THE RELATIVE PRESSURES WITHIN CUTANEOUS LYMPHATIC CAPILLARIES AND THE TISSUES

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The formation of lymph takes place in the main through the walls of the minute lymphatic capillaries, the larger channels acting chiefly, if not wholly, as conveyors of this fluid to the blood. To learn something about the forces which tend to promote the formation and flow of lymph in the smallest vessels, the lymph pressure existing within cutaneous lymphatic capillaries in the ears of mice has been compared in the present work with the pressure prevailing in the tissue immediately outside them. This has been done both under normal conditions and during the development of rapidly forming edema which greatly increases lymph formation, as is well known.

Techniques

Most of the techniques employed have been those of earlier work in this laboratory (1–5). Improved methods were used for the measurement of edema fluid pressure (5) and for the estimation (4) of the approximate pressure in the normal cutaneous tissues, which do not contain enough free fluid for the interstitial fluid pressure to be estimated directly (6–8). Doubtless because of the fact that lymphatic capillaries, unlike blood capillaries, are not readily visible under the microscope, no direct measurements of the lymph pressure in lymphatic capillaries of the skin have been reported previously. However, in the course of other studies (10–15) from this laboratory, the difficulty had been overcome by the development of methods whereby the lymphatic capillaries are rendered visible as they pass through uninjured living skin. As a result, all that was lacking for the study of the pressure relationships inside the lymphatic capillaries and round about them was a suitable method for the measurement of fluid pressure in these channels.

The Determination of Pressure

The work was carried out on visualized lymphatic capillaries in the skin of the ears of mice lightly anesthetized with nembutal,—0.5 mg. for each 10 gm. of body weight,—as in the previous studies (4). Under these conditions the circulation of the ears appears to be excellent, although the blood pressure is slightly reduced (9).

Fine glass tubes were inserted directly into the channels and the capillary pressures were measured manometrically. In the ear of the mouse lymphatic capillaries are larger as a rule than blood capillaries; channels suitable for intubation can be found varying between 25 and

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100 μ in width. One can introduce into these channels glass tubes larger than the micro pipettes required for the measurement of pressure in blood capillaries, and for this reason they will be termed micro cannulae instead of micro pipettes. Despite their size, the measure of pressure in lymphatic capillaries by means of them is not simple for they must be large enough to fill the lumina of the channels so completely that no leakage of lymph occurs, and it is difficult to insert them without tearing the delicate walls of the capillaries, which are often flat and ribbon-like though relatively broad.

Method and Apparatus.—Soft glass micro cannulae were blown in various sizes, from 10 to 100 μ in external diameter. A single cannula, chosen as will be described, was firmly fixed horizontally in the holder of a Chambers micro manipulator and connected by flexible metal tubing to one end of a horizontally placed glass tube 6 mm. in diameter and about 10 cm. long, equipped with a stop-cock in its mid portion. The other end of the tube communicated by heavy rubber pressure tubing with a broad bottomed 300 cc. glass reservoir connected with two manometers. The bottle could be raised or lowered by a rack-and-pinion device. This part of the manometric apparatus has been fully described and illustrated in Text-fig. 1 and Figs. 1 and 2 of a previous paper (1). Suffice it to say that one of the manometers consisted of a glass tube about 2 mm. in interior diameter fixed at an angle of 15° with the horizontal, and filled with kerosene. Small differences in pressure could be measured with it. The second manometer was a simple vertical tube for measurement of pressures greater than that of a column of water 2 cm. high. This second manometer, the reservoir, the glass tube with the stop-cock, and the micro cannula were all filled with the fluid to be introduced into the lymphatic capillaries or into the tissues during the course of the test. Two identical apparatuses of this sort were used. The first was filled with an 0.2 per cent solution of vital red in Locke's solution, a mixture so diluted with water as to render it isotonic with blood. The other contained a mixture of 0.5 per cent of the blue dye, pontamine sky blue, in Locke's solution, to which there had been added 10 units of heparin to each 100 cc. This too was isotonic with blood, and as will be described below it served as a relatively unabsorbable fluid with which to make pressure measurements.

The Visualization of Lymphatic Capillaries.—The lymphatic capillaries had to be rendered visible before the pressure of the lymph could be determined. Two methods were used for this purpose. The first was suggested by some earlier unpublished studies in which large mice under nembutal anesthesia had been injected intravenously with fluid containing the highly indiffusible dye, vital red, in amounts too small to color the skin visibly to the naked eye. Many of the mice had small bites or other injuries at the periphery of their ears, and a local escape of dye took place from the blood vessels into the tissue about these wounds, giving rise to a narrow band of red at the ear margin. Animals in which this had occurred were placed on plastaline molds with the ears spread on porcelain plaques (16) so that the minute blood vessels and lymphatics could be viewed microscopically in the living animal. It was noted that after a few minutes some of the color passed from the tissues into the adjacent lymphatic capillaries rendering them visible, and thereafter drained to others with result that they too could now be seen. Lymphatics thus accidentally rendered visible were utilized for some of the present experiments while for others the margins of the ears of young animals were purposely injured by pinching them with forceps and the dye was injected into the blood several days later.

Unfortunately visualization of the lymphatics did not always take place under the circumstances outlined. For this reason another technique was frequently used: a modification of one already described in many previous papers (10–16). Mice were anesthetized with nembutal, their ears were spread on porcelain plaques, and under the binocular microscope the skin of the ear was punctured with an especially sharp micro cannula, attached to the apparatus that had been filled with the solution containing vital red. After the micro cannula had been pushed through the skin it was run parallel to the surface for a few millimeters in

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order to tear a few lymphatic capillaries of the exceedingly rich plexus which lies in the subpapillary layer of the corium (10, 11). Next, just enough pressure was put upon the red fluid in the apparatus to force about 1.0 c. mm. of it slowly into the tissues. Within a few minutes thereafter it generally entered one or several of the torn lymphatic capillaries and flowed 2 or 3 mm. further into other lymphatics separated from the torn ones by valves and receiving lymph from capillaries draining uninjured portions of the ear. One of these latter channels was selected for cannulation in the way now to be described:—

The Pressure Measurement.—The anesthetized mouse, with lymphatic capillaries visualized by one or the other method, was placed on a plastaline mold on a mechanical stage that could be moved in any direction and the visualized lymphatics were inspected under the microscope to find a segment containing one or more valveless tributaries. Having found one, the observer selected a micro cannula large enough to fit it so snugly that leakage of lymph would not occur after the insertion. This cannula was then attached to the manometric apparatus containing the mixture of blue dye, heparin, and diluted Locke's solution, filled with this fluid, and clamped in place so that its tip came into focus in the center of the microscope field. Its position was not altered thereafter. Next, with the manometers set at zero and the blue fluid in the apparatus and the cannula at atmospheric pressure, the stop-cock behind the cannula was closed. By appropriate movements of the mechanical stage and gentle traction on the margins of the ear, the stationary tip of the cannula was forced through the epidermis and into the chosen segment of the lymphatic capillary, in the direction of lymph flow. When leakage from the capillary occurred about it, and this was frequent, the escape of pink fluid could readily be seen under the microscope, and the preparation was discarded. If the procedure was successful the micro cannula filled the capillary and obstructed lymph flow. Then by further movement of the mechanical stage or gentle traction on the ear the tip of the cannula was thrust further, to a point just above, and distal, to the entrance of the valveless tributary into the cannulated vessel. The tributary normally supplied lymph to the portion of the cannulated channel below the cannula's tip, and lymph flow through it continued. Consequently, the pressures measured are to be regarded as lateral pressures.

As soon as the cannula was in place the stop-cock of the manometric apparatus was opened, establishing fluid continuity at zero pressure. Almost invariably the blue contents of the cannula were forced backwards towards the manometer by the pale pink fluid in the lymphatics. Pressure was then put upon the contents of the cannula by slowly raising the reservoir of the manometric apparatus by means of its rack-and-pinion device (1) until the blue fluid moved out into the lymphatic capillary. As soon as this had happened the pressure was quickly lowered a little, and by trial and error a point was found at which the boundary of the pink and blue fluids lay at the cannula's tip. The pressure in the apparatus at this moment was recorded. Next, the pressure was lowered to zero, and as the blue fluid in the cannula was forced backwards by the contents of the lymphatic sufficient pressure was again applied to bring it back to the cannula's tip. The required pressure was again recorded. The procedure was repeated several times and the average of the measurements was taken as the intralymphatic pressure. Finally, the pressure in the apparatus was raised slightly until the blue fluid began to move into the capillary. The pressure which was just sufficient to maintain flow was recorded. During the measurement of the pressure the ears did not alter in appearance and there was no edema visible under the microscope except in those instances of which special mention will be made later.

The Pressures within Lymphatic Capillaries

The pressure in lymphatic capillaries of the ears of twenty-four mice, normal except for anesthesia and intubation, varied between zero and 2.7 cm. of water

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and averaged slightly over 1.2 cm. In 80 per cent of the instances it was below 2.0 cm. In Table I the pressures are arranged in order of magnitude. In each experiment an increased pressure of 0.1 to 0.5 cm. of water produced flow into the intubated channel.

The pressure in the cutaneous lymphatic capillaries was usually lower than that in edema fluid as previously ascertained; it was even lower than many of our measurements of the normal cutaneous interstitial resistance,—which is higher than the interstitial pressure of skin (4). This finding brought up the question of whether there is a gradient of pressure existing between the interstitial fluid and the lymphatic capillaries which tends to promote lymph formation and flow. To answer this question the pressures inside and outside of the cutaneous lymphatic capillary walls were compared.

TABLE I

Lymph Pressure as Such and the Pressure Required to Initiate Flow in the Lymph Capillary

Experiment No.	Intralymphatic pressure	Pressure to yield flow	Experiment No.	Intralymphatic pressure	Pressure to yield flow
	cm. of water	*cm. of water*		*cm. of water*	*cm. of water*
1	0.0	0.0	13	1.2	1.6
2	0.0	0.0	14	1.3	1.5–1.6
3	0.5	0.9	15	1.4	1.8
4	0.5	0.8	16	1.5	1.7
5	0.8	1.2	17	1.5	1.7
6	0.8	1.1	18	1.5	2.0
7	0.9	1.2	19	1.8	2.2
8	1.0	1.4	20	2.0	2.4
9	1.0	1.2	21	2.0	2.5
10	1.0	1.5	22	2.1	2.3
11	1.1	1.2	23	2.7	3.1
12	1.1	1.4	24	2.7 ± 0.1	3.0
Average of all				1.2–1.3	1.6

The Relative Pressures within Cutaneous Lymphatic Capillaries and the Surrounding Tissues

As has been brought out in a preceding paper (4), the true interstitial pressure in normal skin cannot be measured directly because there is not enough free interstitial fluid to allow one to make accurate manometric measurements. Nevertheless, we have estimated the true interstitial pressure closely by other means (4, 5). It was found that minute amounts of a test fluid composed of Locke's solution and 0.5 per cent of pontamine sky blue, on introduction into the skin acted as a mildly edema-forming agent, the edema increasing the bulk

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of the introduced fluid very slowly. As result, for periods of 15 to 20 minutes the dye solution behaved like a relatively unabsorbable fluid. To estimate the interstitial pressure in normal skin very minute amounts of the solution were introduced into the tissues at zero pressure and in such a manner that neither blood nor lymphatic vessels were entered directly. Pressure was then gradually put upon the introduced test fluid until there occurred the slightest inward movement of it against the tissue resistance that could be measured with the techniques employed,—that is to say, an inflow averaging 0.06 c.mm. per 5 minutes. The pressure required to maintain this rate of flow was termed the "interstitial resistance" (4). It is not a measure of the interstitial pressure, for it is very slightly higher because of the pressure required to overcome the tissue resistance to the slow passage of the minute amounts of fluid employed. Both the interstitial resistance and the interstitial pressure can be measured directly (4) in edematous skin when there is free fluid present. The difference is only about 0.5 cm. of water and in normal tissues it cannot be far from this figure (4).

Since it seemed possible to learn much about the pressure conditions on both sides of the lymphatic capillary wall in normal tissues by comparing the intralymphatic capillary pressure with measurements of the interstitial resistance, experiments of the sort were carried out on the ears.

The Determination of Interstitial Resistance with the Micro Cannula Apparatus.—In the preceding work (4) the interstitial resistance was measured by the introduction of the mixture of dye and Locke's solution into the tissues through the smallest hypodermic needle available. For the present work the method has been simplified and improved by the use of a micro technique. After measuring the intralymphatic pressure with the micro cannula filled with the relatively unabsorbable dye-Locke's solution, the interstitial resistance was measured with the same apparatus. Under these circumstances the latter determination was accomplished with the introduction of even less fluid than was required when a hypodermic needle was employed. As result, the figures for the interstitial resistance which are here reported should lie even closer to the true interstitial pressure than those obtained in the earlier studies.

In each of eleven experiments the intralymphatic pressure was measured as described above. When several good measurements had been obtained preparations were made to determine the interstitial resistance outside of the channel by introducing the relatively unabsorbable test fluid into the tissues through the same micro cannula. While the latter remained within the channel, the pressure in the injecting apparatus was reduced to zero and at the same time the stop-cock connecting the manometers with the cannula was closed. This maneuver prevented lymph from entering the cannula as the pressure was reduced in it, and as result the cannula remained filled with the relatively unabsorbable dye-Locke's solution, undiluted by lymph, and consequently suitable for the measurement of the interstitial resistance which was to follow. Next, the mechanical stage supporting the mouse was shifted so that the lymphatic became disengaged from the cannula and the tip of the latter lay in the connective tissue close

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to the channel but as far from the point of intubation as it could be moved without tearing blood capillaries. As the shift was slight, less than a quarter of millimeter, no other lymphatics were touched, and one could determine by direct observation whether or not blood capillaries were torn—in which case the experiment was abandoned. In the absence of visible injury the interstitial resistance was then measured:—

The stop-cock of the manometric apparatus was opened and a pressure of 0.5 cm. of water was put upon the fluid in the micro cannula. If no blue coloration appeared in the tissues at its tip,—and none appeared usually,—the pressure was raised to 1.0 cm. of water, and thereafter, if no flow occurred, by increments of 0.2 cm., until blue fluid began to appear in the tissues in microscopic amount. The pressure was then held at this point until one could determine whether or not the test fluid continued to enter the connective tissue and whether it entered directly into injured blood or lymphatic capillaries. If spread occurred the pressure

TABLE II

A Comparison of Intralymphatic Capillary Pressure and Interstitial Resistance in the Skin of the Ear

Experiment No.	Intralymphatic pressure	Interstitial resistance	Difference
	cm. of water	*cm. of water*	*cm. of water*
1	Not measurable	1.5	1.5
2	0.5	1.7	1.2
3	0.7	1.3	0.6
4	0.9	1.7	0.8
5	1.0	2.1	1.1
6	1.1	1.9	0.8
7	1.5	1.5	0.0
8	1.5	1.8	0.3
9	1.8	2.5	0.7
10	2.0	2.3	0.3
11	2.6 ± 0.1	3.0	0.3–0.5
Average..........	1.2	1.9	0.7

in the apparatus was recorded as the interstitial resistance. If no spread occurred the pressure was raised by stages of 0.1 or 0.2 cm. of water until it appeared.

It is to be recalled that any mixing of the test fluid with lymph was prevented before removing the micro cannula from the lymphatic. As result the dye coloration of the test fluid remained sufficiently intense to render it visible in torn or injured blood and lymphatic capillaries. If it appeared in them the preparation was discarded. Further, the test fluid by retaining its initial composition also retained its ability to behave within the tissues like a relatively unabsorbable fluid during the period in which the measurements of interstitial resistance were made.

The findings are summarized in Table II which gives the lymph pressure within the capillaries, the interstitial resistance, and the difference between them.

In none of the eleven comparisons was the interstitial resistance lower than the lymph pressure. The latter varied more than the interstitial resistance

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and the greatest differences between them were observed when the pressure in the lymphatics was low. In the first six experiments of Table II and in the ninth, the differences between the lymph pressure and the interstitial resistance were greater than 0.5 cm. of water. It seems probable that in these instances the pressure in the tissues was higher than that of the lymph within the capillaries. In Experiments 8, 10, and 11, the interstitial resistance and the lymph pressure differed by only 0.3 to 0.5 cm. of water. In all probability the pressures on both sides of the lymphatic capillary wall were approximately equal in these instances or, as in Experiment 7 in which the pressure measurements were identical, the pressure in the tissues may have been actually less than that of the lymph.

Visual observations made during these tests yielded some enlightening additional data.

In Experiment 1, Table II, the intralymphatic pressure was too low to be measured by the technique employed; the apparatus, as set up, did not permit one to measure negative pressures and at the moment the test was made the pressure may have been negative. Yet there was an obvious flow of lymph into the cannulated channel from the tributary vessel close to the tip of the micro cannula. Whenever the least pressure was put upon the contents of the cannula, blue fluid emerged from it and was gently swept away along the capillary. Evidently flow was taking place in the latter either at zero or at negative pressure. There must have been a strong pressure gradient from the tissues to the lymph.

In Experiment 7, as already mentioned, the interstitial resistance equalled the lymph pressure, indicating that the latter was higher than the true pressure in the tissues. It was noticed while measuring the pressure that the blue fluid which left the cannula proceeded in the channel only as far as the nearest valve. Just beyond this point, the lymphatic capillary passed over a vein and was squeezed shut between the vein and the skin. A pressure of 5.0 cm. of water was required to force fluid past the point of occlusion. Evidently there existed in the segment of the lymphatic capillary in which pressure had been measured a physiological blockade resulting in stagnation of lymph. This state of affairs could conceivably have produced a slightly higher pressure within the channel than in the tissues.

The manipulations involved in measuring the pressure within the capillaries often led to the appearance of edema by the time a reading of interstitial resistance could be undertaken. All instances showing frank edema under such circumstances, as manifested by a smooth and cloudy appearance of the skin or thickening of it, were excluded from Table II, but it is possible that, in the last two instances in the table, an imperceptible edema had begun to form. The lymphatic capillary pressures were higher than the average and the behavior of the fluid emerging from the micro cannula during the measurement of the interstitial resistance was not typical of that usually observed; the coloring matter forced from the cannula into the tissues spread with greater rapidity than usual. Shortly after the measurements had been made the ears became frankly edematous. In these two experiments the interstitial resistances may have been measured during an unrecognizable stage of oncoming

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edema. The fact that the intralymphatic capillary pressures were high would tend to support this view. If it is correct, then in these two instances the pressures outside the capillary walls would soon have become significantly greater than the lymph pressures, judging from the other tabulated instances.

Comparison of the Lymph Pressure and Pressure of the Fluid in Edematous Skin

It is well known that lymph formation and flow are often greatly enhanced by the pressure of inflammatory edema (14, 15, 17–19). Further, in states of edema, free fluid is usually present in the skin and consequently the pressure existing outside the lymphatic capillaries can be accurately determined (4, 5). Accordingly edema fluid pressure was compared with that of the lymph in the capillaries.

The Induction of Edema.—Painting the mouse ear with xylol or heating its surface to 46°C. by means of a lamp (11) renders the skin intensely hyperemic almost at once and 5 to 15 minutes later an obvious edema appears (4, 6, 11, 20). As it develops the wrinkles of the cutaneous surface become smoother, as viewed under the microscope, and the skin itself looks clouded. Often, especially if xylol has been used, the ears assume a ground glass appearance. When pressed with a blunt instrument they "pit on pressure" or if pricked with a sharp needle droplets of fluid usually exude. When micro pipettes have already been placed in the interstitial tissues (4) to make pressure measurements, free fluid passes into the pipettes in many instances within a few minutes after the application of either heat or xylol, that is to say at about the same time that direct inspection indicates the onset of edema. Measurements of the edema fluid pressure have shown that it generally increases steadily for 2 hours or more and then slowly falls (4).

Intralymphatic Capillary Pressure and Edema Fluid Pressure.—In seventeen experiments lymph pressure was first measured in the lymphatic capillaries in the ears of normal mice anesthetized with nembutal in the usual way. Then, with the micro cannula still in place, a rapidly forming edema was induced by one of the methods just described. Experience gained on hundreds of edematous ears (4, 6, 11, 20) made it possible to recognize edema early, when it formed rapidly, by simple inspection under the microscope without resorting to "pitting on pressure" or other manipulations which might disturb the position of the micro cannula. At various times up to 2 hours after heating or painting the ears, that is to say during the period of rapid edema formation, the lymph pressure was again measured, often repeatedly. Just after the last measurement the stop-cock of the manometric apparatus was closed, leaving the pressure within the apparatus at the level of the intralymphatic pressure. By means of the mechanical stage the lymphatic in which pressure had just been measured was disengaged from the micro cannula, in the manner employed in the previous experiments on normal ears. A new position for the cannula was found in the connective tissue close to the channel, and the stop-cock was slowly opened. Usually colorless edema fluid began to enter the cannula, contrasting clearly with the blue fluid already there. At once the pressure in the apparatus was adjusted until the boundary of the two fluids lay at the cannula's tip. The reading then represented the edema fluid pressure. The first measurements were usually completed within 2 to 4 minutes after shifting the position of the pipette.

Table III presents the data from six experiments in which mild heat was used to produce edema. Table IV summarizes the data from eleven other experiments (Nos. 1 to 11, inclusive) in which the ear was painted with xylol.

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In an additional experiment, No. 12, edema was produced 24 hours before pressure measurements were made, and consequently the lymph pressure just prior to the onset of edema is unknown.

In each of the experiments the first determination of lymph pressure, made prior to the application of heat or xylol, left a little blue fluid in the cannulated

TABLE III

Intralymphatic Capillary Pressure and Edema Fluid Pressure in Ears Subjected to Mild Heat

Experiment No.	Pressure before edema	Time after application of heat		Pressure during edema		Difference	Remarks
	Intralymphatic			Intralymphatic	Edema fluid (interstitial fluid)		
	cm. of water	*hrs.*	*min.*	*cm. of water*	*cm. of water*	*cm. of water*	
1	0.0	0	30	2.0	5.5	3.5	
2	1.3	0	15	2.0			After the 15th minute gross swelling of the ear became visible. Now for the first time the blue fluid expelled from the micro cannula into the lymphatic while the pressure readings were taken was rapidly swept away along the channel
			20	2.6			
			32	4.0			
		1	3	3.0			
		1	6	—	6.0	3.0	
3	1.4	0	40	1.2	4.2	3.0	
4	1.5	0	20	1.5	2.2	0.7	
5	2.0	0	30	2.0	2.2	0.2	
6	2.0–2.3	0	0	2.0			Swelling of the ear appeared by the 6th minute and increased rapidly. Concurrently the blue fluid introduced into the lymphatic paled and was swept away
		0	12	1.0			
		0	21	1.5			
		0	23	—	5.0	3.5	

lymphatic. The onset of edema was frequently heralded by a rapid disappearance of the color, and through the microscope one could often observe replacement of the blue fluid by clear lymph draining into the cannulated channel through some tributary vessel. At other times the blue fluid simply paled and faded out as if dilution had taken place. As these phenomena occurred, the lymph capillary pressure rose steadily in some of the experiments and fluctuated in others. Often, as the clearance of the channels occurred, there was a temporary fall in lymph pressure.

In ten experiments, Nos. 2 and 6, Table III, and Nos. 1 to 4, 6 to 8, and 10,

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TABLE IV

The Intralymphatic Capillary Pressure and the Edema Fluid Pressure in Ears Painted with Xylol

Experiment No.	Pressure before edema	Time after application of xylol	Pressure during edema		Difference	Remarks
	Intralymphatic		Intralymphatic	Edema fluid (interstitial fluid)		
	cm. of water	*hrs. min.*	*cm. of water*	*cm. of water*	*cm. of water*	
1	1.0	0 0	1.0			Short experiment: cannula became dislodged from the lymphatic when edema began to form. The blue test fluid introduced into the channel in taking the preliminary pressure reading was rapidly flushed away along the lymphatic when it no longer entered after the pressure in the apparatus was reduced to zero. It follows that lymph flow was taking place though the pressure was low. 45 minutes after the first edema fluid pressure measurement had been made "gel formation" occurred (see text).
		10–14	1.3–1.4			
		16	—	2.4	1.1–1.0	
		1 4	—	16.0		
2	1.5	0 5	1.5			
		11	3.3			
		27	4.8			
		46	4.8			
		48	—	7.3	2.5	
		1 35	—	7.1	—	
3	1.5	0 6	2.7			Very rapid edema formation
		12–15	3.5			Blue fluid in channel cleared away
		25	4.3			
		36	4.1			
		39	—	9.6	5.5	
		46	—	14.0	—	
		1 2	—	18.0	—	"Gel-formation"
		1 15	—	25.0	—	
	1.8	0 3	1.8			At the 14th minute, with the pressure in the cannula at 2.2 cm. of water, blue fluid introduced into the lymphatic was rapidly flushed away although as this happened inflow from the cannula was continuing. The intralymphatic pressure fell and rose a few minutes later
		14	2.2			
		17	1.0			
		23	2.0			
		32	2.5			
		47	3.3			
		1 1	2.5			
		1 4	—	3.0–3.5	0.5–1.0	
		1 41	—	4.8	—	
5	1.9	0 30	2.4 ± 0.1	2.2 ± 0.1	0.0 to −0.4	The only instance in which edema fluid pressure was lower than the intralymphatic pressure

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TABLE IV—*Concluded*

Experiment No.	Pressure before edema	Time after application of xylol		Pressure during edema		Difference	Remarks
	Intralymphatic			Intralymphatic	Edema fluid (interstitial fluid)		
	cm. of water	*hrs.*	*min.*	*cm. of water*	*cm. of water*	*cm. of water*	
6	2.0	0	3–5	2.2			At about the 6th minute after the application of xylol the blue test fluid was flushed along the channel and the pressure fell, as in Experiment 4. At the 32nd minute free fluid was still present in the tissues. At the 45th minute the ear assumed a "ground glass" appearance, the "gel-state" had appeared, and in consequence free fluid no longer entered the cannula
			7–12	1.5			
			30	1.5			
			32	—	4.7–6.0	3.2–4.5	
			45	—	17.0		
		1	12	—	24.0		
7	2.2	0	5	3.2			
			9	3.2			Rapidly forming edema which remained fluid
			16	6.0			
			29	7.5			
			33	—	9.0	1.5	
		1	26	—	8.6	—	
8	2.4	0	0	2.4			At the 5th minute intense hyperemia: the lymph pressure rose, then fell 5 minutes later and rose again at the 17th minute. At the 35th minute so little free fluid was present in the tissues that there was difficulty in obtaining the correct edema fluid pressure. At the 47th minute the "gel state" was present
			6	3.9			
			11	2.5			
			17	3.1			
			32	3.3			
			35	—	4.1 ± 0.2	0.6–1.0	
			47	—	19.0	—	
9	2.5	1	4	3.8	7.0	3.2	
10	2.6	0	2	2.8			Between the 30th and 45th minutes a temporary fall in lymph pressure took place, with rapid flushing of the test fluid from the channel
			15–20	3.6–4.4			
			26	4.0			
			30–45	3.1–3.3			
		1	1	3.5			
		1	4	—	9.4	5.9	
		2	00	—	8.0	—	
11	2.8	0	5–10	3.0	3.0	0.0	Short experiment; leakage about the cannula developed at the 10th minute
12	No measurement taken prior to edema	24	0	10.0	10.0	0.0	Ear still swollen and edematous 24 hours after xylol application. Free fluid entered the cannula against a pressure of 9.6 cm. of water; lymph pressure and edema fluid pressure equal

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Table IV, lymph pressure measurements were made in series as edema developed. Generally the pressure increased as time passed, but in all these instances the edema fluid pressure rose still higher. The difference in pressure between the outside and the inside of the lymphatic capillaries varied from −0.4 to 5.9 cm. of water. This being so it is easy to see why lymph flow greatly increases in acutely edematous skin. In eight of the experiments (Nos. 1, 3 to 5, Table III, and Nos. 5, 9, 11, and 12, Table IV), the lymph and edema fluid pressures were measured but once. Pressure gradients of 3.0 cm. of water or more were found in eight instances (Nos. 1 to 3, 6, Table III, and Nos. 3, 6, 8, 10, Table IV) while in several others in both tables the pressure of the edema fluid approximated the lymph pressure, though it was very slightly higher. Three of the tests deserve special mention. In Experiment 5, Table IV, the edema fluid pressure was found to be equal to or 0.3 cm. of water lower than the lymph pressure, a difference so slight that the pressures may be regarded as equal. In two experiments, Nos. 11 and 12, Table IV, the measurements were identical. However, in one of these, No. 11, the cannula became dislodged from the lymphatic only 10 minutes after the experiment was begun and the edema had only just become visible. In the other experiment, No. 12, the measurements were made 24 hours after the application of xylol and the fully formed edema was probably receding.

The Occasional Appearance of a Gel-Like Edema in Skin.—A peculiar phenomenon occasionally appeared during the development of the xylol-induced edema in Experiments 1, 3, 6, and 8, Table IV. In these instances, as in all the others, the edema fluid pressure had been measured in the usual way, by first lowering the pressure within the apparatus until the edema fluid began to flow into the cannula and then finding the pressure required to stop the flow. Quite suddenly, as these measurements were repeated at time intervals ranging from 16 to 45 minutes after the application of xylol, the edema fluid failed to enter the cannula when the pressure was lowered. At first it was believed that the mouth of the cannula had become obstructed by bits of tissue or by lymph clots, and indeed this was the case in several experiments which have been ruled out. In other instances clots or obstructions were sought but not found. Since edema fluid did not enter the micro cannula, pressure was put upon its contents until the blue test fluid began to move into the tissue. To accomplish this, very high pressures, 14.0 to 25.0 cm. of water, were required, as shown by the boxed figures in column 5 of Table IV. When the positions of the micro cannulae were changed and they were inserted into the tissues at other points, the same phenomenon was encountered and no free edema fluid oozed from puncture wounds made in the skin. It was as if the free fluid had undergone the change to a gel, perhaps forming a clot as lymph often does. The phenomenon appeared only in the ears painted with xylol and may conceivably have resulted from a high degree of inflammatory irritation.

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It has been mentioned earlier that the difference between the interstitial pressure and the interstitial resistance in edematous skin has been found to be approximately 0.5 cm. of water (4). In this connection it is to be noted that the high pressures shown in the boxed figures in Table IV are not to be considered as measurements of the interstitial resistance. Instead they represent the pressure necessary to disrupt the presumptive gel or clot present in those instances.

DISCUSSION

The pressure in the lymphatic capillaries of the mouse ear has proved to be the same or slightly less than that in the relatively large draining channels of other animals. In the cervical lymphatics of dogs lateral pressures ranged from 0.8 to 2.6 cm. of a sodium bicarbonate solution, 0.5 to 2.0 cm. of a soda solution (sp. gra. 1.080), and −2.8 to +3.2 cm. of water when measured by Noll (21), Weiss (22, 23), and McCarrell (24) respectively. Drinker and Field (18) found the lymph under no pressure in a resting leg of a dog, and Lee (25, 26), studying the mesenteric vessels of cats, reported pressures of 0.5 to 6.7 cm. of water in vessels proximal to the mesenteric node, and pressures of 3.0 to 6.8 in the channels distal to it. Higher pressures have been found in the larger lymphatics draining inflamed areas (17, 19) or actively moving organs (19, 24, 27), as also, of course, when end pressure measurements have been made in obstructed lymphatics (19, 24, 28), that is to say under conditions that differ widely from those considered in the present work. High pressures in minute peripheral lymphatics have been reported only by Königes and Ottó (29) who found an average pressure of 24.5 mm. of mercury in the highly specialized terminal chylous vessels of the intestinal villae of cats.

One of the early workers on the mechanism of lymph flow, Weiss (22, 23), reasoned that the pressure in lymphatics must increase in the vessels as they approach the periphery. This seemed a justifiable assumption since Rudbeck (30) had shown, as early as 1653, that ligated lymphatic vessels become distended on the peripheral side of a ligature and collapse on its proximal side. Two hundred years later Donders (31) concluded that the lymphatics would collapse if the pressures in the tissues should become higher than that in the channels. It remained for Gaskell (32) and later for Starling (33, 34) to suggest that the pressure within small lymphatics need not be higher than that in the tissues since the small lymphatics are connected to the formed elements of the connective tissues by fibrils. As result, an increase of the pressure in the tissues with consequent distention of the latter should serve to pull the lymphatic walls still farther apart rather than to collapse them. The suggestions of Starling and of Gaskell were made at a period when, as the result of writings of von Recklinghausen (35) the lymphatic capillaries were supposed to have open ends, but they have been corroborated by Clark and Clark (36), by

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Pullinger and Florey (37), by work from this laboratory (7, 8, 14, 20), and by some observations made in the present studies. For example, in several of the experiments in which, after measuring the lymph pressure, edema of the ears was induced by painting the skin with xylol (Table IV) the lymphatic capillary walls suddenly became loose around the micro cannulae; as the edema developed and pressure in the tissues rose, the channels were widened and lymph began to leak out around the shafts of the cannulae, preventing further measurements of the lymph pressure. Clearly the high fluid pressure in the tissues during the early stages of edema formation did not collapse the lymphatics, but on the contrary increased their diameter, and under the circumstances it must have acted to force fluid through the capillary walls to form more lymph.

The existence of an effective pressure difference between the edema fluid and the capillary lymph, as shown in the present work, not only discloses a mechanism which will account at least partially for the flow of lymph but it furnishes evidence for the generally accepted view that the lymph capillaries have walls devoid of fenestrations. This evidence corroborates the anatomical studies of others (38–42). It also confirms work from this laboratory (7, 10, 11, 20) in which the behavior of dyes injected into lymphatic capillaries indicated an unbroken continuity of the vessel walls.

Despite their continuity the walls of the lymphatic capillaries are extremely permeable, even more so than blood capillary walls. This is evident in the fact that dyes escape from lymphatic capillaries into the surrounding tissue about as fast as from blood capillaries, although the hydrostatic pressure existing in the lymphatic channels and tending to force fluid through their walls is far lower than in the capillaries carrying blood (10, 11). Since tissue fluid gets into the lymphatics so readily, and lymph flow is relatively slow, what can be responsible for the retention of the fluid in the lymph capillaries until it reaches the larger, relatively thick-walled channels from which it cannot escape? It seems reasonable to suppose that the retention is due to the pressure of the interstitial tissue fluid. Undoubtedly this is the case in the skin of the ears of mice during the rapid formation of edema, but does it hold as well in normal ears in which the true interstitial pressure cannot be measured? As has already been brought out above, the interstitial resistance, measured in the present work by micro methods, must have been very close to the true interstitial pressure. The average of all the measurements of interstitial resistance shown in Table II was 1.9 cm. of water as compared with 1.2 for the lymph pressure. In more than half the experiments on normal ears there must have been a gradient of pressure from the tissues to the lymph, for the interstitial resistance was higher than the lymph pressure by more than 0.6 cm. of water. In the remaining tests the interstitial pressure and the lymph pressure, as judged by measurements of the interstitial resistance, were probably

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about equal. Under these circumstances there was little reason for the fluid in the lymphatic capillaries to escape before an alteration in local conditions caused it be moved into the thick-walled drainage channels.

SUMMARY

The pressure in the cutaneous lymphatic capillaries of normal mice anesthetized with nembutal ranged between 0.0 and 2.7 cm. of water. Measurements of the interstitial pressure in the tissue immediately next the lymphatics showed that, in more than half the instances studied, there was a slight gradient of pressure from the tissues to the lymph. In nearly all the other instances the pressures inside and outside the lymphatic capillaries were approximately equal. In two cases in which lymph flow in the capillaries was rapid, the lymph pressure may have been negative. Under these circumstances there must have been a considerable gradient of pressure from the tissues to the lymph.

In skin which was rapidly becoming, or had recently become, edematous as result of the application of xylol or of heat, the intralymphatic capillary pressure generally was increased, yet when compared with the pressure prevailing in the edema fluid outside of the capillaries it was usually found to be relatively much lower, at times by as much as 5.9 cm. of water. The findings indicate that a pressure gradient is an important factor in lymph formation under normal and pathological circumstances.

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