THE EFFECT OF THE PULSE UPON THE FORMATION AND FLOW OF LYMPH

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It is well known that massage and muscular movement increase the formation and flow of lymph (1–4). Undoubtedly pressure changes in the tissue are largely responsible. Nevertheless, the mechanism of lymph formation under such circumstances is not understood. Clearly the solution of the problem waits upon a better understanding of the factors influencing the extravascular transport of fluids through the tissues.

It has seemed wise in view of these findings to investigate the influence of the most natural of mechanical effects, that of the pulse wave, upon the formation and flow of lymph and the spread of substances through the tissues. The thought has suggested itself that the pulsations of vessels must serve, not only to facilitate the escape of substances from the blood, but to increase their spread through the tissues following their escape and, by an internal massage, as it were, to promote lymph formation and flow.

Previous Findings.—Earlier work has shown that changes in cutaneous lymph flow can be observed with the aid of vital dyes (4–6). On intradermal injection dye enters the superficial plexus of lymphatics through channels torn or ruptured by the injecting needle. As little as 0.01 cc. of a solution of pontamine sky blue or patent blue V will suffice for this purpose and renders them sharply visible at once. Later the dye can be seen, diluted and pale, draining away in the deeper channels appearing through the skin as colored streamers (4, 5). The streamers increase or decrease in length and intensity under conditions known to increase or decrease lymph flow respectively (4, 5). Intradermal injections of dye at the tip of the rabbit's ear (7) lead to the immediate appearance of color in the lymphatics there which extends rapidly to the base of the ear.

It has seemed possible to us that the effects of mechanical forces on the formation and flow of lymph could be determined with vital

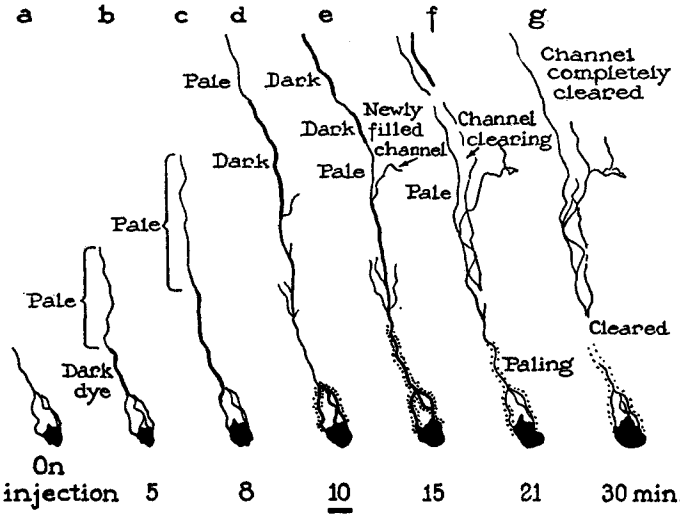
dyes. To do this we have perfused the ears of rabbits with either a pulsating or a constant flow of blood at comparable pressure and have followed, in the manner to be described below, the movements of the dyes through the lymphatics. Before attempting the perfusion experiments, studies of the intralymphatic movement of the dye solutions were made in the ears of normal rabbits before and after subjecting them to conditions known to increase or decrease the flow of lymph respectively.

Preliminary Experiments.—At the tips of the ears of 25 normal rabbits 0.01 to 0.02 cc. of a solution of a vital dye, pontamine sky blue, the most indiffusible of many tested, was intradermally injected on the dorsal surface of the ear with a gauge 30 platinum-iridium needle, using as little pressure as possible. The dye, a 21.6 per cent aqueous solution isotonic with blood, diluted to 2 per cent with Locke's solution, has been employed by us before (8–10) in studies on the permeability of the lymphatic capillaries. As in our previous work on man (4–6), mice (8–10), and rabbits (7), the dye solution injected in this way entered the lymphatics through the superficial channels torn or ruptured by the injecting needle. As soon as it appeared in the channels the injection was stopped. As already shown (4–6), lymphatics injected in this way are not forcibly dilated or stretched, as in making fixed anatomical preparations of them. The deeply colored fluid is not driven into the entire channel to fill it, but instead diluted with lymph, it gradually progresses from the injection site toward the base of the ear. The movement, indicating lymph flow, was watched under a binocular microscope and with hand lenses. Tracings of the observed changes were made upon pieces of celluloid held over the ears.

In our first experiments this movement of the dye in the lymphatics was observed in the intact ear at rest, that is to say when lymph flow was least (1, 3, 4). Similar injections were then made in the opposite ears of the same animals under conditions attended by changes in lymph flow, that is to say following massage (1, 3, 4), after elevating the ear to a vertical position to promote lymph drainage (4), after irritation by paintings with xylol (9), or following the induction of hyperemia by heat (5, 9). For brevity only one of 25 experiments, typical of all, will be outlined, for the results were like those already reported in work on human skin (4, 5) and on the ear of the mouse (8, 9).

In the manner described, approximately 0.01 cc. of the 2 per cent dye solution was injected, within a few seconds, at the tip of one ear of a normal adult rabbit of the same size as those used in the perfusion experiments to be described later.

During the experiment the ear was held in a horizontal position. As soon as the dye solution appeared in the nearest lymphatic capillaries the injection was stopped. Natural sized tracings, showing the state of affairs immediately after the injection and the progress of the dye in the lymphatics at intervals thereafter, are shown in Text-figs. 1*a* to 1*g*. In this figure and in the others the irregular black areas represent the bleb of dye intradermally injected ½ cm. from the tip of the ear. The upper limits of the diagrams in Text-figs. 1 and 2 represent

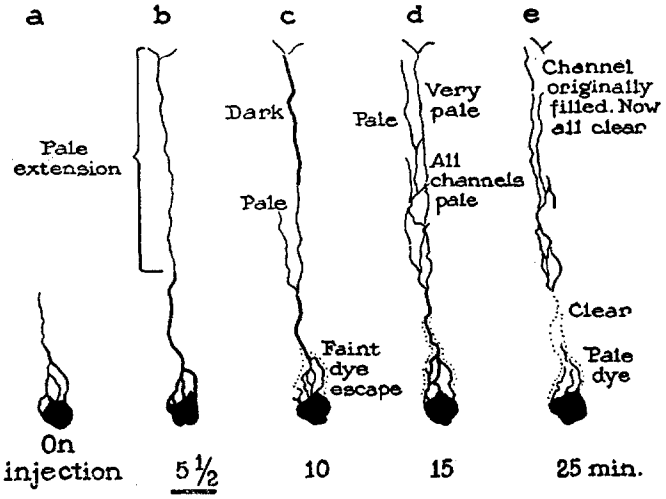


TEXT-FIG. 1. Tracings of the movement of color in the lymphatics of a normal quiet ear.

TEXT-FIGS. 1 to 8. In these text-figures the irregular black areas represent the bleb of dye intradermally injected ½ cm. from the tip of the ear. The upper limits of the diagrams in Text-figs. 1 and 2 represent the base of the ear. The upper limits of the remaining diagrams represent the cut edge of the amputated ears. These points were chosen so that the distance traversed by the dye would be approximately the same in all the experiments. In each text-figure the time interval, required by the dye to reach the base of the ear, has been underlined.

the base of the ear, as the tracings were made with the observer looking down upon the dorsal surface with the tip nearest to the body. The upper limits of the remaining diagrams represent the cut edge of the amputated ears. These points were chosen so that the distance traversed by the dye would be approximately the same in all the experiments. In each chart the time interval required for the dye to reach the base of the ear has been underlined. The first diagram, *a* in Text-fig. 1, shows the dye in the lymphatics immediately after the injection, which required but a few seconds. Within 5 minutes the column of colored fluid

in the lymphatic accompanying the central artery of the ear had reached the point shown in Text-fig. 1 *b*, dark where originally injected and pale in the extension. 3 minutes later a pale extension reached the point shown in Text-fig. 1 *c*. Dye in this channel, in the outer and middle thirds of the ear then became darker, after the 8th minute, while at the same time a few small tributary channels appeared along the course of the large central one, bearing pale, diluted color as indicated in Text-fig. 1 *d*. By the 10th minute pale colored fluid reached the base of the ear, while in the channel in the inner third of the ear, just behind the pale tip, lay the original dark column of fluid. Lymphatics in the middle and outer thirds of the ear bore fluid which was not quite as dark as before. Dye escape from the channels near the injection site had occurred secondarily. We have



TEXT-FIG. 2. Tracings of the movement of color in the lymphatics of an hyperemic, quiet ear.

indicated this state of affairs in Text-fig. 1 *d* by the stippling. 5 minutes later, 15 minutes after the injection, paling of dye had taken place in the inner third of the ear just above a newly visible tributary channel, as though clear lymph were entering from tributaries themselves unseen. At the same time the dark column of dye reached the ear base. This is shown in Text-fig. 1 *e*. By the 21st minute, Text-fig. 1 *f*, pale blue fluid reached the ear base in another newly visible channel. Many new channels carrying pale dye had appeared, and the color in the lymphatic originally filled had completely cleared in certain portions within the inner third of the ear. Enough newly formed lymph had entered the lymphatic to sweep out all the colored fluid. The main dye-containing channels close to the site of dye injection also had become much paler. By the 30th minute that por-

tion of the lymphatic which first became visible and which lay in the inner third of the ear, had become completely cleared, as also that segment lying close to the dye injection, Text-fig. 1 *g*.

The course of events was typical of that in the majority of the experiments done upon normal ears. However, large individual differences occurred, due in part to variations in the size of the original injections and to the extent to which dye ran toward the ear base before the injection ceased. It must be stressed again that large injections forcibly made can drive undiluted dye solution immediately to the base of the ear. From such injections there can result no indications of the state of lymph flow. In our experiments variations in the rate of progress of dye in the lymphatics were dependent upon the state of the circulation of the ear. For example in one instance, in a flushed, warm ear, dye reached the base in 5 minutes, while in cold ears with contracted blood vessels it required 16 to 20 minutes in 3 instances, and even 40 minutes in one animal. In the remaining 21 experiments done on normal ears it required from 9 to 15 minutes.

In the perfusion experiments to be detailed below no such variations as this occurred, for the conditions of flow and pressure of circulating blood were controlled and much more uniform.

In each of the 25 experiments of the sort just described the animal's other ear was subjected to procedures known to increase lymph flow, that is to say the ears were warmed to produce hyperemia, or massaged, or irritated by chemicals.

For example, an intradermal injection of about 0.01 cc. of the dye solution was given at the tip of an ear after a 50 cc. centrifuge tube containing water at 55°C. had been held against the lower surface until a marked hyperemia appeared. Following the injection, to avoid manipulation of the ear and to maintain hyperemia, light from a 100 watt electric bulb 20 cm. distant was allowed to shine on the upper surface. A thermometer held on the ear surface registered 46°C. Dye appearing in the lymphatics progressed toward the base much more rapidly than in normal ears. As Text-figs. 2 *a* and *b* show, the pale and much diluted column of color reached the base in only 5½ minutes. In the 12 other experiments of this type, dye reached the base of the ear in 5 minutes on the average, with variations from 2 minutes and 30 seconds to 9 minutes. In the test under consideration the color in the central lymphatic became darker for a few minutes, but by the 10th minute, Text-fig. 2 *c*, a definitely pale segment developed in that part of the channel lying in the middle third of the ear. As no escape of dye had occurred

into the tissues the paling must have been brought about by colorless lymph coming into the channel from tributaries. The pale fluid in the middle portion of the lymphatic pushed the darkly colored material toward the ear base. At the same time that the paling increased, diluted dye appeared in a channel which had previously been invisible. By the 15th minute many new channels, one running almost to the base of the ear, had filled with pale blue fluid. The channel first filled with dye had become almost invisible in the inner third of the ear; for the dark blue fluid had been driven out of the organ. By the 25th minute this channel had completely cleared, Text-fig. 2 *e*, and so had those in the outer third of the ear. Dye escape, as shown by the stippling, was far less than in normal ears.

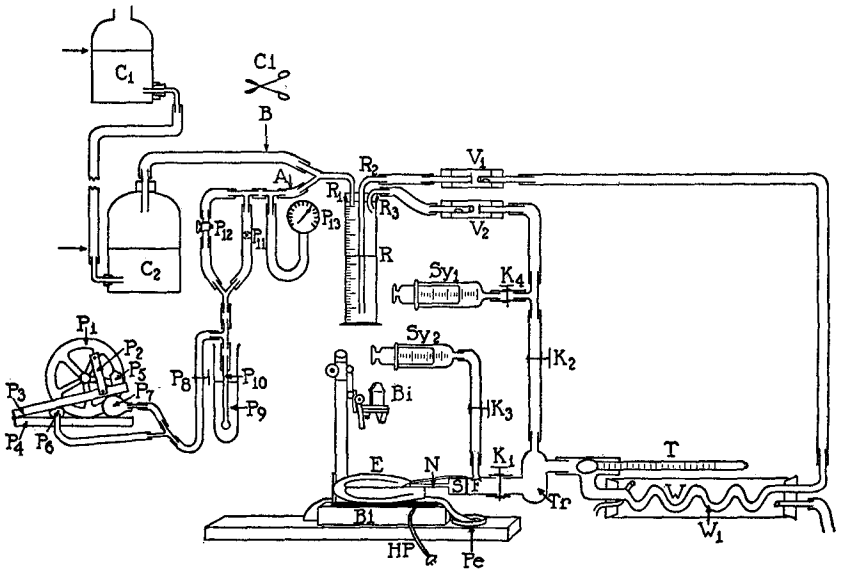
In all the 25 experiments in which the ears were subjected to one influence or another increasing the flow of lymph, as mentioned above, the rate of dye movement in the lymphatics was greatly accelerated. For example, during massage of the ears dye solution in the lymphatics reached the base in a few seconds. In ears with the tip held vertically above the base, color reached the latter in 3 to 5 minutes. In horizontal ears painted 2 to 5 minutes previously with xylol, dye reached the base, from the tip, in periods varying from $1\frac{1}{2}$ to $3\frac{1}{2}$ minutes, whereas in the ears at rest it required, as just reported, 9 to 15 minutes on the average, and never less than 5 minutes. In all these experiments, too, the color in the channels paled or cleared in far less time than in the tests done on the normal, resting ears. In view of these results we felt that the method could be used in the rabbit's ear, as it has been employed in human skin, to indicate changes in the rate of lymph flow. The clearance of color in the channels was indubitable evidence of a rapid formation of lymph and its transport in the lymphatics.

Such observations were next made in the ears of rabbits perfused with either a pulsatile or a constant flow of defibrinated rabbit blood under controlled pressure conditions.

Methods

A perfusion apparatus was devised by means of which warmed, aerated blood could be passed through organs under constant or pulsatile pressures and at alterable rates of flow. The operation of the device is best described by reference to Text-fig. 3.

Defibrinated blood, under pressure in a reservoir, R, was driven by pulsatile or by constant pressure, as will be detailed presently, through a one-way valve V_1 and thence through a warming chamber W into the ear E. Emerging from the



TEXT-FIG. 3. Diagram of the apparatus for perfusion of whole blood under constant or pulsatile pressure. See text for description.

C_1 and C_2, aspiration bottles; P_1, wheel which is connected through reducing gears and pulleys to ¼ h.p. motor; P_2, connecting rod; P_3 and P_4, boards hinged at one end; P_5, 750 gm. weight; P_6, atomizer bulb; P_7, 250 cc. rubber bulb; P_8, screw clamp, to assist in control of volume of air; P_9, mercury in 25 mm. test tube; P_{10}, glass tubing with closely woven silk over lower end to prevent reflux of mercury; P_{11}, one-way ball valve from atomizer bulb; P_{12}, adjustable air valve for controlling "diastolic" pressure; P_{13}, Tycos blood pressure gage; *Cl*, clamp to be placed at *A* for constant pressure or *B* for pulsating pressure; *R*, blood reservoir; 100 cc. graduate closed with 3 hole rubber stopper, sealed with de Khotinsky's cement; R_1, air inlet; R_2, blood outlet; R_3, reflux tube for aeration of blood; V_1 and V_2, glass and rubber flap valves to control circulation of blood; *W*, water bath for warming blood; W_1, glass coils in water bath; *T*, thermometer; *Tr*, glass bubble trap and reflux connection; *S*, serum vial cap with 23 gauge platinum needle (without shank) pressed through it; *E*, rabbit's ear with needle tied into central artery; *HP*, electric connection to heating pad; *Bl*, wooden block; *Pe*, Petri dish to catch venous blood; *Bi*, binocular dissecting microscope on stand; K_1, screw clamp for control of blood flow; K_2, screw clamp for control of circulation of blood; K_3, spring clamp; K_4, spring clamp; Sy_1 and Sy_2, 20 cc. Luer syringes; *N*, needle—23 gauge platinum-iridium; *F*, T tube.

veins it was collected, filtered through absorbent cotton moistened with Tyrode's solution, and returned to the apparatus. Large aspiration bottles, C_1 and C_2, partly filled with water and connected with the blood reservoir, as shown in the figure, imposed a constant pressure upon the perfusate. This could be increased or decreased by changing the level of the water in the bottles. Pulsatile perfusion pressures were generated, when desired, by the bellows-like action of two boards, P_3 and P_4, upon two rubber bulbs, P_6 and P_7. Compression of the bulbs forced air into a T tube, one limb of which led into the circuit of the perfusion apparatus while the other led through a tube to the bottom of a vessel partly filled with mercury, P_9. This device served as an overflow valve, and the mercury level could be changed at will, air bubbling out into the container when the air pressure rose above the desired level. The portion of the air column not bubbling through this valve was led through a Y tube into a double passage, one branch of which contained a one-way ball valve, P_{11}, through which it passed to exert the "systolic phase" of its pressure on the reservoir R. The other passage could be partially closed by the two-way stopcock, P_{12}. With the release of pressure on the bulbs, valve P_{11} closed while P_{12} was properly adjusted to allow just the right amount of air to leak backwards through it to maintain the desired "diastolic" pressure in the reservoir. A Tycos gage, P_{13}, in the circuit indicated the pressures and pressure changes in the reservoir.

The pressure in the reservoir forced blood through a one-way valve V_1 into a tube enclosed by a warming chamber for the adjustment of its temperature. Hot water flowed rapidly through the chamber and about the tube containing the blood. The flow of hot water was so regulated that the temperature of the blood passing the thermometer T was about 3°C. above that desired in the perfused organ. The warmed blood passed next into an air trap, Tr, and thence took one of two routes. Some, passing from the air trap through a short rubber connection, with a screw clamp, K_1, and then through a T tube, entered a 23 gauge platinum-iridium needle, capable of transmitting 18 cc. of blood per minute at a pressure of 141 mm. of mercury and at 38°C. The latter had been introduced into the artery of the organ to be perfused, in our experiments the central artery of the rabbit's ear.

The amount of blood entering the needle was controlled by the screw clamp, K_1. The remainder, passing out at the top of the air trap, returned through another one-way valve, V_2, to the reservoir R, flowing in through a flattened inlet tube R_3. This latter device directed the stream of blood toward the wall of the reservoir so that it spread out in a thin film on its passage down the glass walls of the container, while reoxidation occurred. The circulation and aeration of blood in the apparatus, as just described, was automatic only when pulsatile pressures were used. When constant pressures were used, blood was taken from the system at frequent intervals with the syringe, Sy_1, and returned to the reservoir by a slow reinjection after closing the clamp K_2, thus forcing it first through the valve V_2, then through the flattened inlet tube R_3 so that it ran down the inner surface of the reservoir and was reoxidized. The apparatus permitted the use of either constant

or pulsatile pressures when the clamp, C_1, was placed at either A or B, respectively, as shown in the diagram.

As already stated constant pressure was maintained in the blood reservoir R by the fluid in the bottle C_1 and varied by raising or lowering this bottle. The difference in the levels of the fluids in the two bottles C_1 and C_2 gave the measurement of the pressure. The pulsatile pressures were registered on a pressure gage P_{13}, and could be varied by changing the positions of the bulbs, P_6 and P_7, or by changing the level of the mercury, P_9 in the overflow valve. But the pressure at which blood entered the artery after leaving the needle tip was not known. To estimate it a series of preliminary tests were made. With the needle held horizontally, 30 cm. above the table, a constant flow of defibrinated blood was forced through at various known constant pressures. Beginning with a pressure of 160 mm. of mercury, we measured the distance that the stream of blood was thrown from the needle tip. The pressure was then lowered by 10 mm. of mercury and the distance spanned by the stream of blood again measured. This process was repeated, lowering the pressure by stages, 10 mm. of mercury at a time, until a pressure of only 30 mm. of mercury was exerted. Then, using the pulsating perfusion apparatus, different "systolic" and "diastolic" pressures were applied to the reservoir while we measured the distances that blood was thrown from the needle. For example, after it was found that the stream of blood was thrown 50 cm. from the needle tip by a constant pressure of 110 mm. of Hg, we connected the pulsation perfusion apparatus with the same needle and experimentally varied the pressure until blood was thrown equally far during "systole." The "systolic" pressure in the reservoir necessary to do this was found to register a pressure of about 145 mm. of Hg on the gage. Thereafter when a "systolic" pressure of 110 mm. Hg was desired at the needle tip, pressure was raised within the reservoir of the apparatus until the gage connected with it recorded 145 mm. of mercury. Similarly we noted the readings of the pressure gage when pressures were varied in the pulsation perfusion apparatus in such a way that the stream of blood emerging from the needle was thrown various distances corresponding to those determined when different constant pressures were applied. The "diastolic" pressures of the blood delivered at the needle tip were determined in the same way.

Perfusion Experiments.—Under ether anesthesia, the ears of adult albino rabbits were shaved. Through a small incision, directly over the central artery at the base of one ear, the vessel was isolated and ligated, leaving the ligature long. Now the ear was amputated at the base, weighed, placed on the warming pad (*HP* in the diagram), and at once the large-bored, 23 gauge needle was inserted into the artery and tied in place under a binocular microscope, *Bi* (Text-fig. 3). The needle, N, was fixed in a rubber stopper, S, which in turn fitted over a glass cannula connecting by rubber tubing with a syringe. Neither of the latter is shown in the diagram, but by means of them warmed Locke's solution was flushed gently through the ear as soon as the needle was in place. 20 or 30 cc. of the solution usually sufficed to clear the ear of blood, after which the stopper was taken from the cannula and fitted to the T tube, F. A syringe containing Locke's

solution was joined to the other end of the T tube as shown in the diagram at Sy_2. The long ends of the ligature on the artery were tied over the stopper S and the T tube tightly. The ear was placed horizontally with its under surface on a warming pad supported by gauze sponges so that the flat portion of the outer side could be observed and injected.

Except where specially mentioned, pooled, defibrinated blood, freshly taken from two or three rabbits, was used in all the experiments. Before each, cross agglutination tests were done by the method of Rous and Turner (11), using samples of the pooled blood and that of the animal from which the ear had come. No instances of agglutination were found. Early in our experiments it appeared that whole, defibrinated blood, however gently it was introduced into the ear after amputation, frequently caused an intense, transient spasm of the arterial tree. This phenomenon, well known to those who have perfused organs, was successfully reduced or even avoided by adding small amounts of blood to the warm Locke's solution after the first 20 or 30 cc. of the latter flushed out the animal's own blood already there. Following this, the screw clamp, K_1, was gradually opened, while 10 to 20 cc. of additional Locke's solution was gently injected at a uniform rate into the artery from the syringe, Sy_2. In this way the perfusate gradually became wholly blood, and the blood vessels of the ear relaxed until flow became apparently normal. The maneuver required 5 or 10 minutes, after which closure of the pinch cock, K_3, assured that only blood could reach the ear thereafter. The desired volume of flow was then attained by adjustment of pinch cock K_1. The blood emerging from the cut marginal veins, after its passage through the ear, was drained away by rubber dams, collected, filtered through cotton and gauze, and reinjected, as needed, into the perfusion apparatus through the syringe Sy_1. At varying intervals of 1 and 5 minutes during the experiments the temperature of the perfusate was recorded, together with the rate of blood flow, and adjustments were made if required.

The perfusion pressures employed differed in some experiments and were similar in others for reasons appearing in the following paper. As is well known, the volume of blood flow in the rabbit's ear varies greatly, the vessels being dilated at one time and constricted at another. For our experiments we determined to use those pressures which would yield sufficient flow of blood to the amputated organ to give it an appearance, under the binocular microscope, like that of a normal ear in which dye spots had been placed, when employing arbitrarily a "diastolic" pressure of 60 mm. of mercury. To obtain the desired blood flow it was found necessary in about half the pulsatile perfusions to employ a "systolic" pressure of 141 mm. of mercury. As it was further desired to maintain the constant pressure as high as the "systolic" pressures, we matched this figure in many experiments employing constant pressure. Of the experiments employing constant pressures, one was done at a pressure of 152 mm. of mercury, 9 at 141 mm., 4 at 131 mm., and 3 at 120 mm. of mercury. 6 of the perfusions with a pulsating flow of blood were done at pressures of 141/60 mm. of mercury, 5 at 131/60, 2 at 120/60, 1 at 115/60, 2 at 100/60, and 2 at 95/60. Some of these systolic pressures are higher than

those existing in the living rabbit (12–14), others probably equal to the normal pressures, or lower.

Regardless of the pressure differences, as will be seen below, lymph flow was faster in every ear perfused with a pulsatile flow of blood than in any of the ears perfused at constant pressure.

As soon as the proper flow of blood had become established, minute amounts of the dye solution were intradermally injected on the outer surface at the tip of the ear. The movement of dye in the lymphatics was then watched, and tracings made. Throughout the perfusions a lookout was kept for the development of edema. Under the binocular microscope, and with a fine needle, evidences of pitting on pressure were sought from time to time. The findings in all those instances showing edema will be considered separately.

When the last observations had been made in each experiment, 0.2 cc. of strong 21.6 per cent aqueous isotonic pontamine sky blue solution was injected through the stopper S of the perfusion apparatus (Text-fig. 3) so that the dye passed immediately through the needle into the circulating blood of the ear. The rate and character of its distribution about the lymphatics was noted, as was the rate of the blood flow through the ear. At the end of the experiment the ear was weighed again to determine the gain in fluid, if any, and sections were taken from various spots.

Save where especially mentioned, the data in this paper are based on perfusions with whole defibrinated rabbit's blood, the most satisfactory of the perfusates tested with a view to the maintenance of the tissues in an approximately normal state for an hour. These included Tyrode's solution, Locke's solution, Locke's solution containing 2 per cent gelatin, 7 per cent acacia, horse serum, whole or diluted with Locke's solution, beef serum, whole or diluted, beef plasma, whole or diluted with citrated Locke's solution, defibrinated beef blood, and heparinized or citrated rabbit's blood. Only with freshly defibrinated rabbit's blood were we able to perfuse the ear for an hour or more without the development of edema. In about half the experiments none occurred. The other perfusates containing plasma showed a strong tendency to form clots in the vessels, and hence their use had to be abandoned.

Findings

The data employed for comparisons of the rate of lymph flow were derived from experiments in which the perfusions with pulsating pressure involved a smaller volume of blood flow than those at constant pressure. The differences in the rate of flow are described in each instance. As more experiments were done at pressures of 141/60 and 141 mm. of mercury than at any other pressures the data charted have all been taken from such instances. Yet, as has been described, lymph formation and flow were much greater, in every instance, in

the ears perfused with a pulsatile pressure, that is to say with a pulsating current, regardless of pressure differences.

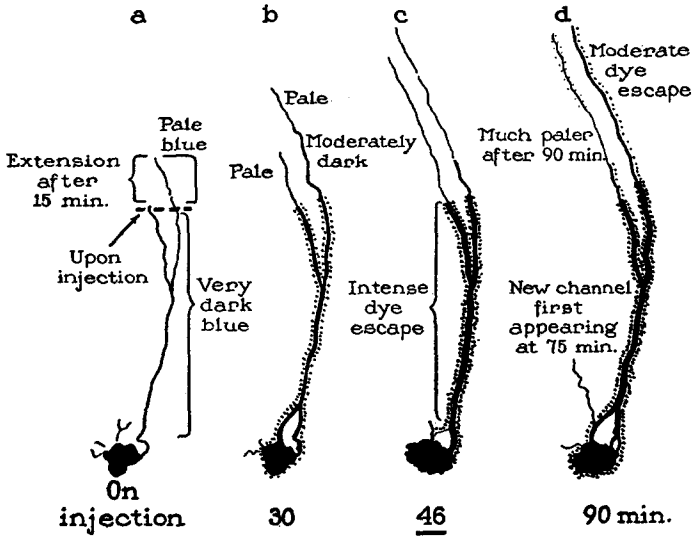
The Effect of Pulsatile Blood Flow upon the Formation and Flow of Lymph

In 17 experiments ears of rabbits were perfused at constant pressure, and in 18 at pulsatile pressure. The differences in the rate of lymph flow in the two types of experiment were so clear cut and constant that a brief description of one experiment of each kind will suffice. The injections were made, as already described, at various intervals after beginning the perfusions. The intervals had no effect upon the results. As in the experiments upon normal ears, the moment dye appeared in the superficial lymphatic capillaries, the injections were stopped. The progress of the dye along the draining lymphatic channels which accompany the central and marginal veins was timed and watched under the binocular microscope and traced on pieces of celluloid, as already described. The slight degree of pressure used to introduce the dye was the same in every instance. The differences to be described below cannot be ascribed to pressure changes.

The experiment in which the greatest movement occurred in an ear perfused at constant pressure will be compared with a typical experiment made with a pulsatile current of blood. As will be seen, the minute volume flow of the pulsating perfusate was far less and the "systolic" pressure far lower than the constant pressure of the former.

The right ear of a normal rabbit was amputated and perfused with defibrinated blood at a constant pressure of 141 mm. of mercury, in the manner already described. The ear weighed 12.2 gm. For 15 to 20 minutes before the dye injection of the lymphatics and for half an hour thereafter, the rate of blood flow averaged 4.01 cc. per minute or 0.33 cc. per gm. of ear. During the remainder of the experimental period, the flow increased to 4.65 cc. or 0.38 cc. per minute per gm. of ear. As usual, in these experiments approximately 0.01 cc. of dye solution was injected, requiring but a few seconds. By the time the injection was halted the color had already extended 5½ and 6 cm. in the central lymphatics (Text-fig. 4), much farther, that is to say, than in any of all the other experiments. This can be easily seen on comparison with the other diagrams. Secondary extension was slow nevertheless, the blue color extending less than 1 cm. in the

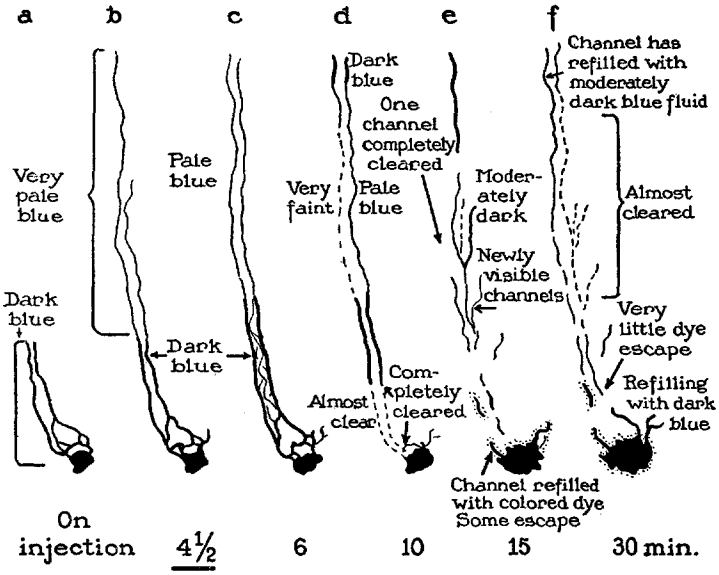
next 15 minutes (Text-fig. 4 *a*). After half an hour it had progressed 1 cm. further in one channel and about 1½ cm. in another (Text-fig. 4 *b*). The color in these extensions was pale and there was no escape of it into the surrounding tissue. Yet dye had escaped profusely from those portions of the channels originally filled by injection, as shown by the stippling in Text-fig. 4 *b*. In the next 16 minutes the movement was but 1 cm. more so that the colored fluid just reached the cut edge of the ear 46 minutes after making the injection. It had moved in all but 3½ cm. in three-quarters of an hour (Text-fig. 4 *c*). Flow in the second channel was slightly faster than that in the first, both vessels containing very pale blue fluid. Dye escape from the segment of the channel first filled became intense,



TEXT-FIG. 4. Tracings of the movement of color in the lymphatics of an amputated ear perfused at constant pressure.

leaving a great, broad band of dark blue about it. The dye in the lymphatics also remained dark blue and showed no evidence of becoming lighter during the experiment. From this it is evident that paling of the channels, as mentioned above, did not result from dye escape from them. After 1½ hours, a small segment of one of the channels containing pale blue fluid, as indicated in Text-fig. 4 *d*, became almost colorless, and a new channel appeared near the site of the injection carrying pale blue fluid. At the end of the experiment there was no detectable edema and the ear weighed only 0.2 gm. more than at the beginning. Although most of the experiments lasted an hour, this was the only one in which the color reached the cut edge of the ear in the lymphatics during a perfusion made with constant pressure, in an ear which did not become edematous.

Very different were the findings in ears perfused with pulsating blood. In a typical experiment now to be detailed, an ear weighing 13.2 gm. was perfused with a current of defibrinated blood pulsating 115 times a minute. Blood was discharged from the needle at "systolic" and "diastolic" pressures estimated to be 141 and 60 mm. of mercury respectively. It flowed at the rate of 1.54 cc. per minute for 15 minutes preceding the dye injection and at the same rate during the period of observation, that is to say, at about 0.12 cc. per minute per gm. of ear. The flow was, therefore, only one-third as fast as



TEXT-FIG. 5. Tracings of the movement of color in the lymphatics of an amputated ear perfused at pulsating pressure.

that in the experiment just cited, and the "systolic" pressure just equal to the constant pressure. Despite this, lymph flow was 15 to 20 times quicker, as estimated by the rate at which color reached the base of the ear.

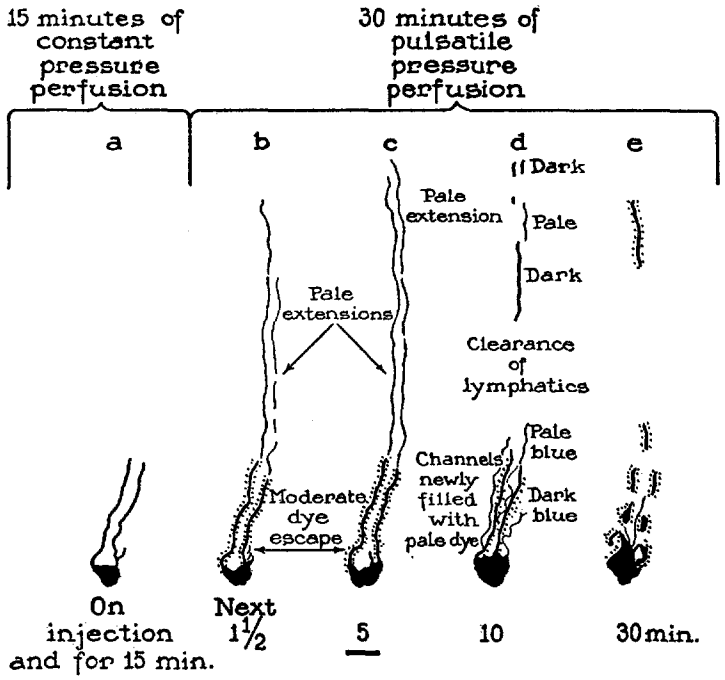
As in the experiment just cited, about 0.01 cc. of dye was injected intradermally near the tip of the ear in about 20 seconds. Text-fig. 5 *a* shows diagrammatically the dye in the lymphatics at the end of the injection. It is to be noted in this experiment that dye had not progressed as far down the ear upon injection as in

the case just cited. Nevertheless one streamer, pale and much diluted, reached the base of the ear, an additional distance of 5.5 cm., in 4½ minutes (Text-fig. 5 *b*), and a second one arrived there in 6 minutes (Text-fig. 5 *c*). At this time, too, many intercommunicating channels had filled with pale blue color between the two darkly colored lymphatics originally filled upon injection. The deep blue in these latter had extended toward the ear base. By the 10th minute the dye had become much paler in two of the lymphatics close to the injection site and had cleared completely in a third. In the meantime, some of the deeply colored material had been swept to the base of the ear, leaving the color very pale in the inner and middle thirds of the organ (Text-fig. 5 *d*). All the color had almost disappeared from one of these lymphatics, as indicated by the dotted lines. There were no signs of dye escape. Text-fig. 5 *e* shows that, during the next 15 minutes, the clearance of dye from the lymphatics near the tip of the ear had continued, the channels having become invisible in places. The more deeply colored fluid in the lymphatics continued toward the base of the ear in one channel, forced there by pale colored lymph now present in the lymphatics of the middle third of the ear. In this region pale colored fluid had entered many lymphatics hitherto invisible. A second channel in the inner third of the ear had now become invisible. There was still no evidence of dye escape from the channels except close to the injection site (stippling in Text-fig. 5 *e*). Within half an hour almost complete clearance in one of the channels in the mid third of the ear had occurred (Text-fig. 5 *f*), leaving a short segment of more deeply colored fluid near the cut edge of the ear. In the meantime, that channel near the cut edge which had been invisible 15 minutes before now contained deeply colored fluid. The observations gave clear evidence of rapid lymph flow.

The findings just described are in every way typical of those obtained during pulsating perfusion experiments. There was ample evidence of rapid lymph formation and flow, far exceeding that in the most favorable experiment upon an ear perfused at constant pressure. A comparison of typical experiments would have yielded even greater differences. Indeed no constant pressure perfusion resulted in as much, or as rapid, movement of color as the least movement that took place in the experiments with pulsatile perfusion. The effect of pulsation upon lymph flow is seen to better advantage in another type of experiment.

A rabbit's ear was perfused at a constant pressure of 141 mm. of mercury, the rate of flow being 2.7 cc. per minute. An intradermal injection of dye made in the usual manner colored two or three lymphatics blue, to the point shown in Text-fig. 6 *a*. For 12 to 14 minutes there was almost no movement of dye in the channels but much escaped through their walls as indicated by the stippling in

Text-fig. 6 *b*. This is characteristic of the constant pressure perfusions. For the constant pressure we then substituted "systolic" and "diastolic" pressures of approximately 141 and 60 mm. of mercury, respectively, exercising care to maintain the same rate of blood flow. Within 1½ minutes the color had advanced 4 cm. in one lymphatic, and 5 cm. in another (Text-fig. 6 *b*). The colored fluid in one channel reached the cut edge of the ear in less than 5 minutes, and in the second channel did so by the 6th minute (Text-fig. 6 *c*). As indicated in all the



TEXT-FIG. 6. Tracings of the movement of color in the lymphatics of an amputated ear. (*a*) The lack of lymph flow while the perfusion was done for 15 minutes with constant pressure. (*b*) Active lymph flow 1½ minutes after changing to a pulsating pressure. (*c*, *d*, and *e*) Subsequent lymph flow and clearance of the lymphatics as the pulsatile current is continued.

figures, the colored extensions were far paler than those portions of the channels originally injected with dye. At the 10th minute after the shift to pulsatile flow, the color in the channels of the middle third of the ear had disappeared (Text-fig. 6 *d*). There was no evidence of dye escape from the lymphatics save in the regions close to the injection site, as indicated by the stippling.

The change from constant pressure to pulsating pressure in the perfusate brought about a great difference in lymph formation and

flow. All through the experiment, the rate of flow of the perfused blood remained approximately constant.

In all the ears which were perfused at pulsatile pressure, and which remained edema-free, the column of blue fluid in the lymphatics advanced rapidly toward the base, and reached it on the average in about 7 or 8 minutes, with variations between 3 and 11 minutes. By contrast, in only one of the experiments done at constant pressure in edema-free ears, did dye ever reach the base of the ear at all, and then only after three-quarters of an hour (Text-fig. 4). On the average, the color moved but 2 to 4 cm. in the half hour period usually allotted for the experiment. The average distance from the injection site to the cut edge of the ear was 8.5 cm.

In ears perfused with a pulsatile flow of blood, as also in normal and in hyperemic ears, the color of the draining lymphatics paled rapidly. Since the escape of dye from lymphatics to the tissues can readily be seen (6–8), it follows that the paling of the contents of the channels and their clearance of color was not due to the escape of dye through the lymphatics' walls but resulted from formation and flow of colorless lymph along the lymphatics. In all the ears perfused at constant pressure, the dark column of dye-stained fluid in the lymphatics advanced very slowly, and there was much more escape of dye from the channels into the tissue, yet the colored fluid did not become as pale within the lymphatics themselves as it did in the pulsatile perfusion experiments. In the latter there was less evidence of dye escape. It is of interest further that the paling of dye within the lymphatics and the subsequent disappearance of color began usually at the outer or middle third of the ear; the pale fluid entering the channels from tributaries, displaced the darker fluid, rapidly forcing it out at the proximal cut ends of the lymphatics. This latter phenomenon was never seen in ears perfused at constant pressure, save in one, during the formation of edema, as will be described below. On the average, in the experiments in which pulsatile perfusions were done, and no edema appeared, obvious paling of the lymphatics of the middle third of the ear began 8 to 15 minutes after dye reached that region; complete clearance of portions of the channels (shown in Text-figs. 5 and 6) began to occur in about 15 to 20 minutes. There was almost no clearance of the lymphatics when constant flow was used.

The differences described were found consistent and they constitute clear evidence that the presence of the pulse is of primary importance for the formation of lymph and, by corollary, in the maintenance of lymph flow. In ears perfused at constant pressure, there is almost no lymph flow.

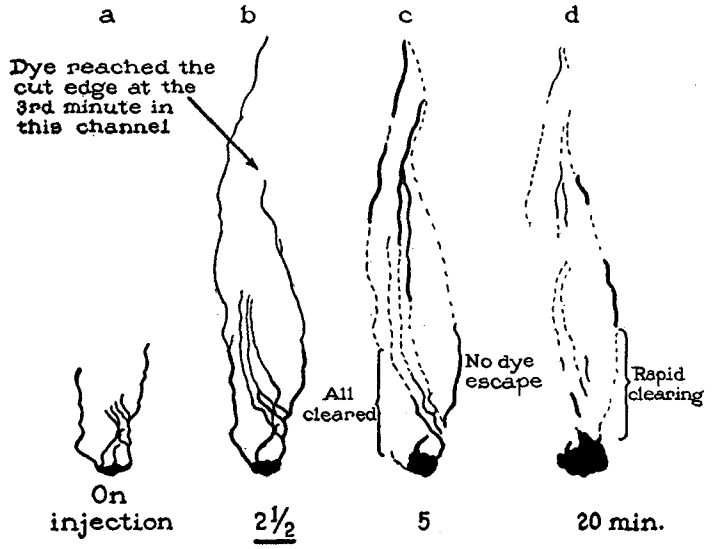
In a few experiments, tracings were made of the movement of colored fluid in superficial lymphatic capillaries draining micropuncture wounds which had been filled with dye solution by a method to be described in the following paper. When the ear was perfused at pulsating pressure, colored streamers extended 2 to 5 cm. from many of these dye spots. Similar phenomena were never seen in ears perfused at constant pressure.

The Effect upon Lymph Flow of a Developing Edema in Tissues Perfused with Pulsatile and Non-Pulsatile Current

The phenomena just described were noted in ears free from demonstrable edema; but the occurrence of edema in many instances offered an opportunity to study its effects upon the formation and flow of lymph. Edema developed in 8 out of 17 experiments, during perfusion at constant pressure, and in 6 out of 18 during pulsatile perfusion. In these cases edema was already demonstrable in the ear and was increasing when the dye was introduced into the lymphatics. The rate of dye movement within the lymphatics also increased while this was happening and this effect was much more evident when pulsating currents were used.

In the ears becoming edematous while perfused with a pulsating current of blood, dye reached the cut edge of the base of the ear within $2\frac{1}{2}$ minutes, on the average, with variations between $1\frac{1}{2}$ and 7 minutes. The dye movement was more rapid than that occurring within the lymphatics of non-edematous ears perfused in the same way, and much quicker than in normal resting ears. In the ears becoming edematous a paling of the colored contents of the lymphatics and complete clearance of portions of them took place with great rapidity, that is to say within $5\frac{1}{2}$ to 11 minutes, and 7 to 25 minutes respectively. The clear segments enlarged and extended rapidly toward the base of the ear, forcing the darkly colored fluid out of the cut lymphatics there.

Tracings of a typical experiment are shown in Text-figs. 7 *a* to 7 *d*. The characteristic progress of the colored streamers is obvious and may be compared with that in Text-fig. 5. As in most of the experiments done at pulsatile pressure, the rate of flow of the perfused blood per gram of ear was slower than in the perfusions done at constant pressure. In this instance it varied between 2.0 and 1.5 cc. per minute or 0.2 and 0.15 cc. per gm. of ear per minute respectively, for the ear weighed about 10.0 gm. "Systolic" and "diastolic" pressures of 141 and 60 mm. of mercury were used. The usual small intradermal injection of dye solution at the tip of the ear filled the channels for 2.5 to 3.0 cm. Pale streamers of dye reached the cut edge of the ear with great speed, in 2½ and 3 minutes (Text-fig. 7 *b*).



TEXT-FIG. 7. Tracings of the movement of color in the lymphatics of an amputated ear which became edematous while perfused at pulsating pressure.

By the 5th minute a large segment of one channel near the base showed complete clearance of dye-colored fluid. The lymphatics in the middle third of the ear had become very pale, as indicated in the figure by the dotted lines, while the dark colored fluid had been displaced toward the base of the ear and could be seen draining from the lymphatics at their cut ends. There was almost no dye escape into the tissues. By the 20th minute clearance of the lymphatics was marked, and there was still practically no dye escape into the tissues.

In 3 of the 8 constant pressure perfusions in which edema of the ears developed, the dye in the lymphatics reached the cut end of the

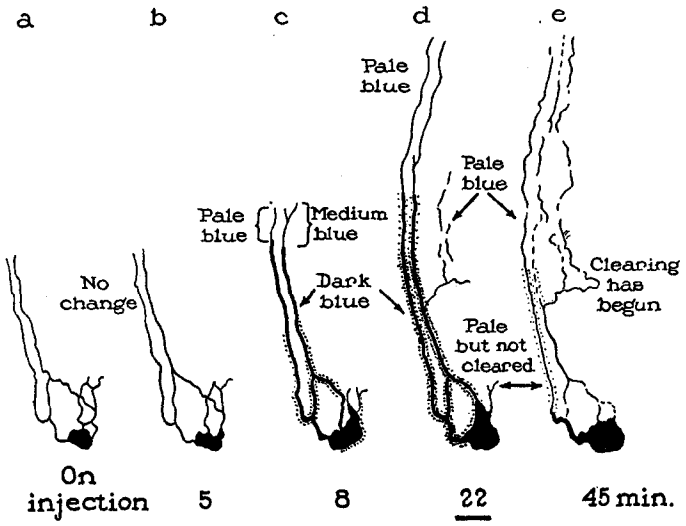
channels, an average distance of 8.5 cm., in 22, 26, and 30 minutes, respectively, at an average time of 26 minutes. In the remaining experiments it progressed only 4 or 5 cm. toward the cut base of the ear, moving that is to say almost as slowly as in the non-edematous ears which were also perfused at a non-pulsatile pressure. It is to be recalled, however, that in only one of the latter instances did dye reach the base and then it required 46 minutes to do so. Furthermore, in the absence of edema, the dye solutions diffused from the lymphatics into the tissue. Paling of the colored contents of the lymphatics did not occur, save in one instance (Text-fig. 4), and then it required 1½ hours. In the 8 instances in which edema appeared, there was a slow but definite paling of the channels in 3 instances, but it required 30 minutes to 1 hour to become definite: it was in no way comparable with that seen in the experiments at pulsating pressures or in the intact, hyperemic, or normal ear.

For a direct comparison of the most marked findings obtained during perfusion at constant pressure while edema was developing, with typical, average findings in the pulsatile perfusion experiments involving edema, or in the tests on normal ears, we have reproduced the tracings from the constant pressure experiment in which color moved the fastest and paling of a lymph channel occurred soonest (Text-fig. 8). The pressure in this instance was 141 mm. of mercury. The rate of blood flow through the ear was faster than in any of the experiments done with pulsatile flow,—0.33 and 0.4 cc. per gm. of tissue per minute for the first and second half hour periods of the experiment respectively. The flow was therefore approximately double that of the experiment just described and charted in Text-fig. 7.

As Text-figs. 8 *a* and 8 *b* indicate, there was no movement of the colored solution in the lymphatics during the first 5 minutes following the injection, which required the usual few seconds. There was little progress of dye in the lymphatics in the next 3 minutes (Text-fig. 8 *c*), but some escape of dye, as shown by the stippling. At the 22nd minute pale extensions of color in two channels had reached the cut edge of the ear (Text-fig. 8 *d*). Already there had been much dye escape from the portions of these lymphatics which first became colored during the injection, and now they had become slightly paler, although still well colored. This fact cannot be shown in the diagrams. At about the 40th minute the paling increased and there was partial clearance of these channels in the regions

indicated by the dotted lines in Text-fig. 8 *e*. By the 45th minute the clearance was definite. Several new channels had appeared containing pale blue fluid, one of them reaching the cut edge of the ear, and there was some clearance close to the injection site.

The findings demonstrate that lymph formation and flow is only slightly greater than that observed in the constant pressure perfusions in which there was no edema. In spite of the presence of edema and excess fluid in the tissues, lymph flow is not as rapid as



TEXT-FIG. 8. Tracings of the movement of color in the lymphatics of an amputated ear which became edematous while perfused at constant pressure.

in normal ears, or in non-edematous ears perfused by pulsating blood, and not nearly as rapid as in edematous ears perfused with pulsating blood.

It is to be stressed here that we have been able to compare the changes in lymph flow only during the formation of edema. Since it increased progressively as long as the perfusions were continued we were unable to ascertain the state of lymph flow when edema is fully formed or receding.

DISCUSSION

The method employed showed itself sensitive enough for the demonstration of changes in lymph flow in the rabbit's ear under conditions of hyperemia, change in position, and irritation. In all these states there was readily demonstrable the increased lymph flow which is known to take place (1–4). Color moved along the lymphatics more quickly under these conditions and the channels cleared of dye more rapidly.

One must infer from our experiences that there was little or no formation of lymph in the non-edematous ears perfused at constant pressure when edema failed to occur. Under these latter circumstances, however, with excess fluid in the tissues, there was evidence of a slow fluid movement in the lymphatics. It was far less than in normal ears or in ears perfused at pulsatile pressure, especially those which became edematous. Always during perfusion at pulsatile pressure, lymph flow was active whether or not edema formed.

It is well known too that tissues perfused with a pulsatile flow do far better than those perfused without this advantage. Our experiments disclose one reason why this is so. What happens through the agency of the pulse that has so great an effect to increase the formation and flow of lymph? In many of the pulsatile perfusion experiments blood flow was but 1/4 or even but 1/6 that of the non-pulsatile experiments, yet in the latter far less lymph was formed. In not a few of the experiments with constant pressure the ears became edematous, demonstrating that large amounts of fluid had escaped from the vessels. Nevertheless lymph flow was not promoted to any great degree. Obviously our findings must be ascribed to the mechanical effects of the pulse. The great flow of lymph in ears that were becoming edematous under pulsatile pressure, as compared with the slight flow when pressure was constant, may very well be ascribed to the massaging effect of the pulse. The influence of this last to increase the formation of lymph is more difficult to understand; and consideration of the problem presented will be deferred until further evidence has been set forth in later papers.

One further point requires mention. In previous work (5) it was shown that in the edematous skin of resting horizontal legs of aged patients suffering from cardiac edema there was no flow of lymph,

whereas in the edematous legs of youthful patients with nephritis, lymph flow was excessive. The presumptive effect in the cardiac patients of increased venous pressure obstructing lymph flow was ruled out, by the demonstration (5) that normal lymph flow took place in the non-edematous arms of these patients, when the arms and legs were at the same level in relation to the point of entrance of the thoracic duct into the great veins of the neck. No explanation for the excessive lymph flow in the nephritic patients was forthcoming.

The present experiments cast some light upon the state of affairs. In the rabbit ears perfused with pulsatile current, and in which edema occurred, lymph flow was excessive. In edematous ears perfused without pulsation it was very slight. The state of affairs in the ears under the circumstances first mentioned was in some ways comparable to that of the skin of the youthful nephritic patients in whom the action of the pulse was excellent (5), whereas that under the second set of circumstances may be likened to the condition in the cardiac patients in whom the pulse was not so good. In these latter patients, of course, the flow of blood was often reduced as well.

SUMMARY

The ears of rabbits were perfused with defibrinated rabbit's blood in such a way that pulsation could be imparted to the perfusate or withheld from it at will. In the absence of pulsation there was almost no lymph flow, whereas when it was present lymph flow was rapid despite the fact that the "systolic" pressure of the perfusate never exceeded the constant pressure in the non-pulsatile instances and the volume flow was far less.

Non-pulsatile perfusion led to a slight flow of lymph in ears that were becoming edematous, whereas when it was pulsatile the lymph flow was enormous.

The pulse exercises an influence to move fluid into the lymphatics and along them.

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