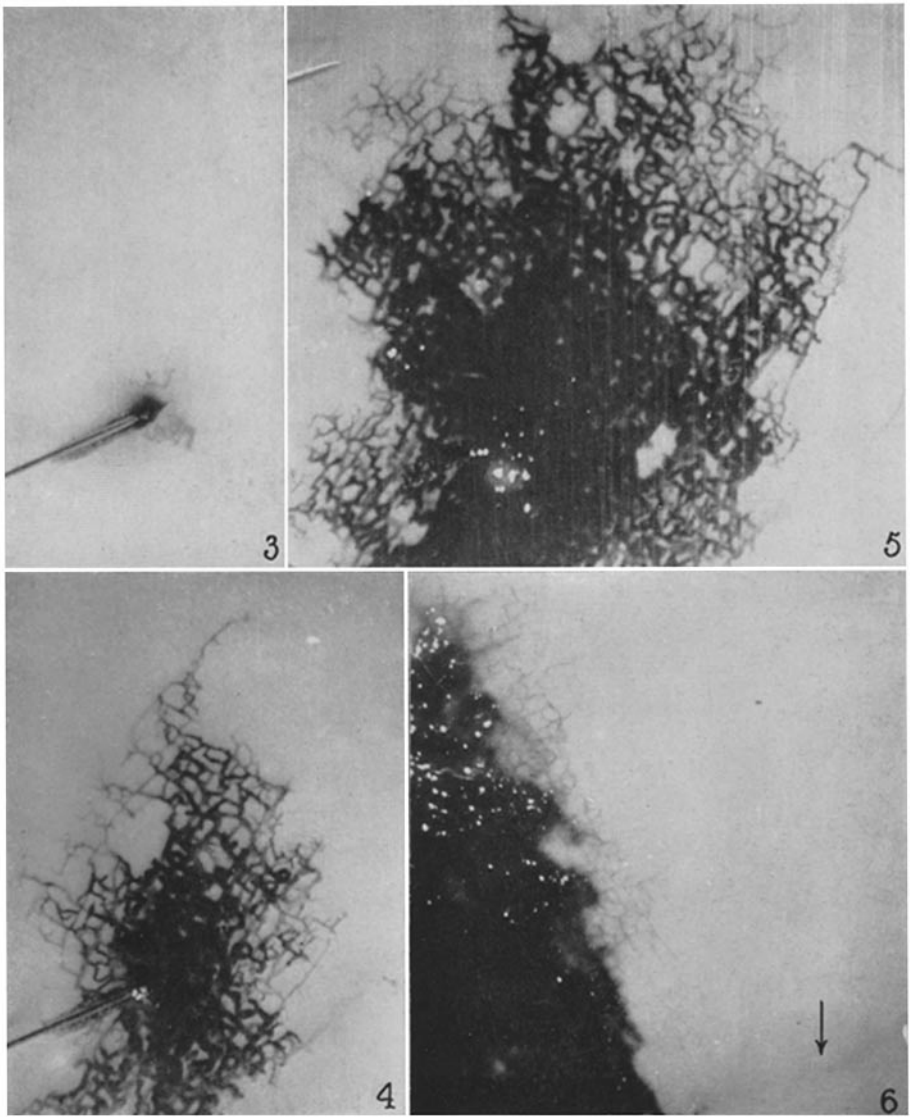
The hyperemic areas receiving the dye are now of a deep blue color owing to its rapid secondary escape into the tissue from the lymphatic capillaries into which it had at first passed. The injected control areas are much lighter in color, for in these the dye was more widely distributed through the lymphatics before its escape.

FIG. 9*c*. The same arm 3 hours and 10 minutes later. The findings are now wholly different, the control areas being much the darker, owing to an almost complete decolorization of the inflamed ones.



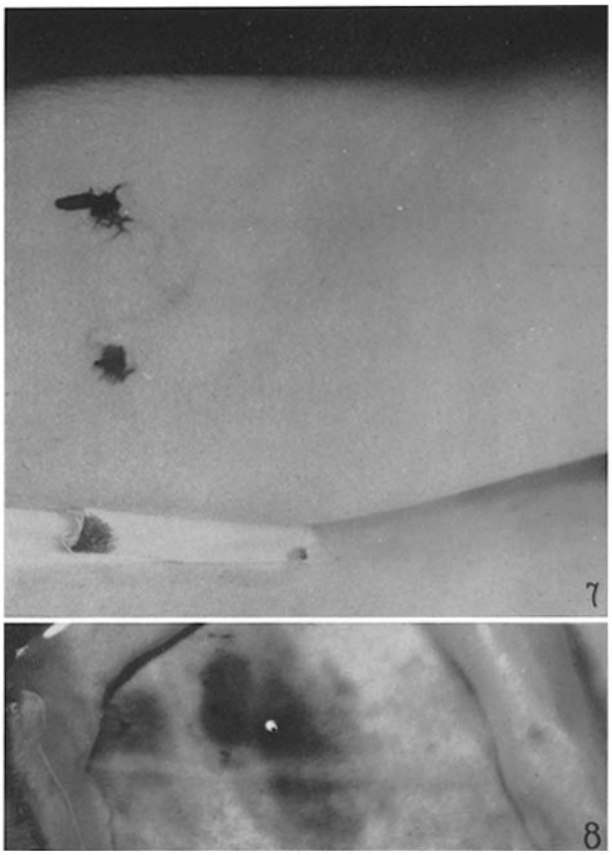
Photographed by Louis Schmidt

(Hudack and McMaster: Lymphatics of living human skin)

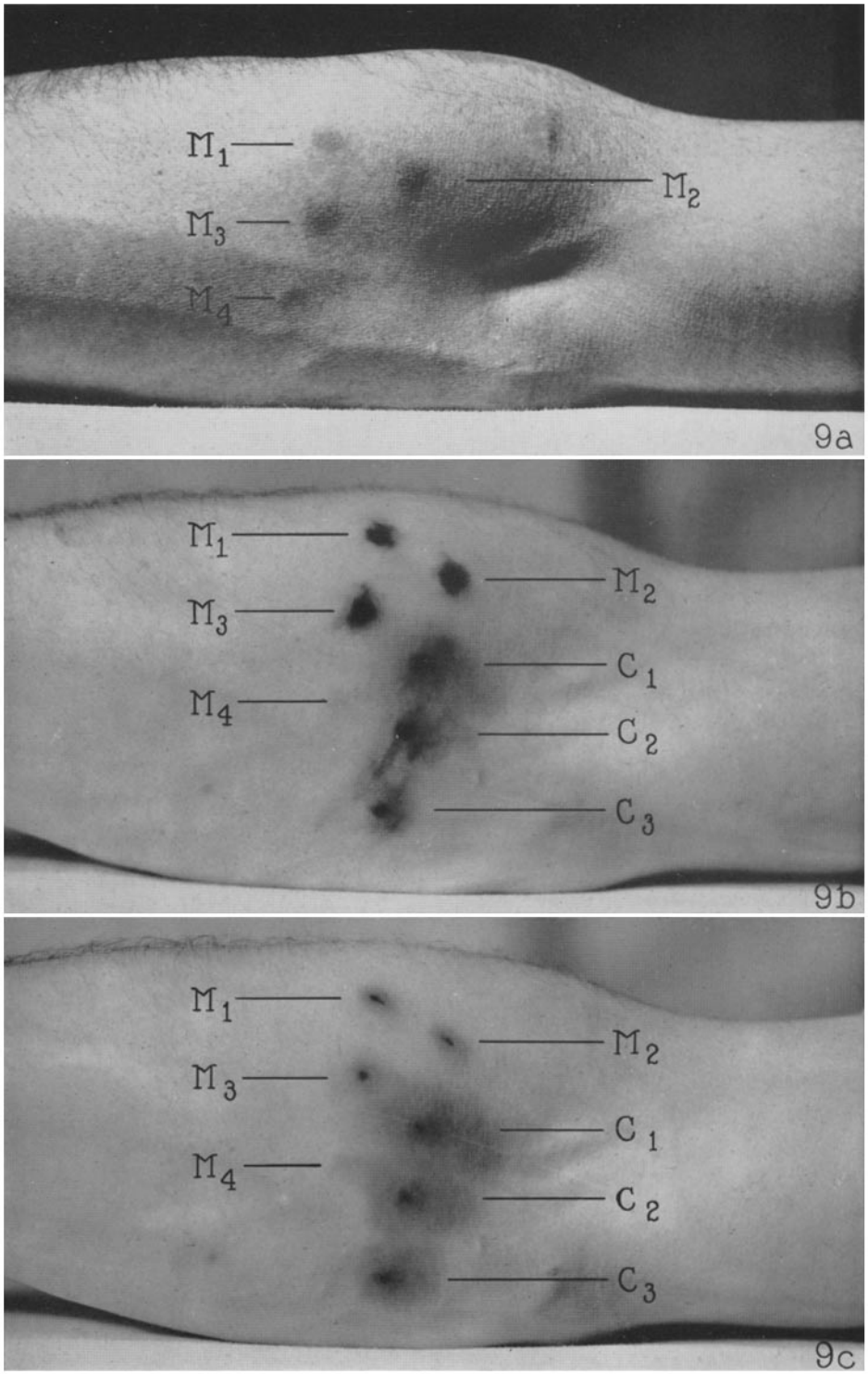


Photographed by Louis Schmidt

(Hudack and McMaster: Lymphatics of living human skin)



Photographed by Louis Schmidt



Photographed by Louis Schmidt

(Hudack and McMaster: Lymphatics of living human skin)