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OBSERVATIONS ON LIVING MAMMALIAN LYMPHATIC CAPILLARIES—THEIR RELATION TO THE BLOOD VESSELS [1]

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THIRTEEN FIGURES

The microscopic observations of lymphatic capillaries in the living mammal has been carried out for the past 5 years by means of transparent chambers permanently installed in the ears of rabbits. In the 'round table' chamber, in which a hole is cut clear through the ear and a celluloid table inserted in the gap, the regeneration of new tissue takes place in a space approximately 6.5 mm. in diameter, the thickness of which is controlled by means of small celluloid buffers glued to the surface of the table on which the mica cover slip rests (Clark et al., '30). Since in such chambers the thickness of the new tissue with its circulating blood vessels (40 μ to 75μ) plus that of the mica cover slip (60 μ to 70 μ) is about equal to the thickness of a no. 1 glass cover slip, the finest cytological details can be studied at high microscopic magnifications.

Descriptions of the manner in which new blood vessels invade the table space and of the remodelling of the primitive capillary plexus into a stable vascular pattern, have been given (Clark et al., '31). The growth of new lymphatics into the table space and the morphological characteristics of lymphatic capillaries in the living mammal have also been described (Clark and Clark, '32, '33). By means of prolonged

[1] These studies have been aided by a grant from the Rockefeller Foundation. They were carried out, in part, at the Marine Biological Laboratory, Woods Hole, Massachusetts.

observation of individual vessels with the oil immersion lens, with frequent camera lucida sketches, it was possible to demonstrate positively that the regeneration of lymphatics in the adult mammal occurred by a process of sprouting from the preexisting endothelium of lymphatic vessels outside the table area, accompanied by division of endothelial nuclei, in the same manner as in the lymphatic vessels of amphibian larvae (Clark and Clark, '32; Clark, '09, '12).

In a former study a description was given of the relationship between the lymphatic vessels in the transparent chambers and the connective tissue and intervascular substance (Clark and Clark, '33). The growth pattern of the regenerating lymphatics was found to be modified by differences in the density of the connective tissue present in the region of invasion. Following the invasion of the table area by lymphatics their further extension was frequently impeded by the development of connective tissue fibers, and in such cases the lymphatics ceased to grow, lost the pointed tips characteristic of growing capillaries, and acquired rounded, bulbous endings. In the region at the edge of the table the growth of connective tissue frequently became so dense as to block the lumen of lymphatics which previously had grown well out across the table area. A description was also given of the modification in form of lymphatic capillaries following the development of unyielding fibers and bands of connective tissue around them, which constricted the lymphatic vessels at various points. Although the growth and form of the regenerating lymphatic capillaries were influenced in these ways by the character of the connective tissue outside, our studies showed clearly that the lymphatic endothelium always remained distinct from connective tissue cells. Moreover, fibroblasts showed no tendency to adhere to the lymphatic wall and to form adventitial cells as they did in the case of the newly formed blood capillaries. The question whether the 'reticulum and collagen fibrils' of the surrounding tissue adhere to the outside of the lymphatic endothelium as recently described by Pullinger and Florey ('35) we have not investigated.

Our studies on the relation of living mammalian capillaries to the 'tissue spaces' showed that normally the endothelial wall of these lymphatics, like that of amphibian lymphatics is closed, and that their contents remain fluid, while under usual conditions there is no free fluid present in the outside tissue; that, however, under certain conditions, such as in inflammations, free fluid makes its appearance in the intervascular tissue and that in such cases occasionally a lymphatic may be broken open and remain open for from 1 to 10 days, during which time there is a free passage of fluid and blood cells back and forth between the outside tissue and the lumen of the injured vessel (Clark and Clark, '33; Henry, '33). Such persistent holes in the endothelium of injured blood capillaries were never seen.

In the present study we desire to present further observations which have been made upon living lymphatic capillaries in the rabbit, especially upon their relation to the blood vessels.

GROWTH AND DISTRIBUTION OF LYMPHATICS IN RELATION TO BLOOD VESSELS

In previous studies on regenerating lymphatics in the 'round table' chambers we reported the tendency of lymphatics to follow the course of the larger blood vessels in their growth and showed this to be due to mechanical factors rather than to a special affinity of the two types of vessels (Clark and Clark, '32). In chambers in which dense connective tissue developed between the blood vessels before any lymphatics had invaded the table area a loose perivascular space was frequently present beside the larger veins and arteries and it was into this space that a new lymphatic capillary, which invaded the growth area after it had been completely covered by new connective tissue and a network of blood vessels, advanced (fig. 1). Recently it has been found that lymphatics which followed the course of larger blood vessels in their growth showed a greater tendency to maintain persistent open connections with the preformed vessels outside the thin space

over the table, than did the lymphatics which had grown in early and followed more random paths between the vessels. This was evidently due to the support and protection from squeezing afforded by such an accompanying blood vessel with its perivascular space, in the region at the edge of the table where the secondary formation of dense intervascular connective tissue frequently blocked the lumen of the lymphatics not so located (Clark and Clark, '33).

Thus according to our observations, the presence of a looser tissue around blood vessels determined the course of regenerating lymphatics which entered a region of dense intervascular tissue and helped to preserve the connections of

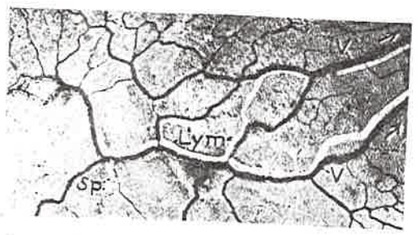


Fig. 1 Photomicrograph of part of the table area of a chamber installed in the ear of a rabbit 8 months previously. Table area completely vascularized 21 days after operation. Lym., lymphatic which grew onto table 1 month before taking photograph and extended along the course of a vein (V.) and its branches. See text page 255. Sp., perivascular space surrounding branch of vein, which was not invaded by lymphatic. × 16.

lymphatic vessels which had followed such a course, in thin regions which were largely free from movement ('massage'). Although the perivascular space, or loose tissue, surrounding larger blood vessels in the transparent chambers afforded an easy path for the growth of new lymphatics, and consequently came to be occupied in many cases by such vessels, there were, in all chambers studied, a number of large vessels with no companion lymph vessel, while in most cases, the lymphatic accompanied a blood vessel on one side only, although the loose tissue or space on the opposite side was apparently identical.

Some of the problems relating to the growth and behavior of lymphatics in close proximity to blood vessels have been clarified by the recent 15-month study of a splinted chamber the base of which was reinforced to prevent warping. The table area was completely vascularized at 21 days after the operation, after which the whole observation area and the pattern of the blood vessels remained remarkably stable with no signs of inflammation or mechanical disturbance for 6 months, and the formation of connective tissue was greatly retarded. Not only were connective tissue fibers between the vessels less numerous but a wide clear space free of connective tissue cells and fibers was present around an unusual number of blood vessels in the central area of the chamber. Figure 2 is a photomicrograph of a venule in this chamber which shows such a clear space outside its wall. These perivascular spaces were found to be the remains of clear spaces which appeared following dissolution of fibrin outside the growing blood capillaries in the early growth stages in all the chambers studied and which in this specimen had not been encroached upon by the new growing connective tissue. In many places the connective tissue at the outer border of these persistent perivascular spaces formed a continuous longitudinal arrangement of fibers, with occasional flattened cell bodies, which at cursory examination had the appearance of a definite wall. Very probably such a space in fixed tissue might be mistaken for a lymphatic. However, by observation in the living with high microscopic magnifications, such perivascular spaces could be distinguished from the true lymphatics, with their continuous endothelial walls and typical lens-shaped nuclei, which frequently occupied the same position adjacent to a blood vessel. (Compare fig. 2 and fig. 3.)

The clear spaces beside the blood vessels contained occasional macrophages, leukocytes and erythrocytes, but were free from connective tissue cells or fibers. At times localized portions of such a space contained free fluid as evidenced by the bobbing of blood cells or small particles present in it, while at other points the cells were motionless, indicating the

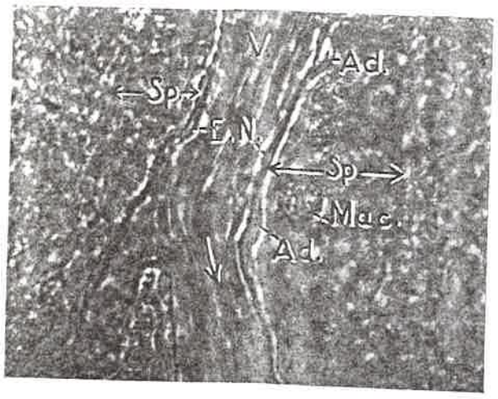


Fig. 2 Oil immersion photomicrograph taken 1 month after insertion of chamber, showing perivascular spaces (Sp.) surrounding a venule (V.) near the center of the table which persisted for several months and were not invaded by new lymphatics. Ad., adventitial cells on wall of blood vessel. Mac., macrophage in perivascular space. Compare with figures 3 and 4. × 650.

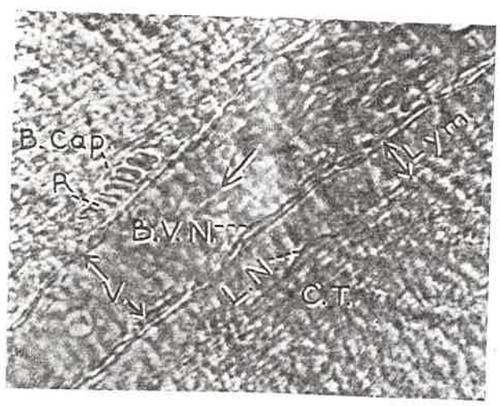


Fig. 3 Oil immersion photomicrograph of a more proximal portion of the same blood vessel shown in figure 2, taken on the same day, showing new lymphatic capillary (Lym.) occupying space at one side of venule (V.). B.V.N., endothelial nucleus of blood vessel. L.N., endothelial nucleus of lymphatic. B. Cap., blood capillary on opposite side of venule. R., red blood cells in rouleaux formation inside blood capillary. C.T., connective tissue. × 650.

presence of a transparent gelatinous substance similar to that present between the growing capillaries at early stages of invasion (Clark and Clark, '33). The fluid areas in the clear space varied from day to day; cells which were freely movable at one time of observation were seen to be motionless on the following day or at a later period of the same day.

Several lymphatic capillaries invaded the table area in this specimen. As was the case with all growing lymphatics previously studied the new vessels extended as outgrowths from preexisting lymphatic vessels in the preformed tissue surrounding the table by the process of endothelial sprouting already described (Clark, '09, '12; Clark and Clark, '32). In the case of one of the lymphatic capillaries watched intensively the growing vessel extended from the periphery of the table for some distance across the table area, advancing in the clear space beside one of the blood vessels. For the first part of its course in the peripheral part of the chamber where the perivascular space was narrower, the growing lymphatic filled the space so that the endothelial walls of the two vessels were practically in contact (fig. 3). More distally, where the space was wider, the lymphatic occupied only its outer portion. Later, after the lymphatic had extended further out into the central area of the chamber, its growing tip diverged from the blood vessel and the perivascular space (fig. 4). At this time the new connective tissue which had developed in this area formed two sheets, one on the celluloid table and one immediately beneath the mica cover slip, leaving a thin, loose region between, filled with gelatinous, intercellular substance, into which the growing lymphatic capillary continued to extend. The clear space next the venule, beyond the point at which the accompanying lymphatic diverged from it, remained unchanged for several months with no sign of a lymphatic appearing therein (fig. 2). Similar clear spaces were also present, surrounding a number of other blood vessels in the central table area which likewise persisted for months, and which were never invaded by a lymphatic capillary.

At the end of 6 months an extensive growth of connective tissue took place so that this chamber then acquired the appearance usually seen after the first month. No new lymphatics had invaded the table area during this period. The lymphatics which had grown in previously were still present but had ceased to advance and had acquired bulbous ends.

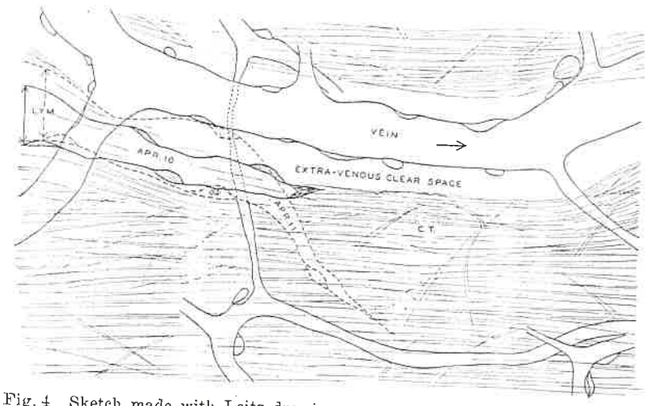


Fig. 4 Sketch made with Leitz drawing eye-piece of the distal portion of the same lymphatic and venule shown in figure 3. Shows relation of growing tip of lymphatic capillary (Lym.) on 2 successive days to the extra-venous clear space. April 10th, lymphatic (solid outline) occupied outer portion of extra-venous space. April 11th, lymphatic (broken outline) enlarged and filled entire space in this region and its growing tip had diverged from the vein and extended into the extravascular substance, growing between two layers of connective tissue fibers (C.T.). See page 259. The extra-vascular clear space beyond the point of divergence of the lymphatic persisted for several months and was not invaded by a lymphatic. See figure 2, a photomicrograph of more distal portion of same blood vessel. Oil immersion. × 285.

At this time the development of an infected spot outside the chamber area resulted in several days of increased circulation through the blood vessels of the ear, including those in the observation area, which was accompanied by an increase in extravascular fluid and emigration of leukocytes from many of the vessels. This disturbance to the stability of the tissue in the chamber was followed by changes in the vascular

pattern of the nature previously described (Clark et al., '31). New blood vessel sprouts grew out, several formerly small vessels enlarged to form veins, while other vessels located in portions of the chamber subjected to pressure disappeared. Two lymphatics, located in a compressed area, were obliterated, one of them entirely and the other one for all but a short stretch near the table edge. Following this, a new lymphatic began to grow into the observation space as an extension from a preformed lymphatic outside the table area. Simultaneously and in the same region, a new medullated nerve, the first up to this time, grew onto the table. This new lymphatic extended rapidly toward the center of the table growing close beside a large vein. A number of branches from this lymph vessel also followed the course of the venous branches (fig. 1). During its period of extension the new lymphatic grew so close to the vein that it filled the loose space beside it and the two endothelial walls were practically in contact.

Three months later, the circulation had diminished through the blood vessels of the observation area, including the large vein which was accompanied for part of its course by the lymphatic, and following this the caliber of the vein decreased and a wide clear shrinkage space appeared surrounding it. On the side next the lymphatic, a clear space now appeared between the endothelial walls of the two vessels. The wide clear space on the opposite side of this vein persisted for several weeks but was not invaded by a lymphatic. The outer border of the perivascular space was seen to be formed by a dense, refractile band of connective tssue which ran parallel with the vein, marking the former outline of the vessel. Similar 'shrinkage spaces' developed simultaneously outside several other vessels in the table area. One of these was especially marked in the case of a former vein which received an arterial blood supply following the disturbance to the circulation in the chamber, and subsequently acquired a typical thick wall and the narrower caliber distinctive of arteries as compared to veins of the same importance.

In this chamber in which, owing to its greater rigidity, the formation of an intervascular connective tissue network was delayed, the perivascular spaces usually present around most of the new growing blood vessels persisted longer than in cases previously studied. Again, following the development of dense connective tissue several months later, a secondary type of perivascular space was observed which was in the nature of a shrinkage space. In both instances an excellent opportunity was afforded for the study of the growth of new lymphatic capillaries in relation to such perivascular spaces. It was clear from these observations that such spaces were not concerned with the formation of new lymphatics except in affording convenient pathways for their extension into the growth area, since many spaces outside vessels were never invaded by lymphatics, while others into which lymphatics grew became wider secondarily, owing to shrinkage of the blood vessel, leaving an extra space between the two vessels. Moreover, a lymphatic which grew along a perivascular space in the proximal part of the chamber was seen to diverge from the space distally and to grow out into an area of loose tissue away from the blood vessel.

Aside from the relationship just described in which a new lymphatic grew close beside a blood vessel for the greater part of its course across the table space, there were numerous instances, in many of the chambers studied, in which growing lymphatic capillaries which followed more random paths came in contact with blood vessels, For example, in a number of cases, the tip of a growing lymphatic, extending in the intervascular tissue, was seen to approach a large blood vessel which crossed its growth path and filled the thin space between cover slip and table. In certain of these cases the lymphatic then sent out a sprout laterally and followed the course of the blood vessel in its further growth, while in others it ceased to advance, withdrew its solid sprout and acquired a bulbous ending which remained in contact with the large blood vessel (fig. 10).

At other times lymphatics have been seen to cross smaller blood vessels which did not fill the space completely, and to remain thereafter in contact at the point of crossing. In some of the latter cases, the blood vessel was seen to enlarge subsequently, following circulatory changes, until it compressed the lumen of the lymphatic at the point of crossing to such an extent that no cells were able to pass between the distal and proximal portions. Occasionally the lymphatic was completely severed at such a crossing point, following the enlargement of the former blood capillary into a vein or artery, after which the two isolated ends of the formerly continuous lymphatic remained as rounded bulbs one on each side of the intervening blood vessel. The instances in which growth of lymphatics was interfered with by the presence or later development of large blood vessels in their path occurred chiefly in thin chambers in which the space over the table was maintained at a uniform thickness of 40 μ to 75 μ. In thicker chambers the lymphatics often formed an interlacing plexus with the blood vessel network, sometimes crossing superficially to the blood vessels and sometimes beneath them (Clark and Clark, '32). No instance of anastomosis between a lymphatic and a blood vessel with which it came in contact in these various ways was ever seen.

In all of the chambers in which the growth of new lymphatics was studied, it could be clearly seen that the new lymphatic capillaries grew out independently as sprouts from preexisting lymphatic vessels and that their endothelium remained specific, with no addition from connective tissue cells or spaces or from perivascular spaces and although frequently running in such close proximity to blood vessels that the walls of the two vessels were in contact, they never anastomosed with them.

BEHAVIOR OF LYMPHATICS IN RELATION TO BLOOD VESSELS

Although the lymphatic vessels which grew into the space over the central table area in the round table chambers, as well as those present in the preformed tissue chambers and in

the intact ear formed a specific system of endothelial-lined vessels which remained morphologically distinct from the blood vessels their tendency to run for long distances in such close proximity to blood vessels introduced interesting physiological relationships between the two sets of vessels.

In the case of lymphatics which follow the course of veins and their branches and the smaller, thin-walled vessels the two endothelial walls are frequently separated merely by a clear space which is free from connective tissue cells and fibers and contains either no cells at all or an occasional leukocyte or macrophage while in many instances the two walls are practically in contact, with no other type of cell intervening except the scattered adventitial cells on the blood vessels. Figures 5, 6 and 8 illustrate this relationship. It is clear that in the case of lymphatic vessels and portions of such vessels which run for considerable distances beside thin-walled blood

Figures 5 to 10. Photomicrographs of vessels in seven different transparent chambers showing relationship of lymphatic capillaries to thin-walled blood vessels.

Fig. 5 Venule (V.) and companion lymphatic (Lym.). No intervening tissue. Blood vessel endothelium in phase I (p. 269). Lymphatic filled with clear fluid. Oil immersion. × 450.

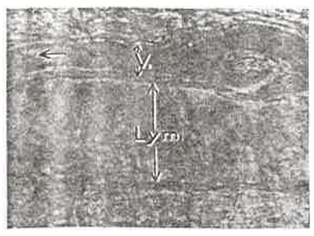
Fig. 6 Venule (V.) with endothelium in phase II (p. 269). Leu., leukocytes clinging momentarily to wall. No emigration of leukocytes. Lym., lymphatic capillary, with clear fluid, in close proximity to vein. Oil immersion, × 450.

Fig. 7 Vein (V.) and accompanying lymphatic (Lym.) with narrow tissue space between. Poly., polymorphs in lumen of lymphatic which emigrated from the adjacent vein on previous day, following change in its endothelium to phase IV (p. 269). At time of photograph venous endothelium had reverted to phase II, with no further emigration of leukocytes. Oil immersion. × 450.

Fig. 8 Vein (V.) and accompanying lymphatic (Lym.). Leuk., leukocytes sticking to wall of blood vessel. Mac., macrophage in lumen of lymphatic surrounded by blood cells which entered it from adjacent vein several days previously following endothelial change to phases IV and V (p. 270). × 400.

Fig. 9 Vein (V.) and accompanying lymphatic (Lym.) photographed on the day after a hemorrhage from blood vessel to lymphatic caused by mechanical pressure (p. 268). Ery., erythrocytes packed in lumen of lymphatic. Art., arteriole crossing vein. × 400.

Fig. 10 Bulbous end of lymphatic (Lym.) in contact with vein (V.). Dw.P., dwindled polymorphs in end of lymphatic, which emigrated from vein to lymphatic and remained in end of lymphatic where they underwent degenerative changes. Compare size with that of recently emigrated polymorphs in figure 7. Oil immersion, × 450.



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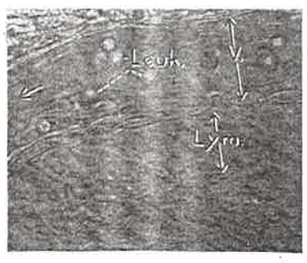

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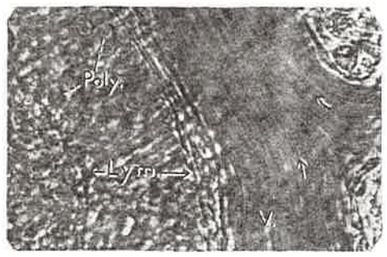

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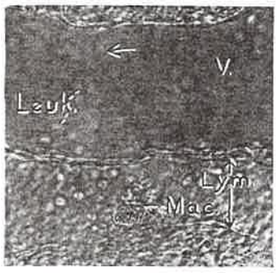

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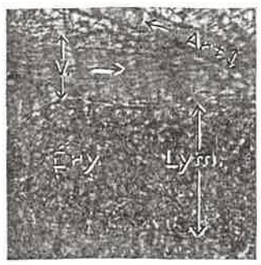

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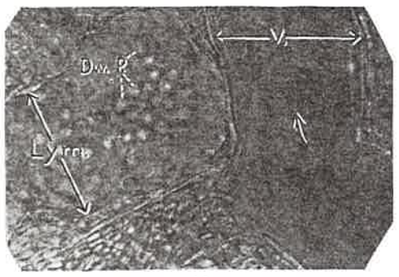

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vessels in this manner, the fluid which enters them must represent in no small degree what may be termed a direct leakage from the blood stream, rather than a product of absorption from tissue spaces. The term 'leakage' as here used is not intended to imply the passage of fluid through definite openings between the two, but rather the diffusion of fluid through first the blood vascular and then through the lymphatic endothelial layers without bathing cells of outside tissues.

Demonstration of such fluid leakage by direct observation under normal circulatory conditions is difficult, owing to the ease with which the lumen of the lymphatic may be compressed by the surrounding tissue in the thin, semi-rigid chamber space and also to the extremely meager spontaneous flow of lymph in the peripheral terminal vessels (Clark and Clark, '32; Henry, '33).

In all of the chambers studied, an increase in blood flow through the chamber, following local heating of the ear for example, resulted as a rule in the lymphatics' becoming temporarily more prominent. When the increased blood flow was prolonged for several hours, resulting in a change in the blood vascular endothelium characterized by pronounced sticking of leukocytes to the vessel wall, increased passage of fluid from the blood vessels was evidenced by the appearance of free fluid outside the vessels and over the surface of the tissue. Whether or not there was actual increased absorption on the part of the neighboring lymphatics in such cases corresponding to their apparent increase in diameter, or whether this appearance was due to change in pressure conditions caused by raising of the thin and somewhat elastic mica cover (p. 253) was difficult to determine. Henry ('33) found no increase in the flow of lymph from the whole ear, following the use of heat.

When the circulation in a chamber was increased for 24 hours or more—as was the case in inflammation due to irritation or local infection near the chamber area—a definite increase in the caliber of lymphatic vessels situated near veins was observed, which followed the widening of the actively

circulating blood vessels and which persisted for about 24 hours or longer after the blood vessels had returned to their former caliber. Moreover, in the 'preformed tissue' chambers, in which the original ear vessels and tissue are included in the chamber (Clark and Clark, '32), the lymphatics were usually markedly enlarged during the first few days after the operation for installation of the chamber. In both of these cases the enlargement of lymphatics in inflammation or edema could not be attributed definitely to increased absorption of fluid from veins or surrounding tissue as it was observed that the normally sluggish flow in the lymphatics became even slower or was absent under such conditions. For example, in preformed tissue chambers clumps of red blood cells have frequently been seen in the lumen of distended lymphatics for the first few days following the operation, which moved back and forth within the lumen with no forward progression, so that the same group of cells could be identified from day to day. After 4 or 5 days, when the blood vessels in the chamber area had reached a stable circulatory condition, the clumps of blood cells in the lymphatics were gradually washed away from the observation area.

However, in one instance, ocular demonstration of direct leakage from a vein to a contiguous lymphatic was obtained, during the course of an experimental procedure in one of the round table chambers. In this experiment methylene blue (5 cc. of a 1:200 solution in 0.9% sodium chloride) was injected into the large border vein of the ear, at a point near the chamber, with the purpose of obtaining a vital stain of the nerves in the chamber area. A description of this experiment has already been given (Clark, Clark and Williams, '34) in which the deep blue vital staining of the nerves, which took place 7 minutes after the injection and persisted for 40 minutes, was emphasized. The amount of methylene blue solution injected intravenously was sufficient to replace temporarily the blood in the vessels of the chamber area. Following the injection, which lasted for 1 minute, the stain was held in the ear vessels by pressure at the base of the ear

for 2 minutes more, after which the ear was placed on the stage of the compound microscope for observation. When first examined (3 minutes after the injection) a pale blue stain was noticeable in the tissue just outside many of the veins and capillaries, which quickly faded. A lymphatic on the table, which ran parallel with and in close proximity to one of the larger veins, was conspicuous by reason of the distinctly blue stain of its fluid contents. Aside from a few macrophages lying close to one of the veins which also took a blue coloration, there was no stain present elsewhere in the chamber for 4 minutes, at which time the nerves of the chamber became stained in the manner described. The blue in the lymphatic disappeared within a few minutes, but the promptness with which it appeared in this vessel in close proximity to the vein afforded a visual demonstration of 'leakage' of fluid from vein to lymphatic.

In addition to the leakage of fluid from vein to lymphatic, the close proximity of the two vessels results in the passage of formed elements from vein or capillary to lymphatic under certain conditions. Local direct hemorrhage from vein to accompanying lymphatic has been produced mechanically, by slight pressure on the cover slip (fig. 9). This mechanical forcing of blood cells from a blood vessel into the lumen of an adjacent lymphatic was noted on a number of occasions when the rabbit struggled slightly and caused the cover slip to come into sudden contact with the objective of the microscope. Small extravasations into the tissue in regions where no lymphatic was present were produced in the same way. On the other hand, when care was taken to avoid such accidents during the observation period, lymphatics usually remained clear with only a very few widely scattered cells in their interior for many weeks, in chambers free from inflammation, since the detached celluloid splints effectively protected the chamber areas from mechanical pressure or strain between observation periods when the rabbit was in its cage.

Aside from mechanical pressure, another condition in which cells from the blood stream were seen to enter lymphatics was

that following changes in the consistency of the blood vascular endothelium. A description of the progressive changes which endothelium of blood vessels may undergo in response to various external and internal stimuli has already been given (Clark and Clark, '35). The six different phases in the consistency of blood vascular endothelium were shown to form a progressive series of changes from the smooth elastic lining of normal vessels (phase I) through phases II and III in which the endothelium became increasingly sticky for leukocytes and apparently more permeable to fluid, to a softer consistency (phase IV) in which leukocytes could penetrate the walls, on to phase V in which the endothelium showed weakening and loss of elasticity, evidenced by the occurrence of hemorrhages and bulging of the wall, and finally to phase VI in which it broke into segments and disintegrated. The changes to phases II and III were produced so easily and by such slight stimuli that they could not be considered pathological. Reversals in consistency from phases II, III and IV took place abruptly. When the vessel wall had undergone the change to phase V, an appreciable time of days or even weeks intervened before its complete recovery. There was no recovery from phase VI.

On many occasions mild mechanical, thermal and chemical stimuli resulted in change in consistency of the vascular endothelium of single vessels or parts thereof or of many or all of the vessels, to phases II or III, in which the leukocytes adhered slightly or more tightly without migrating through to the wall. With a stronger stimulus, such as prolonged heating or the presence of a small abscess outside the chamber, the endothelium apparently became softer and leukocytes penetrated the wall (phase IV). When such a change occurred in a vein or capillary with an accompanying lymphatic, the process of migration of individual leukocytes through the wall of the blood vessel and of the lymphatic into the lumen of the latter was observed. In certain instances in which the lymphatic concerned was blocked at the table edge or isolated (rounded at both ends) the leukocytes which entered it frequently remained imprisoned in the lymphatic for many

weeks. The changes which such a group of leukocytes, most of which were polymorphs, underwent into a collection of small, round cells with round nuclei have been described elsewhere (Clark, Clark and Rex, '36). In the case of a lymphatic possessing an open connection with other vessels outside the table area, a group of leukocytes which had entered it in the manner just described, remained for a day or longer in the bulbous end, and later moved back and forth in the vessel before they finally drifted off the table area into the communicating preformed lymphatics. It was frequently found that following periods of inflammation in which leukocytes had emigrated from many of the blood vessels in the chamber, new collections of leukocytes were present in the lumen of a lymphatic located close to a vein while the contents of other lymphatic vessels not so situated, remained clear. Conversely, we have observed cases of emigration of leukocytes into the tissue in one region of a chamber, following which a lymphatic, located close to a vein but in another portion of the chamber in which no endothelial change had occurred, remained clear.

In chambers in which the inflammatory reaction continued longer or was more intense the endothelium of some of the blood vessels or portions thereof underwent the further change in consistency characterized by loss of elasticity and increased tenderness and the occurrence of small hemorrhages (phase V). In cases in which a thin-walled blood vessel (vein or capillary) located close to a lymphatic underwent such an endothelial change an extravasation of erythrocytes into the lumen of the lymphatic was frequently observed. As in the case of hemorrhages into the tissue the endothelium closed immediately after the extrusion of cells as no break in the wall of the blood vessel could be seen at the point where the extrusion of blood cells had occurred (Clark and Clark, '35). Similarly, no hole in the lymphatic wall could be perceived at the point at which the blood cells had been squeezed through it. Following such an occurrence the fate of the blood cells which entered the lymphatic from the adjacent blood vessel varied

according to the following factors: 1) The presence or absence of an open connection of the lymphatic concerned with vessels outside the table area, 2) the amount of movement in the lymphatic, 3) the amount of blood in the extravasation in proportion to the size of the lymphatic, and 4) the presence or absence of macrophages in the lumen of the lymphatic.

In most cases in which open connections with other preformed lymphatic vessels had been maintained the blood cells from such a hemorrhage remained for several days bobbing back and forth in the lymphatic near their point of entrance from the neighboring blood vessel, before moving off the table into the connecting vessels. This was also true of the cases in which blood cells entered the lymphatic vessels of preformed tissue chambers at the time of operation. In those instances in which the lymphatic was blocked at the table edge, the erythrocytes from such as extravasation often remained for indefinite periods—several weeks—in the lumen, as did the emigrated polymorphs in the cases already described (p. 270).

In the specimen described on page 270, 40 minutes after the injection of methylene blue, numerous small hemorrhages in the tissue appeared and at the same time a hemorrhage from one of the larger veins of the table area, into its accompanying lymphatic was observed. The blood cells were seen to move jerkily along this vessel in the direction of the table edge and within a few minutes to enter the connecting preformed lymphatics. At this time there was a very rapid flow through all the blood vessels of the chamber, accompanied by a great increase in intervascular fluid which elevated the cover slip, thus temperarily increasing the space over the table. On the following days, although the inflammatory condition was still present and the whole ear edematous, the circulation in the blood vessels of the chamber was somewhat less rapid and there was evidence of squeezing from the increased pressure around the table area. At this time, a few erythrocytes entered the lymphatic from the same vein and in this case they were not washed out, but remained

bobbing back and forth in a short stretch of the lymphatic for 3 days before finally moving out of the observation area.

In some instances the hemorrhage from vein to adjacent lymphatic was so massive as to pack the lumen out to its bulbous end. In a number of such cases the blood cells were so closely pressed together that no cell outlines could be distinguished, and the blood then gave the appearance of being laked. That this appearance was caused by crowding of the cells together and loss of fluid from the lymphatic was shown when such vessels were studied continuously. In a number of these cases some of the blood was squeezed out of the end of such lymphatics into the tissue and it could then be seen that the erythrocytes were still distinct haemoglobin-containing cells. In the course of days such lymphatics frequently became wider and contained more fluid and the masses of blood then became broken up into individual cells which bobbed back and forth in the lumen. In two cases of hemorrhage into a completely isolated lymphatic from an adjacent venule, slight pressure over the region resulted in hemorrhage in the reverse direction—from lymphatic back into the vein.

When a hemorrhage consisting of a moderate number of erythrocytes first entered a lymphatic from a nearby vein the blood cells could be seen to move freely back and forth, either singly or in rouleaux, with no tendency to stick to each other, to the lymphatic endothelium, or to any leukocytes which might be present. On succeeding days erythrocytes inside the lymphatic were frequently seen to cling together in clumps, which moved back and forth in the lumen. At times and in certain localized places, such agglutinated masses as well as individual erythrocytes were observed to adhere to the vessel wall. At this time also erythrocytes were seen to stick to macrophages which chanced to be present in the lumen of the lymphatic. This change in the physical character of erythrocytes which have remained for several days in the interior of a lymphatic capillary, evidenced by sticking of such cells to macrophages, to each other and at times to localized

regions of the lymphatic endothelium, was not permanent since the same group of cells, imprisoned in an isolated or blocked lymphatic, which showed this property on one day have been seen on succeeding days to bob back and forth freely, without adhering to each other, to macrophages, or to the vessel wall. At certain times also erythrocytes which stuck to each other or to macrophages inside the lymphatic failed to adhere to the endothelium.

The fate of the blood cells which entered a lymphatic from a nearby blood vessel and lingered for some time in the transparent observation area was influenced to a great extent by the presence or absence of macrophages or monocytes in the lumen of the lymphatic. Monocytes, outside the blood stream, have been shown to change into macrophages after engaging in phagocytosis (Clark and Clark, '30; Lewis and Lewis, '26 and Elliott, '26). In some instances monocytes were included in the extravasation into the lymphatic while in others the hemorrhage consisted of erythrocytes and polymorphonuclear leukocytes alone.

In one specimen, for example, two successive hemorrhages into the bulbous end of a lymphatic from an adjacent vein were observed, which consisted of erythrocytes, polymorphs and a few monocytes. The cells for the first day floated freely back and forth in the lymphatic. After 24 hours many of the erythrocytes were seen to stick together in clumps which adhered temporarily in a few places to the wall of the lymphatic. Three days after this first hemorrhage a number of typical macrophages were present in the lymphatic lumen, some of which may have wandered in from the tissue but others of which from their clearer cytoplasm appeared to be enlarged monocytes. Both erythrocytes and changed polymorphs were seen to cling to the macrophages and many of them to be ingested on this day. On the following day most of the cells had moved back from the end of the lymphatic capillary into the connecting lymphatics located around the periphery of the table. Later, on this day, a new and more massive hemorrhage occurred which distended the end of the lymphatic. On the

following day the erythrocytes inside the lymphatic clung together in clumps, while some of them were forced out of the vessel tip into the tissue where their phagocytosis by tissue macrophages was observed. The blood cells remained inside the terminal portion of the lymphatic for 3 days longer during which time the number of erythrocytes and polymorphs diminished and the macrophages with cell inclusions became larger and more conspicuous. After this the group of cells again moved off the table into the connecting peripheral lymphatics where they could be seen bobbing back and forth. Two days later, a number of cells drifted back onto the table and out toward the tip of the lymphatic and at this time they were seen to consist for the most part of typical macrophages containing pigment and cell fragments (Clark, Clark and Rex, '36, p. 157, fig. 9).

According to our observations, although macrophages from the tissues have been seen to migrate into the lumen of a lymphatic capillary and to phagocytize erythrocytes and degenerated polymorphs present therein, they apparently make their way through the lymphatic wall less frequently than was the case in our studies on Amphibia (Clark and Clark, '28, '30). Hence in many cases studied in which monocytes were absent in the orginal hemorrhage from blood vessels to lymphatic, the erythrocytes in blocked or cut-off lymphatics remained intact for much longer periods than did those which had been extruded at the same time into the tissue. This was undoubtedly due in part at least to the greater number of macrophages present in the region of tissue hemorrhages than in the lumen of the lymphatic. For example, in a chamber studied recently a single hemorrhage into a cut-off lymphatic, rounded at both ends, occurred from an adjacent vein, which consisted of a few polymorphonuclear leukocytes and a large number of erythrocytes. The blood cells were packed very closely together in the lumen of the lymphatic, especially at the two bulbous ends. Several days later some of the erythrocytes were squeezed out through the lymphatic wall into the tissue where they were phagocytized by macrophages which had been present in this region for several

weeks. The erythrocytes left in the lymphatic remained unchanged in color for over a month, during which time the polymorphs in the same vessel underwent the change into small, clear, round cells with round nuclei already described (Clark, Clark and Rex, '36). At the end of this time a macrophage made its way into the lymphatic from the outside tissue and ingested a number of the erythrocytes and degenerated polymorphs.

The foregoing observations showed that, due to the tendency of blood vessels and lymphatics to follow the same course and in such close proximity to each other, not only leakage of fluid but passage of formed elements can occur directly from blood stream to lymphatic. The active migration of leukocytes from blood vessel to lymphatic, following the change in blood vascular endothelium to phase IV, often took place following relatively mild stimuli such as over-heating, while the hemorrhages into the lymphatic either followed a further change in the wall of the blood vessel to a weaker condition (phase V) or direct mechanical pressure over the vessels.

The entrance of red blood cells into the nearby lymphatic capillaries by the process observed frequently in amphibian larvae—i.e., by the growth of lymphatic sprouts to extravasated blood cells and the active taking of such cells into the lumen of the lymphatic (Clark, '09; Clark and Clark, '26) —was never observed in our studies on the rabbit's ear, although numerous extravasations of blood cells were produced in the tissue just outside lymphatic capillaries which were watched carefully with this possibility in view.

As mentioned in our previous study, in the case of the vessels in both Amphibia and the rabbit which have undergone the change in endothelial consistency in which there is an observable weakened condition characterized by distortion in shape, bulging of the wall and the occurence of ecchymoses designated as phase V, an appreciable recovery period intervened (Clark and Clark, '35). Frequently when the recovery from an inflammation was accompanied by the sudden return of an active blood flow through temporarily non-circulating

vessels, marked bulgings or aneurysmal swellings of some of the veins were noticed which persisted for days in the tadpole and for weeks or even months in some of the rabbit's vessels. The localized swellings of arteries and arterioles sometimes present in the preformed tissue chamber following the original operation were apparently due to trauma at the time of operation to the muscle and nerve supply rather than to such an injury to the endothelium (Clark and Clark, '32).

According to our studies, bulges present in the walls of veins or venules whose endothelium had become weakened in this manner were larger and persisted longer in cases in which such vessels were located in close proximity to a lymphatic vessel. Apparently the longitudinally arranged connective tissue fibers surrounding the blood vessels supported the wall of such injured vessels more effectively than did the accompanying lymphatic vessel with its delicate endothelium and relatively low internal pressure. This was also borne out by observations that bulges were less apt to occur in blood vessels surrounded by a dense connective tissue framework than in those with a perivascular space.

In the case of one chamber installed 3 months previously, intensive observations were carried out on a lymphatic vessel which extended to the center of the table and for most of its course in such close proximity to a vein that no connective tissue cells or fibers were present between the endothelial walls of the two vessels. The chamber area from the time of its complete vascularization, 24 days after the operation, had remained stable and free from inflammation. The lymphatic at this time (August 25th) was clear, containing only a few cells—macrophages and dwindled polymorphs—which moved back and forth in the lumen with no forward progression for days at a time.

On August 26th, the appearance of a small infected spot in the ear outside the chamber, associated with a period of hot weather, resulted in an increased circulation through all the ear vessels which continued for 4 days. During the first 48 hours there was rapid circulation through all the vessels in

the chamber, increase in intervascular fluid, and sticking of leukocytes to the walls of most of the veins and capillaries (phases II and III) but no evidence of endothelial injury in the chamber area. On the third day (August 29th) in addition to the above changes all of the blood vessels were markedly distended and emigration of leukocytes occurred from many of them (phase IV), while in some of the veins the relaxed and weakened condition of the endothelium was present (phase V) which was accompanied by the appearance of numerous small extravasations of erythrocytes into the tissue and into the lymphatics at points in which the later were located close beside them. The fast circulation continued for another day during which fresh hemorrhages from veins to lymphatics occurred. On this day (August 30th) several swellings appeared on a vein accompanied by the lymphatic, which were especially marked on the side next the lymphatic (fig. 11).

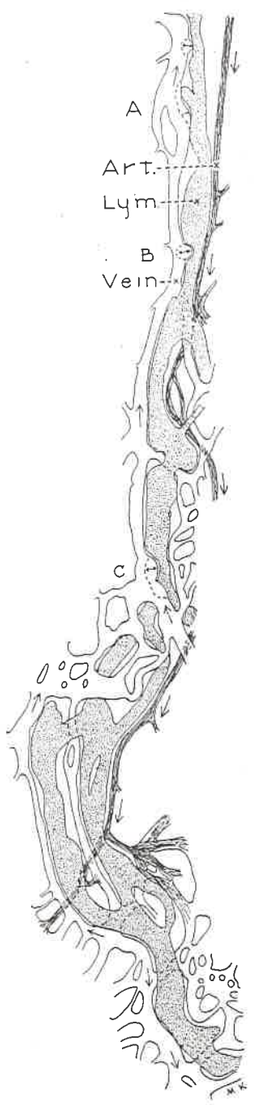
On the following day (August 31st) the infection had subsided, the circulation in the chamber had become more moderate, emigration of leukocytes had ceased and most of the endothelium of the chamber vessels had reverted to phase I, with no leukocyte sticking. However, the bulging places of the wall of the vein next the lymphatic, at the site of the former hemorrhages into the lymphatic, were still conspicuous.

During the next 2 days, as had been noticed previously following periods of inflammation, there was an accumulation of fluid and cells between the vessels and also between the tissue and the cover slip which squeezed the blood out of the vessels over the table at times when the supplying arteries underwent rhythmic contractions. After a day or two, with the removal of the excess fluid, the tissue and vessels again became adjusted to the thin space over the table and normal circulation was resumed. In the regions of the venous dilatations still present next the lymphatic, the spontaneous contractions of the supplying arteries (Clark and Clark, '32) caused interesting changes in the two neighboring vessels. With the artery

dilated and a full blood stream coursing through the vein, the venous bulges indented the lymphatic, encroaching on its lumen to a considerable extent. Following a contraction of the supplying artery, the flow in the vein diminished and the lymphatic, possessing greater obstruction to its outflow than the vein, then bulged into the lumen of the vein at this point (figs. 11 and 12). When the animal was warmed, or heat from an electric light applied locally to the ear, the arterial contractions became more frequent and the periods of dilatation and abundant blood flow greater and correspondingly the flapping back and forth of the double membrane formed by the two endothelial walls occurred oftener and the bulging of the vein into the lymphatic lasted longer. Conversely, when the ear was cooled the arterial contractions lasted longer, the amount of blood flow diminished in the vein, the back and forth movement of the combined venous-lymphatic endothelium occurred less frequently, while the periods of bulging of the lymphatic into the vein were of greater duration.

During the next week, the circulation of the ear was moderate and steady and the chamber area was beautifully clear, showing no signs of the former inflammation aside from the balloon-like swellings between the vein and lymphatic. The bulging places on the vein during this time became less conspicuous, although whenever the circulation was stimulated by heat, they became temporarily as prominent as before. Eleven days after the first inflammation (September 6th) another day of increased blood flow occurred due to unusually high atmospheric temperature. Although this period of increased circulation was not truly inflammatory, since only

Fig. 11 Low power camera lucida sketch of a lymphatic (Lym.) (stippled) located in close proximity to a vein (in outline) the endothelium of which had shown localized injury during a period of inflammation occurring 1 week earlier (see text, p. 277). A, B and C mark three regions in which weakened wall of vein bulged into adjacent lymphatic. Dotted lines in veins at these three places mark the point to which the lymphatic encroached on the lumen of the vein during periods of decreased venous pressure (p. 278). Art., (black) arteriole running parallel with lymphatic on its other side. × 40.

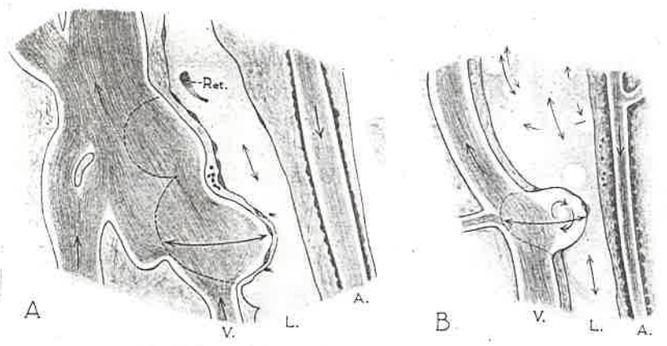

A
Art.
Lym.
B
Vein
C

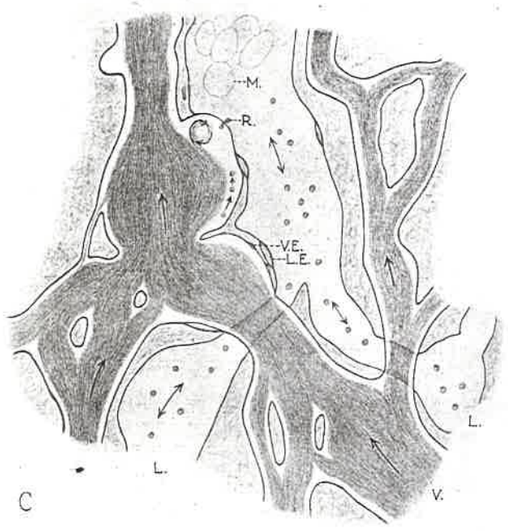
slight leukocyte sticking (phase II) was observed in the majority of the blood vessels in the chamber and no emigration of leukocytes or extravasations of blood cells into the tissue occurred, fresh hemorrhages took place from the weakened vein into the accompanying lymphatic in the region of the bulges. Following a hemorrhage at one of these points, an erythrocyte remained caught in the membrane formed by the venous and lymphatic endothelium, half in the vein and half in the lymphatic (fig. 12, C).

On the following day (September 7th) the circulation had again subsided but the vein next the lymphatic showed signs of further injury following the day of rapid blood flow. The bulges into the lymphatic had become still larger and the venous endothelium in the regions of the bulges appeared to be thinner and to have suffered a still further loss in elasticity. The latter was shown by the fact that at times when the venous pressure fell below that of the lymphatic and the swelling was inverted and bulged into the vein, instead of retaining its former round balloon-like form such as that shown in figure 12, A, it became elongated in the direction of the current in the vein.

For the next 8 days, the circulatory conditions in the chamber remained stable and the venous bulges into the lumen of the lymphatic although still present, gradually became less conspicuous except when the ear was heated. On one day, local heat was applied to the ear continuously for an hour without producing any changes in endothelial consistency of

Fig. 12 High power drawings showing the same points of bulging (A, B, C) of the injured vein into the adjacent lymphatic shown in figure 11. Drawings of regions A and B made 3 weeks after the original injury to the venous endothelium. V., vein; L., lymphatic; A., artery; Ret., reticulum. Dotted lines in vein indicate extent to which lymphatic bulged into vein when the flow through the latter vessel diminished (p. 278). Leitz drawing eye-piece. × 175. Drawing C made 1 week after original injury to vein, on a day of increased circulation in which fresh hemorrhages from vein to lymphatic occurred at the weakened (bulging) points. V., vein; L., lymphatic; V.E., venous endothelial nucleus; L.E., lymphatic endothelial nucleus; R., red blood cell half in vein and half in lymphatic; M., macrophage in lumen of lymphatic. Free hand, approximately × 250.


Ret.
A
V.
L.
A.
B
V.
L.
A.


M.
R.
V.E.
L.E.
L.
L.
C
V.

any of the blood vessels of the observation area beyond phase II (transitory sticking of leukocytes). Although the bulging places became more prominent and the flapping back and forth of the thin walls corresponding to the pressure changes became more frequent, no emigration of leukocytes or extravasation of blood at these points occurred and the bulges remained firm and round as shown in figure 12, instead of elongating in the manner observed following their second period of weakening. This experiment seemed to point to a partial recovery of the venous endothelium from the original injury which resulted in the formation of the swellings.

On September 25th and again on October 4th, the ear again became temporarily inflamed. On both occasions the same phenomena were repeated—i.e., increased blood flow with sticking of leukocytes in most of the blood vessels of the chamber, emigration of leukocytes from many of the vessels, followed by the appearance of small hemorrhages at scattered points in the tissue. On both of these days fresh extravasations of blood cells from the vein under observation into its accompanying lymphatic occurred, and following this the endothelial bulges again enlarged. The bulges gradually became less prominent during the days of moderate circulation which succeeded each of these periods of inflammation. By October 15th, 2 months after the first appearance of the bulges in the wall of the vein, the accompanying lymphatic had grown narrower and the venous bulges were smaller and less conspicuous. A week later, the endothelium of this vein had apparently recovered its original firm and elastic quality.

In another specimen, a number of similar bulging places appeared on the wall of a vein following a period of inflammation caused by a small area of infection outside the chamber area. During the week after the period of active circulation the vein gradually acquired its original contour except for four bulging places which were located at points where a lymphatic crossed the vein. The bulges from the vein into the lymphatic persisted for 2½ months in this specimen.

During this time the same inversion of the bulge—from lymphatic into the lumen of the vein—was noticed at times when the pressure in the vein fell below that in the lymphatic, following contraction of the supplying artery.

These examples demonstrated that the aneurysmal bulgings in the weakened endothelial wall of veins and other thin-walled blood vessels which frequently occur following inflammation tend to be larger and to persist longer when such blood vessels are located close beside a lymphatic and that subsequent increase in blood flow through such injured vessels exaggerates the condition, causing the bulges to enlarge still further and their endothelium to become thinner and more sensitive to changes in consistency, so that hemorrhage may occur at these points in response to stimuli too slight to induce such changes elsewhere. The inversion of such swellings, in which the lymphatic bulges into the vein when its pressure falls, is apparently also due to the weakening of the blood vascular endothelium at these points, rather than to a similar change in the lymphatic wall, since our observations have shown that the lymphatic endothelium is normally thinner, more delicate, and less elastic than that of all but the youngest of blood capillaries, so that lymphatics usually tend to bulge out into sac-like forms in regions in which the surrounding tissue is loose enough to permit their distension. However, our records showed a thinning of the lymphatic as well as of the venous endothelium in the region of venous bulges which have persisted for some time (fig. 12, B, C).

In all of our studies on the relationship of lymphatics to blood vessels, the independence of the two systems both in their growth period and subsequently has been obvious. Although growing lymphatic vessels frequently followed the course of blood vessels for considerable distances and in such close proximity that the walls of the two were practically in contact, no anastomoses of the two were ever seen. In the case of bulging of blood vessels into adjacent lymphatics, in which the two endothelial walls became thinner and so closely

applied as to form a double membrane through which emigration of leukocytes and extravasations of blood cells occurred following comparatively slight circulatory changes, no instance of a fistula developing between the two vessels was observed.

The observations just described on the relation of lymphatics to blood vessels have been concerned with thin-walled vessels—veins and capillaries—in which the two endothelial walls were separated by few or no connective tissue cells. As already remarked, lymphatic capillaries which invade the chamber space also frequently grow beside arteries. According to our studies, the new arteries which form in the chambers especially those which develop active contractility, are frequently surrounded by a loose space into which lymphatics which invade the table area after its vascularization often advance. This space beside the arteries also affords a path of growth for companion veins the development of which as secondary outgrowths has been observed (Clark et al., '31). In some specimens in which a lymphatic grew along one side of an artery in this fashion, a companion vein developed on the opposite side of the same vessel.

The early development of a muscular wall on the newly formed arterioles in the chamber areas modifies the relationship of such blood vessels to an accompanying lymphatic in comparison with that of thin-walled vessels. Emigration of leukocytes through the walls of arteries has not been observed and hemorrhages from arteries occur very rarely in the protected environment of the chambers. Hence in most cases the lymphatics located in close proximity to arteries are clear and contain only a few cells even at times in which emigration of leukocytes and extravasations of blood have occurred from many of the veins and capillaries of the same chamber.

Active contraction of arteries the caliber of which is relatively large for the confined space of the table area (40 μ to 50 μ) was seen to produce passive changes in the caliber of the lymphatics in the observation area and in the movement of their contents. Commonly, the dilatation of such an artery,

aside from allowing more blood to circulate through the vessels of the chamber, lifted the mica cover slip slightly, thereby increasing the space over the table area. At such times the majority of the lymphatics on the table became more distended and fluid and cells from communicating vessels outside the table area might be sucked back into their bulbous ends. When such an artery contracted, a slight squeezing of the tissue over the table occurred, the lymphatics were slightly compressed and fluid and cells were frequently seen to move in the reverse direction.

In the case of lymphatics which occupied a position close beside an actively contracting artery the opposite effect was observed. In such cases when the artery dilated, it filled the loose space around it, thereby encroaching on the accompanying lymphatic, frequently to such an extent as to obliterate its lumen. When it contracted the lymphatic again became filled with fluid which was sucked back into it from communicating vessels outside the thin space. Figure 13, A and B illustrates such a case. Thus contractions of arteries in the thin chamber space apparently exert a massaging effect on lymphatics in the area, which is especially marked when the two vessels are in close proximity. In many of the splinted chambers studied, the newly formed arterioles of the regenerated vascular plexus remained relatively small and frequently failed to display active spontaneous contractions. When such specimens were protected from irritation the thin space over the table remained relatively rigid and uniform in thickness for weeks or months, and the movement inside the lymphatic capillaries present therein was very slight, consisting chiefly of a bobbing of the cells in the vessel lumen with short back and forth excursions, and with no forward progression for days.

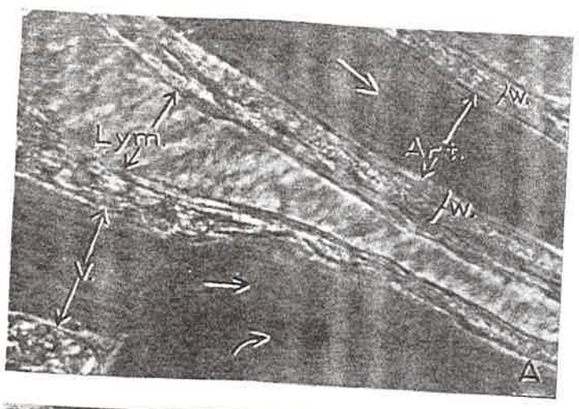


Fig. 13 A and B. Successive photomicrographs of the same field showing relationship of a lymphatic capillary to a newly formed artery which had acquired active contractility (p. 285). Lymphatic (Lym.) located close beside artery (Art.) with a vein (V.) on its other side. A, artery contracted, lymphatic lumen open. B, artery dilated (3 minutes later) lymphatic lumen obliterated. W., muscular wall of artery—note increase in thickness with artery contracted. × 450.

DISCUSSION

The fact that lymphatic vessels frequently occupy a position in close proximity to blood vessels has led some investigators to conclude that the lymphatic system in its embryonic development arises from a transformation of perivascular spaces. This view was advanced at one time by Huntington and McClure ('08) as "the extra-intimal origin of lymphatics." They claimed that the endothelial wall of the lymphatic was derived on one side from the mesenchyme and on the other from the venous endothelium. They later abandoned this theory in favor of the view that lymphatics arise through the coalescence of spaces in the mesenchyme. Recently Zimmermann ('35) has revived the theory of the origin of lymphatics from perivascular spaces. Both of these investigations, as well as those which formed the basis for the view that lymphatics are derived from tissue spaces, were based upon reconstructions of cross sections, a method which has been shown to be inadequate for demonstrating the extent of delicate lymphatic capillaries (Clark, '11, '12). Sabin ('11) showed that many of the so-called extra-intimal spaces around embryonic blood vessels were shrinkage spaces which appeared after fixation.

The question of the origin of the first lymphatic endothelium in the embryo has never been finally settled owing to the lack of a sufficiently transparent region in which fine microscopic details could be studied in the living animal. The supposedly isolated lymph sacs in embryos, which have been thought from the study of cross sections to establish connections with veins after their formation and thus to prove the origin of lymphatics from tissue spaces, have been shown, wherever the same embryonic stage was tested by injection, to be connected with the venous system and to be preceded as a rule at a still earlier stage by a plexus of lymphatic capillaries possessing a great number of venous connections. Thus in the case of the jugular lymph sac in the chick, injections in the living embryo showed definite connections between a lymphatic plexus and the jugular vein in the same region and at the same

stage in which Miller ('11) described an isolated lymph sac (E. L. Clark, '12). The subocular 'lymph sac' of the trout described by McClure ('15) as appearing first as a completely isolated tissue space was demonstrated by Haj, a pupil of Hoyer ('34) to be connected at this stage with the veins.

In the case of the posterior lymph heart of chick embryos, observation in the living and micro-injections supplemented by oil immersion reconstructions of embryos with the blood vessels completely injected, demonstrated the presence of a plexus of definite lymphatic vessels, possessing numerous connections with the veins, 3 days before the stage (8 days) at which Sala ('00) described the origin of the lymph heart as an isolated sac (Clark and Clark, '20). It was further shown that the first lymphatic capillaries appeared at a time when the intervascular tissue was dense and no mesenchymal or extra-intimal spaces were present.

Although on account of the limitations of the methods available the possibility that the first lymphatics may arise from differentiation of mesenchymal cells into angioblasts, which form temporary connections with veins, has not been excluded, the fact that the earliest identifiable lymphatics possess numerous connections with blood vessels while in later stages the number of such connections diminishes instead of increasing, appears to favor the original view of Sabin ('02) that the lymphatic endothelium arises as an outgrowth from the blood vascular endothelium.

Whatever the exact mode of origin of the first lymphatics in the embryo, the fact that they soon acquire specificity and spread through the body by a process of endothelial sprouting, as inferred by Ranvier (1897), has been definitely established. In studies on the transparent tails of amphibian larvae, in which every cell could be clearly seen, prolonged observation of the same cells and vessels demonstrated, beyond question, the growth of lymphatic capillaries by sprouting from preexisting lymphatic vessels without the addition of connective tissue cells or spaces, of blood vascular endothelium or of any type of wandering cell (Clark, '09, '12).

Djiurzinski ('11) using the same method of observation in the living found that the same type of independent growth is characteristic of the regenerating lymphatics of Amphibia. With the development of a method for study of microscopic details in the living rabbit, the same specificity and method of growth has been found to characterize the regenerating lymphatic capillaries in adult mammalian tissue.

In the transparent fins of tadpoles, in which the connective tissue is composed of widely spaced stellate cells and in which no perivascular spaces are present, the new lymphatic capillaries were found to grow for the most part at a distance from blood vessels and to make their way through the homogeneous intercellular substance as far as possible from the bodies of the connective tissue cells (Clark, '12).

In the new tissue which forms over the table area in the chambers inserted in rabbits' ears, clear spaces were regularly present around the newly formed blood capillaries and, in splinted chambers in which the formation of connective tissue fibers was delayed, many such perivascular spaces persisted for several months. After the formation of dense intervascular connective tissue, loose spaces were often present around some of the larger blood vessels, while in one specimen wide, clear, perivascular spaces appeared secondarily, surrounding several of the larger blood vessels, the diameter of which had diminished following a change in circulatory conditions, thus showing that true 'shrinkage spaces' may form in the living adult animal. The pattern which the regenerating lymphatics assumed in the transparent chambers varied with the character of the intervascular tissue present at the time of their invasion of the chamber space. Thus, in chambers in which this tissue consisted mainly of a soft gel, the new lymphatics sometimes advanced along perivascular spaces for part of their course while frequently they grew at random in the intervascular tissue in a manner similar to the growth of such vessels in amphibian larvae. In chambers in which a dense connective tissue had formed prior to their appearance, the new lymphatics tended to advance in the loose space beside the blood vessels (Clark and Clark, '32, '33). However,

in all specimens studied perivascular spaces were present at one time or another which were never invaded by lymphatics.

These observations showed definitely that although perivascular spaces frequently provide convenient pathways for the growth of regenerating lymphatic capillaries they play no essential part in their formation. The opportunity to study regenerating lymphatics in the living mammal in a transparent region in which the finest microscopic details can be seen has enabled us, we hope, to throw further light on their embryonic growth and to explain their true relationship to 'tissue spaces' and to 'extra-intimal spaces.'

Although the lymphatic endothelium, after its primary differentiation, remains specific both in its extension in the embryo and in its regeneration in the adult mammal, the pattern which the lymphatic capillaries finally assume in different regions produces interesting physiological relationships. The frequency with which lymphatic vessels accompany the larger blood vessels in the adult human body is well known. Henry ('33) in his studies of the intact ear of the rabbit showed that not only do most of the larger lymphatics follow the course of veins and arteries, but that many of the lymphatic capillaries of the skin are located in close proximity to veins and capillaries. When the two vessels are so close together as to be practically in contact, with no intervening tissue, the fluid which enters the lymphatic in such a region, whatever its chemical composition in comparison with the blood plasma, undoubtedly represents in part at least a direct 'leakage' from blood vessel to lymphatic rather than a drainage of lymph which has first 'bathed the tissue cells.' The direct passage of fluid from blood vessel to lymphatic was demonstrated in the methylene blue experiment described on page 267 of this paper.

This direct 'leakage' of fluid from blood vessel to lymphatic brings up interesting possibilities for lymphatic behavior in various parts of the body. In the villus of the intestine, the central position of the lymphatic at a distance from the peripherally arranged blood capillaries resembles the arrangement of lymphatics in the tadpole's tail. In the liver, on

the other hand, the lymphatics are located in the interlobular tissue, in close proximity to the portal blood vessels, and separated from the liver cells by the perilobular connective tissue capsule. No lymphatic capillaries have ever been demonstrated which penetrated the liver lobules. Moreover, the vessels in the liver appear to be unusually permeable, since particulate matter injected into the hepatic artery or portal vein appears quickly in the lymphatics of this organ (Mall, '06). Hence, it is probable that much of the fluid in the lymphatics draining the liver—which is generally considered to constitute a large proportion of the lymph entering the thoracic duct—represents a direct 'leakage' from the perilobular portal venules to the accompanying lymphatics and that it has not been in contact with the cells of the liver lobule.

In addition to the direct leakage of fluid from blood vessel to lymphatic made possible by the close proximity of the two thin-walled vessels, the passage of blood cells from vein to accompanying lymphatic is facilitated by such a relationship. Following slight mechanical pressure over vessels so located, the forcing of blood cells into a lymphatic located beside a vein has been noted repeatedly. Changes in consistency of the blood vascular endothelium which occur in response to chemical, thermal and mechanical stimuli (Clark and Clark, '35) have also been seen to result in the passage of formed elements of the blood into lymphatics in the absence of external pressure. Thus with a moderate degree of endothelial softening unaccompanied by visible injury, leukocytes in considerable numbers have frequently been seen to make their way through the wall of a vein or capillary into an adjacent lymphatic (Clark, Clark and Rex, '36), passing through the two endothelial layers in succession, while with a greater degree of change in the vascular endothelium involving loss of elasticity, hemorrhages from vein to lymphatic have been observed. These direct observations on the passage of blood cells from blood vessel to lymphatic at points in which the two vessels lie close together, in addition to the observation reported previously on the entrance of blood cells into lymphatic capillaries which have been broken open either in the

region of inflammatory exudates or during a surgical operation (Clark and Clark, '33), have demonstrated two ways in which some at least of the erythrocytes, which are always found in samples of lymph taken from thoracic duct, may have entered the lymphatic system. The property of actively picking up extruded erythrocytes, displayed so commonly by the lymphatic capillaries of Amphibia (Clark, '09; Clark and Clark, '26) appears to be absent in those of the mammal as judged by the behavior of the lymphatics in the rabbit's ear.

The recent studies of Drinker and his associates ('35) are of interest in this connection. The authors found that pneumococci injected intravenously appeared in the thoracic duct within 1 to 20 hours, depending on the amount injected, and that the bacteria increased in the lymph and continued to be fed into the blood stream during succeeding days. It is probable that the bacteria entered the lymphatics from the blood stream following an endothelial change in the manner observed for blood cells.

The fact that individual red blood cells and clumps of cells which had entered a lymphatic from an adjacent blood vessel were frequently seen to adhere to the wall of the lymphatic at certain points and not at others and on some days and not on others, seems to indicate that lymphatic as well as blood vessel endothelium may undergo changes in consistency. In the case of blood vessels, however, the first change in endothelial consistency is characterized by a tendency of leukocytes to stick to the vessel walls, while erythrocytes rarely if ever adhere to the lining of the blood vessel in any of its phases, although occasionally erythrocytes have been observed to be imprisoned in the endothelium following a change to a pronouncedly softer consistency followed by abrupt reversal (Clark and Clark, '35). On the other hand, in the lymphatic, sticking of either recently emigrated or changed polymorphs to the endothelium, has not been observed. The character of changes in consistency of lymphatic endothelium should receive further investigation but these observations point to a difference in the physical properties of the endothelium of the two sets of vessels.

Unlike the conditions present in Amphibia in which the lymph flow is greatly aided by contractions of the lymph hearts and in which there is continuous absorption of water through the skin (Clark and Clark, '21; Moore, '15) the flow in mammals, at least from peripheral regions of the animal, is very slow. In the resting limbs of dogs the flow has been shown to be so slight as to be negligible (Starling, 1898). Henry ('33) demonstrated the relatively slight flow of lymph from the vessels of the rabbit's ear in proportion to their surface area. In the thin protected areas in the chambers our observations have shown that the flow in the lymphatic capillaries is very slow and is often absent for long periods. Menkin ('31) has found that the lymph flow from areas of inflammation is less than normal and that it may be blocked completely. Our direct observations on the stagnation of the contents of lymphatic capillaries in areas of inflammation confirm Menkin's work in this respect.

SUMMARY

In this and in previous studies in which the regeneration of lymphatic capillaries into the thin transparent space of 'round table' chambers inserted into the ears of rabbits was observed in the living animal under high microscopic magnifications, it was established that the new lymphatic capillaries grow by a process of sprouting from preexisting lymphatic endothelium and that, throughout their period of regeneration and their subsequent life in the chamber, they form a system of specific vessels, independent of connective tissue cells or spaces and of blood vascular endothelium.

Clear perivascular spaces were observed to surround most of the newly formed blood capillaries, and to persist for many weeks in the central table area of especially protected chambers. Such persistent perivascular spaces occasionally acquired an outer border of longitudinally arranged connective tissue fibers which caused them to resemble definite channels. They could, however, be distinguished microscopically from endothelial-lined lymphatic capillaries ad-

jacent to blood vessels. After the development of dense intervascular connective tissue, loose areas were frequently present around the newly formed arteries and veins. The secondary formation of wide clear 'shrinkage spaces' around arteries and veins, the diameter of which had decreased following a change in circulatory conditions, was observed in one specimen.

Lymphatic capillaries which invaded the table area previous to the formation of dense intervascular tissue sometimes followed the course of blood vessels but frequently grew at random in the gelatinous intervascular substance, while lymphatics which appeared on the table after the formation of intervascular connective tissue advanced in the loose space beside an artery or vein.

Although perivascular spaces afforded convenient pathways for the extension of regenerating lymphatics and helped to preserve their open connections with preformed vessels in rigid chambers, they played no essential part in their formation. Around many blood vessels spaces persisted for months which were never invaded by lymphatics. Moreover, lymphatic capillaries were seen to follow a perivascular space for part of their growth and subsequently to diverge from it and invade the intervascular substance. Again, the growing lymphatic in some cases occupied the entire perivascular space at one side of a blood vessel and in others only a portion of it.

Lymphatics which have followed the course of blood vessels in their growth frequently occupy a position, thereafter, in such close proximity that the walls of the two vessels are practically in contact, with no intervening cells or tissue. In instances in which the blood vessel is a capillary or thin-walled vein, direct leakage of fluid from the blood stream can take place. Such leakage has been demonstrated experimentally.

Following a change in endothelial consistency of such a thin-walled blood vessel, emigration of leukocytes from the blood vessel to the interior of the adjacent lymphatic was observed. After a more pronounced endothelial change direct

hemorrhages from blood vessel to lymphatic were seen. Hemorrhages from blood vessel to lymphatic were also produced experimentally by localized pressure on the cover slip. The endothelium of both blood vessel and lymphatic closed immediately after such a hemorrhage as in no case was there a visible break in the wall of either vessel before or after the emigration of leukocytes or extravasation of erythrocytes.

Following periods of inflammation, veins and capillaries which had undergone a change to a softer endothelial consistency often showed localized widenings which persisted for days or even weeks. Such bulges in the vessel wall were relatively larger and persisted longer in veins which were accompanied by lymphatics and on the side next to a lymphatic than was the case in blood vessels or parts thereof not so situated. At times of active blood flow the bulges in the wall of such a blood vessel indented the wall of the accompanying lymphatic, encroaching on its lumen, while with decreased pressure in the blood vessel the 'aneurysm' was inverted and the wall of the lymphatic then protruded into the vein. The weakening of the endothelium of such a blood vessel was further demonstrated by the ease with which repeated hemorrhages occurred at such a bulging place following stimuli too slight to produce them elsewhere in the observation area. Although the walls of both blood vessel and lymphatic in the region of such bulges became thinner than at other points and were in contact for weeks, they remained distinct and no fistula developed.

Owing to the sluggishness of lymph flow in peripheral lymphatics—a flow which is further retarded in the thin semi-rigid chamber areas—cells which entered a lymphatic from a neighboring blood vessel frequently remained for hours or days within its lumen before moving on. During periods of inflammation the movement in the lymphatics was still slower and stagnation of the vessel contents usually persisted for days. In blocked or severed lymphatics the imprisoned blood cells remained in the lumen for several months.

Erythrocytes in the lumen of lymphatics showed agglutination on some days and at times they were observed to adhere temporarily to the lymphatic endothelium. In cases of massive hemorrhage into a lymphatic capillary, squeezing of some of the blood cells through the wall of the lymphatic into the tissue and at times the forcing of erythrocytes back from the lymphatic into the adjacent vein were observed. Phagocytosis of degenerated polymorphonuclear leukocytes and of erythrocytes by macrophages, occasionally present inside the lumen of lymphatic capillaries, was observed.

Passage of leukocytes and erythrocytes from blood vessel to lymphatic was not seen in cases in which the lymphatics followed the course of the thick-walled arteries and arterioles. In those instances in which newly formed arteries had acquired active contractility, an accompanying lymphatic was compressed when the artery dilated and expanded when the artery contracted.

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