OBSERVATIONS ON THE NEW GROWTH OF LYMPHATIC VESSELS AS SEEN IN TRANSPARENT CHAMBERS INTRODUCED INTO THE RABBIT'S EAR[1]

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SIXTEEN FIGURES

In spite of the vast amount of work which has been done during the past thirty years on the development of the lymphatic system, there are still many gaps in our knowledge of it. Previous to 1900, no conclusive studies had been made regarding the question, although several hypotheses had been proposed, which may be classified in two general groups. According to one view, lymphatic endothelium was thought to be a derivative of blood-vascular endothelium, while according to the second and more widely held view lymphatic endothelium differentiated from mesenchyme cells and acquired connections with the vascular system secondarily.

The growth of the differentiated system was generally considered to take place by the continued addition of mesenchyme cells, although Ranvier ('97) pictured lymphatic endothelium as invading the body by a process of out sprouting, as the roots of a tree extend into the ground, while S. Mayer ('85) believed he had evidence for the continued transformation into lymphatic vessels of blood vessels cut off from the remainder of the blood-vascular system.

A new era in our knowledge of the lymphatic system was inaugurated in 1901 by F. R. Sabin's epoch-making studies

[1] The work in this laboratory on the development of the method for studying living cells and tissues in the transparent chamber inserted in the rabbit's ear is being aided by a five-year grant from the Rockefeller Foundation.

on the development of lymphatics in pig embryos, carried out under the stimulation of Prof. F. P. Mall. She discovered that it was possible to inject lymphatics in the embryo, especially those of the skin, and her injections showed gradually extending plexuses which started first at the base of the neck, where they were connected with the venous system at the junction of the anterior and posterior cardinal veins, and a little later in the inguinal region, where they were temporarily connected with the iliac veins. From the early connection with the venous system, Sabin concluded that lymphatic endothelium arises by a process of sprouting from the veins, and from the gradual spread of injectible plexuses, she inferred that growth occurred by sprouting. About the same time, MacCallum ('03) published the results of an important series of studies on the relation between the lymphatic capillary, on the one hand, and the subcutaneous tissue spaces and peritoneal cavity, on the other, in which he found the lymphatic to be a closed system, without preformed openings.

Sabin's discovery started an enormous amount of investigation on the mode of differentiation and growth of the lymphatic endothelium. Among these may be mentioned the numerous studies of Huntington and McClure ('10, '14) and their pupils (Miller, '12; West, '15, and others) on the development of the lymphatics in which the method of reconstruction from serial sections was used and from which the authors concluded that the lymphatic system arises from discontinuous mesenchymal anlage which make secondary connections with the veins; and the extensive studies of Hoyer ('10) and his pupils (Baranski, '10; Dziurzynski, '11; Mierzyewski, '11; Polinski, '10; Federowicz, 13, among others) on the comparative embryology of the lymphatic system, in which the injection technique was used and which all pointed to the origin of lymphatics from venous endothelium by a process of sprouting. The authors (Clark and Clark, '20), from a study of the first identifiable lymphatics in chick embryos, using both of the above methods, concluded that it was

impossible to decide with absolute certainty in regard to the exact mode of origin of lymphatic endothelium from fixed material, although the occurrence of more numerous connections between the earliest lymphatic capillaries and the veins —the majority of which are lost in the first thirty hours of development—make it appear more probable that the first lymphatics are derived from the veins.

Although the exact mode of primary differentiation of lymphatic endothelium still remains an unsettled question, the problem of the manner of growth of lymphatics after the original formation is in a more satisfactory state. Fortunately, in a region such as the transparent tail fin of the amphibian larva, it is possible to observe lymphatic capillaries directly in the living animal with highest microscopic magnifications.

One of the authors (Clark, '09, '12, '22), using chloretone anaesthesia and a special micro-aquarium, was able to make daily long-continued high-power microscopic observations of the same growing lymph vessels over a period of weeks and to establish definitely that, in amphibian larvae, the lymphatic capillaries extend by outgrowth from preexisting lymphatic vessels, which invade the tail in the manner suggested by Ranvier ('97), i.e., by the sending out of fine protoplasmic sprouts which become definitely lumen-containing. Nuclear areas in the lymphatic sprouts were seen to be provided by the in-wandering of nuclear areas from the main stem and by mitotic division. In its growth the lymphatic endothelium remains specific, showing no tendency toward anastomosis with blood-vascular endothelium nor to the addition of any form of connective-tissue cells. It was also observed that new lymphatic capillaries extend as a completely closed system, and that the lymphatic endothelium is syncytial in character, since nuclear areas were observed to move past one another in the vessel wall.

Dziurzynski ('11), using the same method, made a study of lymphatics in the regenerating tails of tadpoles and found that here, too, the lymphatics extended by a repetition of the

same process of sprouting from preexisting lymphatic endothelium shown in the original invasion of the tail fin.

Lymphatics which were isolated experimentally from their connections were observed to retain their vitality and power of growth for three weeks and longer and eventually to be reincorporated in the lymphatic system. The regeneration or healing of such severed lymphatics was found to be much slower than in the case of blood capillaries similarly isolated (Clark, '22). It was also possible to carry out studies on the morphological characteristics and reactive powers of the lymphatic capillaries in the tadpole's tail under a number of experimental conditions (Clark and Clark, '17, '20, '23).

Although the mode of growth of lymphatic capillaries was proved in the case of the amphibian larva, it was not clear just how far the results obtained were applicable to the mammal, since no natural transparent region in the mammal is available for such long-continued, high-power microscopic studies in the living animal under normal conditions. While the studies of Coffin ('06), who found newly formed lymphatics in peritoneal adhesions, and of Evans ('08), who demonstrated lymphatic invasion of experimental sarcomata, indicated that new lymphatics may form in the adult, the conflicting studies on regeneration of lymphatic vessels by Meyer ('06), who found none, and Reichert ('26), who described regeneration, prove the meagerness of the knowledge in this field.

It was with a view to the possibility of making observations on living lymphatic capillaries in the mammal, similar to those already carried out in the tadpole's tail, as well as studies on living blood vessels and various other cells and tissues, that the new method of inserting transparent chambers or 'windows' into rabbits' ears was devised.

The first transparent chambers adapted to such studies were inserted by Sandison ('28) in this laboratory. He was able to make microscopic studies on the same growing blood vessels over a period of four months, and to observe living blood cells both in the circulation and in the outside tissue (Sandison, '28, '31).

Within the past three years a number of workers in this laboratory have collaborated in order to obtain standard chambers, adapted for various kinds of cytological, physiological, and experimental studies, which would remain permanently in the ear and in which an observation space of known and controllable thickness could be retained. Four varieties of successful chamber have been developed, a sufficient number of which have been inserted in rabbits' ears to be certain of their meeting the various requirements (E. R. Clark, Kirby-Smith, Rex, and Williams, '30). Of these four kinds the 'round table' chamber is in most ways the best adapted for the study of new-growing vessels and tissues. The distinctive feature of this chamber is the round central observation table (0.6 mm. in diameter) made of clear celluloid, which is fitted into a hole cut through the ear at the time of operation and across which the new tissue grows. The thickness of the new tissue is regulated by the thickness of four small celloidin buffers, glued to the table at spaced intervals, since the top of the chamber, composed of clear mica through which the observations are made, is screwed down at the time of operation until it comes in contact with these buffers.

A general description of the growth of new mammalian blood vessels as observed in over sixty of the standard 'round table' chambers has been published (Clark, Hitschler, Kirby-Smith, Rex, and Smith, '31). It was possible to make continuous microscopic observations of the living growing blood vessels, together with simultaneous observations of the circulation, in chambers of uniform shape and dimensions and with known and controlled thickness, and to follow the subsequent changes in the vascular pattern over periods of many months—for more than a year in several instances. The growth of new blood vessels, as extensions from preformed vessels, across the observation table and the subsequent remodeling of the vascular network took place in such a definite and regular manner under the relatively uniform conditions present in the standard chambers as to make it possible to

prophesy the general course of events in any new chamber which is inserted.

Lymphatic capillaries were first identified with certainty in February, 1930, in a chamber of the 'round table' variety in which observations were being made upon growing blood vessels. It was then evident that, for the first time, a region was available of sufficient transparency to make possible long-continued observations, with high microscopic magnifications, of the growth, morphological character, and reactive powers of mammalian lymphatic vessels, in the living animal. At the same time it was clear that the previously unsettled question of the capacity for regeneration of the adult mammalian lymphatic system had been definitely answered in the affirmative.

Lymphatic vessels have been seen in the majority of the chambers containing new growing tissue which have been inserted since that time. In eight of these chambers intensive high-power studies of the growing lymphatic capillaries have been carried out. Continuous daily records of the same growing vessels were made with the Leitz drawing eye-piece, similar to those made in former investigations of the living growing lymphatics of amphibian larvae (Clark, E. R., '09, '12), and for many of these the oil-immersion lens was used. In addition, both low- and high-power photomicrographs were taken. For the latter it proved possible, with the Leitz 'Mifilmca' camera and Eastman 'Superpanchromatic' film, to obtain oil-immersion snapshots (1/10″ to 1/50″) of living lymphatic and blood capillaries (figs. 13 to 16). Furthermore, through the kind cooperation and expert assistance of Dr. E. A. Swenson, high-power cinematographic records of lymphatics and blood vessels have been obtained. A preliminary account of observations on living lymphatics was presented at the meetings of the American Association of Anatomists at Chicago, April, 1931 (Anat. Rec., vol. 48, p. 13).

A description of the construction of the 'round table' chamber and of the operation for its insertion in the rabbit's ear has been given (Clark et al., '30, p. 196). The thickness

of the space over the observation table which was invaded by the growing blood vessels and lymphatics was left at approximately 40 μ to 75 μ during the period of intensive

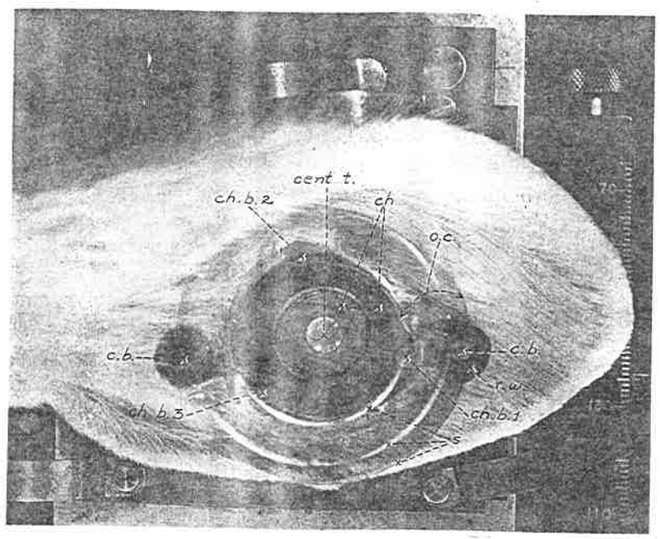


Fig. 1 Photograph of 'round table' chamber with a detached outer collar (*o.c.*) which has been in ear for three months. The outer collar is carried on two individual bolts (*c.b.*) which pass through the ear at a distance from the chamber (*ch*), thus serving as a splint for the chamber. A 'pie-plate'-shaped shield (*s*) of thin (0.24-mm.) Kodaloid is bolted over the top when the chamber is not under observation. The chamber is thus both immobilized and protected from insults. *r.w.*, rubber washer; *ch.b. 1, 2,* and *3*, bolts of chamber proper; *cent.t.*, central table of chamber, in which new tissue has grown. Natural size. We are indebted to Mr. and Mrs. B. B. Varian for the photograph and the photomicrographs.

study in which the records reported here were made. Since the mica covers were only 60 μ in thickness, the whole preparation, including both the new tissue and coverslip, was in most cases thinner than the ordinary no. 1 coverslip. Figure 1 is a photograph, natural size, of a 'round table' chamber

with the detached protective collars (see p. 61), which had been in position for three months, and in which studies of growing lymphatics were carried out.

The living mammalian lymphatic capillaries, as seen with high magnifications in these transparent chambers in rabbits' ears, appear as clear vessels with walls of delicate endothelium in which the nuclear thickenings stand out distinctly as clear lens-shaped structures, or, frequently, as large rounded swellings which bulge into the lumen. The outline of the living mammalian lymphatic is slightly more irregular than that of the blood capillary, but this difference is not nearly so marked as in the tadpole's tail, where the living lymphatic capillaries are continually sending out and withdrawing numerous fine-pointed projections, so that the contour of the wall is constantly changing. The lumen of the lymphatic vessels as seen in these chambers varies in diameter from that of the smallest blood capillaries to that of the larger venules. The thin wall of simple endothelium is characteristic of both narrow and wide lymphatics. Although the lymphatic capillaries frequently lie very close to blood vessels, they never anastomose with them. The fluid content of the lymphatics is clear and colorless, except for occasional blood cells of different types. Such cells are very sparsely distributed, as a rule, although under circumstances, which will be described in a later article, their number may increase tremendously. Figure 9 shows variation in cellular content of the same lymphatic capillary on different days. The characteristic movement of fluid inside the lymphatic capillary of the chamber area—a movement which can be studied directly by means of occasional blood cells which they contain—was seen in most cases to be a bobbing back and forth, synchronous with the heart beat or respiration, with only a sluggish, jerky, forward progression. Cinematograph records were obtained which showed this characteristic back-and-forth movement in the lymphatics, simultaneously with the different types of blood flow in arterioles, veins, and capillaries present in the chamber area.

In the first chambers in which we observed vessels possessing the characteristics just mentioned we followed them very carefully, by means of the oil-immersion lens, and demonstrated clearly their continuity with similar vessels in the preformed tissue outside the table area, and, in addition, we identified the same vessels day after day and made continuous observations of many hours' duration, with simultaneous records of all the neighboring blood vessels, in order to be positive that we were dealing with true lymphatics. This preliminary study, combined with experience from former observations of living lymphatics in amphibian larvae and in chick embryos, now makes it possible for us to distinguish mammalian lymphatic capillaries from blood capillaries with ease, even in the earliest stages of their growth, provided, of course, that the tissue is sufficiently thin and transparent to permit of the use of high microscopic magnifications.

When the growth of individual lymphatic capillaries was studied intensively in the transparent chambers of this type, it was clear that they, like the growing blood capillaries, invaded the area of the central observation table as extensions from vessels already present in the surrounding preformed tissue. However, the appearance of new lymphatic capillaries in the observation area was always secondary to that of the blood capillaries. For example, in a series of eight chambers in which the growth of lymphatics was studied with especial care, it was found that the new vessels invaded the table area on an average of nineteen days after the operation with a time interval varying from ten days to forty days after the operation. In the case of at least two chambers, no lymphatics were seen in the thin space over the table. On the other hand, in the case of the blood vessels, as reported (Clark et al., '31), the time interval for invasion of the table area by new-growing blood capillaries varied from five to ten days after the operation, with an average of seven. In one case in the series, in which lymphatics made their earliest appearance at the periphery of the table ten days affer the operation, the new blood-vessel plexus had extended a third

of the distance toward the center of the table, having first appeared on the edge of the table at slightly under five days after the operation. In a case in which new lymphatics did not grow onto the central table until forty days after the operation, the blood capillaries had started to advance onto the central table at five days after the operation and vascularization of the table area had been complete for twenty-four days before the first appearance of a new lymphatic capillary at the table edge.

We found that the distribution and pattern of the new lymphatics of a given table area usually varied with differences in the relative time at which they invaded the area. For instance, in cases in which lymphatics grew into the observation space at the same time as or soon after blood vessels and fibroblasts, they showed a tendency to invade the table at several points around its periphery, to grow more or less at random in the interstices of the blood-vessel network, and to form a wide-meshed plexus. On the other hand, in cases in which the lymphatic growth lagged conspicuously behind that of the blood capillaries, the new lymphatics showed a tendency to grow close to one or more of the larger blood vessels.

Figure 2 is a photograph of the first type of growth and shows the clear lymphatics forming a wide-meshed plexus. In this chamber lymphatics appeared on the table a week later than the first blood capillaries and for part of the time advanced simultaneously with the latter across the thin space over the table, while at the time the photograph was taken a few of the new lymphatics were invading the area of the central blood clot side by side with the growing zone of blood capillaries. In contrast to this is the condition shown in figure 3, in which the lymphatic hugs the largest artery of the chamber for most of its extent. In this latter case no lymphatics appeared in the observation area until well over a month after the operation and until three weeks after the new blood-vessel plexus had completely covered the table. At that time a single lymphatic capillary invaded the central

Fig. 2 Photomicrograph of table area, twenty-five days after insertion of chamber in rabbit's ear, showing plexus of lymphatics characteristic of early invasion (text, p. 58). Blood vessels started to invade the table from periphery eight days after operation. Vascularization of this area complete at twenty-eight days. Lymphatics grew in fourteen days after operation. *Lym.*, lymphatic; *A.*, artery; *V.*, vein. × 13.

Fig. 3 Photomicrograph of table area of a chamber in ear for fifty days, showing lymphatic growing close to larger blood vessel. This mode of lymphatic growth is characteristic of late invasion in chambers in which dense connective tissue has formed between the blood vessels (text, p. 58). Blood vessels started to invade table from periphery at six days and had anastomosed across the center at sixteen days after the operation. A single lymphatic vessel (*L1*) grew onto the table at thirty-five days after the operation. It advanced in the loose space next the largest arteriole and sent out branches which grew beside two of the larger venules. *L.*, lymphatic next artery; *A.*, artery; *V.*, veins. × 13.

area, as an outgrowth from a preformed lymphatic, and advanced alongside the largest artery of the chamber. All the lymphatics present in the observation area of this chamber were outgrowths from this one lymphatic vessel (fig. 3, *L1*).

We made the observation that in completely vascularized 'round table' chambers (a month after operation and later), the tissue just outside the larger of the newly developed arteries and veins showed a tendency to be looser than the rest of the connective tissue. It was this loose space around the larger vessels which apparently offered such a favorable path for the extension of those lymphatics which invaded the chamber at a comparatively late period.

This observation led us to study more carefully the character of the connective tissue at different stages of invasion, and it soon became evident that the greater richness of the lymphatics, in the case of earlier invasion, is associated with the greater looseness of the connective tissue present in the younger chambers. In the first few weeks after the insertion of the chambers, the interstices of the growing blood-capillary plexus are filled by fluid, or by a semigelatinous intercellular substance, in which the advancing line of fibroblasts, macrophages, and other wandering cells are somewhat separated from one another. On the other hand, in the older chambers, in which the new growth has completely covered the space over the 'table,' the connective tissue between the blood vessels becomes much denser and richer in fibers which are packed closely together, with the exception of the loose space around the larger blood vessels. We have observed repeatedly that the advance of lymphatics is interfered with or even blocked completely by the presence of such dense connective-tissue fibers across its line of growth (p. 64).

The growth of new connective tissue and the character of the intercellular substance are problems which are being studied at present and which will be reported at another time. It is of interest, however, to mention briefly that in thirty 'round table' chambers inserted during the past year, in which a modification of the outer collars has been used, the

character of the connective-tissue growth has been modified, and that this in turn has had an interesting effect on the growth of lymphatic vessels. In these modified chambers, the protective outer collars of the standard 'round table' chamber were left completely detached from the chamber proper and bolted together through two or three extra holes which were punched through the ear a week before the operation and allowed to heal (fig. 1). This modification was introduced in order to serve as a splint to protect the chamber from undue strain and to prevent the gradual migration of the chamber toward the tip of the ear (Clark and Clark, '32, p. 446). In these improved chambers the ingrowth of blood vessels took place at the same time and in the same manner as in the 'round table' chambers with collars attached (Clark et al., '31), but, as a result of the added protection afforded by the detached 'splints,' there was much less extravasation of blood cells from the ends of the delicate new-forming blood capillaries, thus resulting in a clearer picture in the earlier stages, and also the pattern of the blood-vessel network of later stages was more stable. In addition to these hoped-for results, we found that in these chambers, which are more protected from strain, the ingrowth of fibroblasts and the formation of connective-tissue fibers was conspicuously retarded, so that new blood capillaries frequently grew directly out into a space occupied solely by serum, fibrin threads, and extravasated blood cells.

When new lymphatic vessels invaded such chambers early —i.e., in the first week or two after the first appearance of the blood capillaries—they formed the typical wide-meshed plexus already described (p. 58). On the other hand, in two recent cases of chambers with detached collars, in which the connective-tissue growth was retarded, we observed that lymphatic vessels, which entered the chamber relatively late (in one case nineteen days and in the other over a month after the operation), advanced along the space next the larger blood vessels only in those parts of the table area in which the typical densely packed intervascular connective-tissue

fibers had developed. The lymphatics which grew into a region in which the connective tissue was still primitive showed the plexus formation typical of 'younger' stages. Thus the

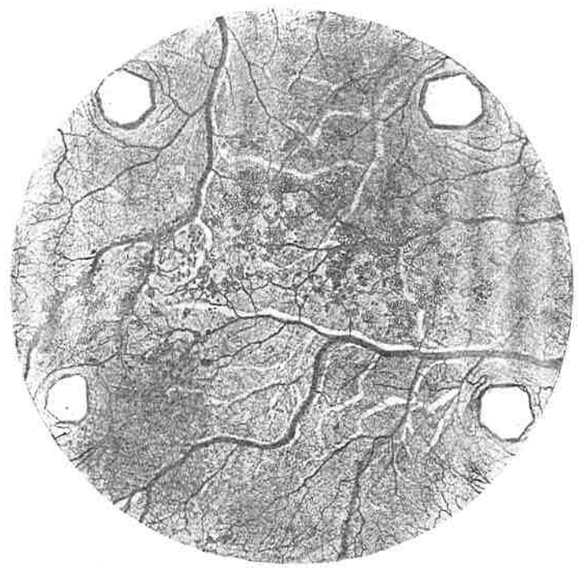


Fig. 4 Photomicrograph of table area of chamber in ear for sixty-seven days, showing mixed pattern characteristic of late lymphatic invasion in a 'splinted' chamber in which connective-tissue differentiation was delayed (text, p. 61). Blood vessels first started onto table at five days after the operation and the table area was completely vascularized at nineteen days. The first lymphatics grew in at thirty-seven days after the operation. They followed along an arteriole and two venules for the greater part of their advance in the peripheral portion of the table, where dense connective tissue had already formed. In the central region, in which connective-tissue differentiation was delayed, they grew out as a plexus. Groups of developing fat-cells show in this chamber. × 16.

two types of growth may be present in the same chamber as shown in figures 4 and 5. In one of these cases, a lymphatic extended in the looser area next a large vein for two-thirds of the distance toward the center of the table and then

branched out and grew more wildly, forming an anastomosing plexus. In this case we noted that the connective-tissue fibers were well formed in a portion of the peripheral region of the

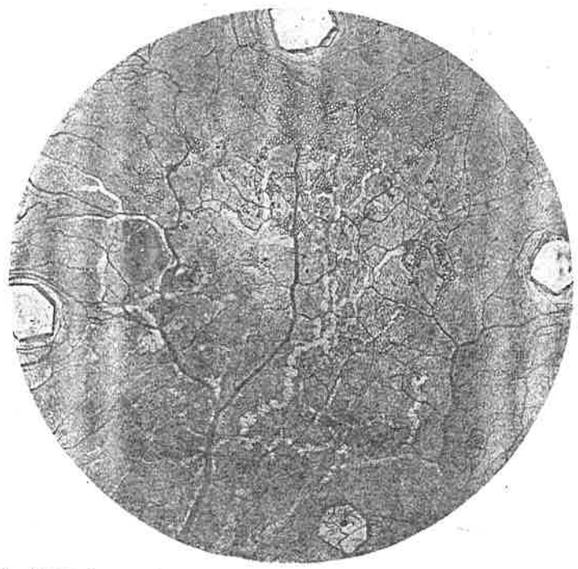


Fig. 5 Photomicrograph of table area, seventy-four days after operation, showing rich lymphatic plexus in a 'splinted' chamber in which connective-tissue development, particularly in the central region, had been greatly delayed. Blood vessels first invaded table six days after operation and anastomosed across the center at twenty-four days. Lymphatics first started to grow in at nineteen days. They grew along a larger vein in one part of the periphery, in which connective-tissue cells were more abundant. Elsewhere they grew at random. Connective-tissue growth was greatly delayed in central area in this specimen, and here the lymphatics formed the richest plexus. Sending out of new lymphatic sprouts in this central area continued for several months. Developing fat shows in upper part of chamber. The dark area at left center is a piece of débris accidentally included at the time of operation. × 16.

table at the time of lymphatic ingrowth, while the primitive connective tissue, with widely separated cells, was still present in the central area and persisted until well after the lymphatic had penetrated it.

These observations appear to demonstrate clearly that the two patterns displayed by the newly formed lymphatics—that of the wide-meshed plexus and of the single lymphatic vessel just adjacent to a larger blood vessel—are associated with the comparative looseness and density of the connective tissue into which the lymphatics grow. They also show that lymphatic extension is more easily interfered with by the greater density of the surrounding medium than is that of the growing blood capillaries. The experiments of Reichert ('26) are of interest in this connection, since he found that the regeneration of lymphatics was delayed or hindered in the presence of a large amount of scar tissue.

In the case of most of the chambers in which a uniform thickness of 40 to 75 μ over the table area was retained throughout the period of invasion, the growth of lymphatics, like that of the blood vessels already described (Clark et al., '31, pp. 142–143), was essentially a growth in one layer, or what might be termed a growth in two dimensions. In chambers in which the space between the mica coverslip and the small buffers on the table was secondarily increased, growth of lymphatics in several planes occurred, somewhat like that described for the blood vessels (Clark et al., '31, p. 143), resulting in a richer plexus which wound in and out in the meshes of the blood-vessel network, sometimes crossing superficial to the blood vessels and sometimes beneath them (fig. 11).

Although the two sets of vessels—blood vessels and lymphatics—may weave in and out in this fashion or run side by side, they invariably remain separate. Just as was the case in the tadpole's tail and in the subcutaneous tissue of chick embryos, the growing blood vessels anastomose freely with other blood vessels, while lymphatics form occasional anastomoses with neighboring lymphatics, but a blood capillary has never been seen to connect with a lymphatic in the region of observation.

In a number of the transparent chambers of the 'round table' type inserted in rabbits' ears, the new tissue was suffi-

ciently thin to make it possible not only to identify and observe new lymphatics as well as blood capillaries in the living mammal and to watch their extension, but to watch the finest microscopic details of their growth, including the growing ends and the endothelial walls with their nuclei. Thus, in a number of specimens it has been possible to make daily camera-lucida records with the oil-immersion lens of the same growing lymphatic vessels and of the neighboring blood vessels over a period of weeks. In short, we have been able to carry out long-continued microscopic observations on mammalian lymphatic capillaries similar to the studies made on the vessels of the tadpole's tail (E. R. Clark, '09, '12).

Figure 6 shows a low-power photomicrograph of the whole table area of a chamber in which such observations were made. Figure 7 shows a portion of the same chamber at a higher magnification. This particular chamber was one in which a very thin space was retained at the left of the central table, thereby forcing the growing vessels to invade the chamber from one side only. (The new growth of blood vessels in this chamber has already been described (Clark et al., '31, pl. 8.)) The lymphatics were late in invading this chamber, a single lymphatic appearing at the edge of the table thirty days after the operation, at a time when blood vessels had extended well beyond the middle of the table. During the succeeding days, new lymphatics invaded the table area at seven points and, in a manner characteristic of such cases of late invasion with much differentiated connective tissue filling the space between the blood vessels, they each advanced in close proximity to one of the larger blood vessels. The ingrowth of four of these separate lymphatic vessels (*L1, L2, L3, and L4*) was watched intensively and daily camera-lucida records of them were made with the oil-immersion lens.

Figure 8 was selected from such a series of daily records of one of these growing lymphatic capillaries (*L2*). The same region was located each day and a careful camera-lucida oil-immersion sketch made of the lymphatic under observation and of several neighboring blood vessels. When several of

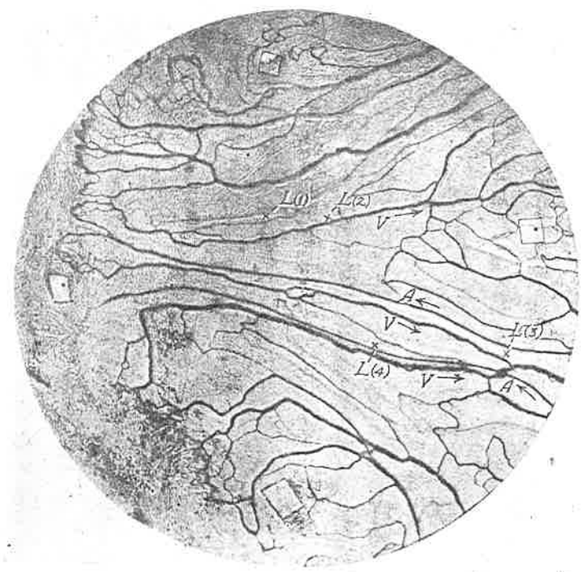


Fig. 6 Photomicrograph of chamber, sixty-six days after operation. In this chamber, space at left of table was very thin and growth occurred from right side only. Shows several lymphatic vessels which have grown well across table, among them four (*L1*, *L2*, *L3*, *L4*) which were studied intensively and whose growth was recorded in daily observations. *L2*, lymphatic from which records, shown in figures 8 and 10, were made. *L1*, lymphatic shown in figure 9. *L3*, lymphatic shown in figure 12. *A.*, artery; *V.*, vein. × 16.

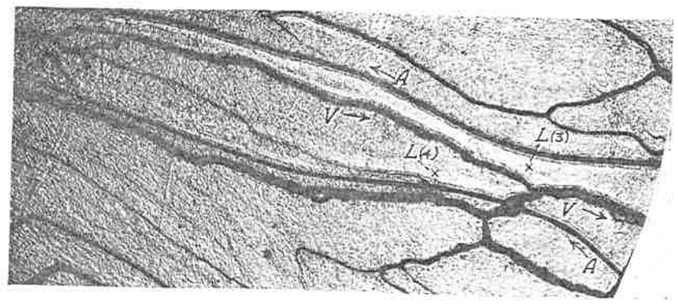


Fig. 7 Photomicrograph of part of same chamber and taken on same day as figure 6. Higher power. *L3* and *L4*, same lymphatics designated in figure 6. *L3* marked at place at which drawing for figure 12 was made. × 64.

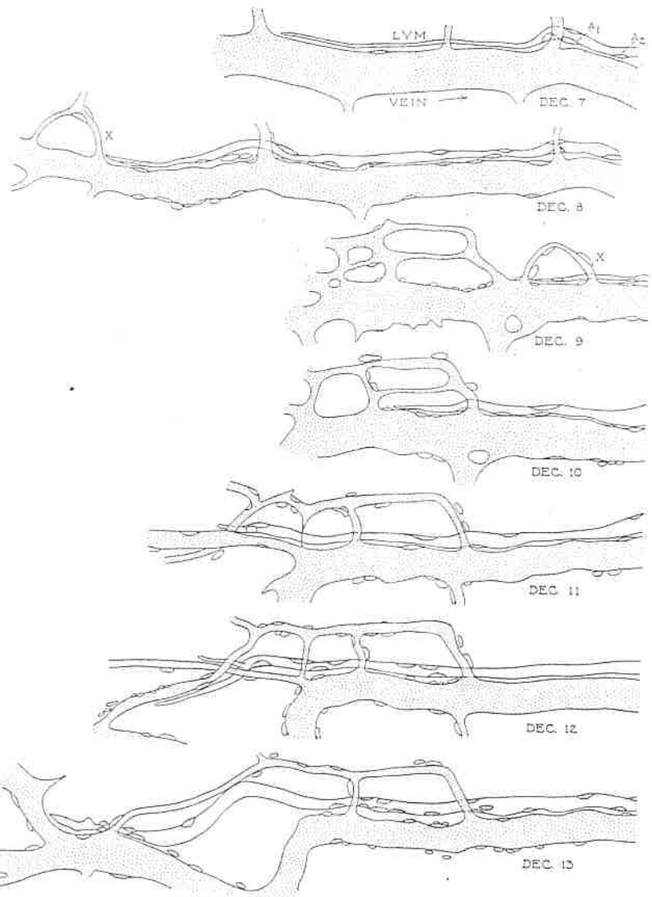


Fig. 8 Series of camera-lucida, oil-immersion records, showing growth of an individual lymphatic capillary, *Lym.* This is one of the lymphatics (*L2*) shown in the photograph in figure 6. Corresponding parts of the lymphatic and vein are placed below one another in drawings of December 7th and 8th, and in drawings of December 9th to 13th. In drawings of December 9th to 13th they have been moved to the right, so that X represents the same spot on December 8th and 9th. During the growth of the lymphatic there was considerable change in the blood vessels, conspicuously in the retraction and disappearance of certain branches and loops. The retraction of a short lymphatic tip is shown between December 11th and 13th. Lymphatic endothelial nuclei stippled. *A1, A2*, the two daughter nuclei shown after mitotic division in figure 10. × 177. (Magnification of original drawing, × 700). The drawings, figures 8 to 12, were made by Miss M. B. Chambers.

such daily records were compared and the relation of the lymphatic tip to the same region in the field—for example, to the particular point of the nearest blood vessel, marked X (December 8th and December 9th) in the series shown here—noted on successive records, the growth of lymphatics by endothelial sprouting showed up in a clear and striking manner.

Throughout the course of this intensive study it was easy to distinguish the endothelial wall of the growing lymphatic from that of the neighboring blood vessels, even when the two vessels lay side by side with practically no intervening tissue, as in the case shown in the photomicrographs, (figures 14 and 16, and to be sure that there was no connection between them. It was also possible in suitably thin preparations of this kind to distinguish the endothelial wall and the growing tip from the surrounding connective-tissue cells and fibers.

When the same growing lymphatic capillary was watched carefully and continuously with the oil-immersion lens, a short, solid protoplasmic thread was seen to extend from the end of the lymphatic under observation, which in the course of hours increased in length and into which a lumen, continuous with that already present, gradually extended and enlarged.

As the new sprout advanced in this manner, nuclear areas eventually moved along the endothelial wall into the newly formed portion. Throughout these observations of growing lymphatic sprouts we were able to see and to keep track of individual endothelial nuclei, and to see that, as was the case in the vessels of the tadpole's tail, the nuclei were not fixed in position, but changed their location in the wall from day to day.

On several occasions during this intensive study of individual growing lymphatic capillaries we observed the mitotic division of a lymphatic nucleus and were able to record the different phases in the process, together with the times at which they occurred. Figure 10 shows such a record of mitosis in a lymphatic endothelial cell (*A*.). The two daugh-

ter cells of the nucleus in which this particular division was observed are *A1* and *A2* on the record made December 7th, figure 8.

As the figures show, the endothelial nucleus which was about to undergo division became swollen. It remained for a half-hour or longer in this prophase, during which it became more granular, and the spireme and later the chromosomes themselves became visible. The separation of the chromosomes was seen to take place rapidly while the nucleus was still swollen. Following this the cutting in of the protoplasm took place, which also occurred quickly, consuming only two or three minutes. A pause of several minutes then intervened, during which the two parts of the cell appeared to flow together slightly, and then occurred the final stage in which the two daughter nuclei elongated, separated, and moved actively away from each other. After division and separation, the daughter nuclei (*A1* and *A2*) remained attached to and a part of the vessel wall.

It was interesting to compare the records of such a mitotic division of the endothelial nucleus of a mammalian lymphatic, as observed in the living animal, with that described by one of the authors for similar nuclear divisions in the amphibian (E. R. Clark, '12, fig. 7, p. 377). It was found that the process was identical even to the relative times consumed in the different phases of the process.

The microscopic details of outgrowth of lymphatics were studied in this intensive fashion in the case of three other capillaries in this same chamber and similar observations were repeated on individual growing lymphatic vessels present in seven other chambers.

At times a growing lymphatic tip was observed to send out more than one of the solid terminal processes, thus forming a fork, which then proceeded to enlarge and extend as two or more branches from the original trunk (fig. 9, *A* and *B*). Occasionally, a new branch of this kind, after persisting for a few days, was eventually seen to retract (fig. 8, December 11th to 13th). However, the formation of branches from

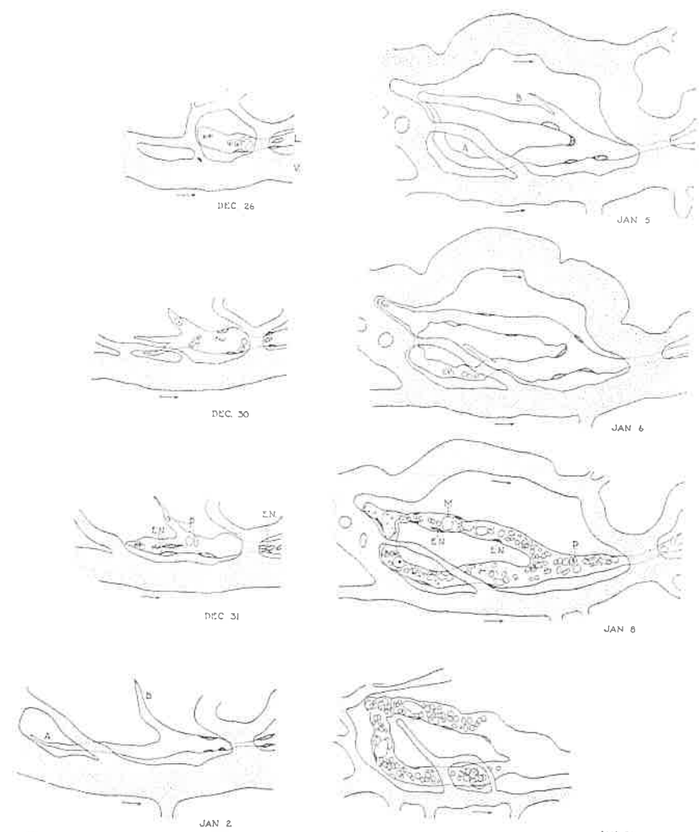


Fig. 9 A series of camera-lucida oil-immersion records of another growing lymphatic, showing branching of the growing tip and later anastomosis of the two branches (A and B). This is the vessel (*L1*) shown in the photograph (fig. 6). Endothelial nuclei, *E.N.*, were best seen in profile, but on certain days several of them could be seen clearly en face. The cell content of the lymphatic lumen varied on different days—the vessel being empty on some days (January 2nd, January 5th) and containing a number of cells on others (January 8th, January 26th). *M*, macrophage in vessel lumen. *P.*, polymorphonuclear leucocyte in vessel lumen. Most of the other cells were leucocytes which had lost their granulation and showed other evidence of degeneration. × 133. Magnification of original drawing, × 700.)

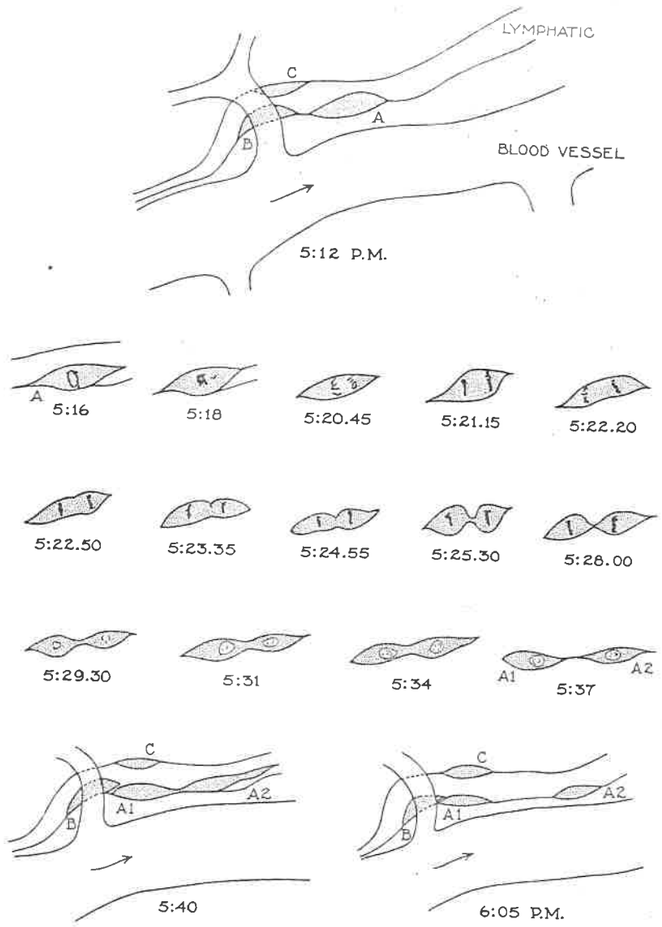


Fig. 10 Series of camera-lucida oil-immersion records of a mitosis in an endothelial nucleus (*A*) of a growing lymphatic capillary. The vessel shown is a portion of *L2* (figs. 6, 7, and 8). The daughter cells *A1* and *A2* are shown in the series of growth records (fig. 8, December 7th). (Compare with record of mitosis in lymphatic endothelial nucleus in tadpole's tail, Clark, '12, Am. J. Anat., vol. 13, p. 377.) × 450. (Magnification of original drawing, × 700.)

the lymphatic trunk, which grew out laterally until they met and anastomosed with similar extensions from a neighboring lymphatic, was observed repeatedly. The process of branch formation and subsequent anastomosis occurred more frequently in chambers in which the new lymphatics had grown in earlier or in regions of chambers in which the growth of connective tissue was retarded. But the sending out of lateral sprouts, which later formed anastomoses, was never so abundant in the case of the lymphatics as in that of the blood vessels.

Although the invasion of the space over the central table by lymphatics is always secondary to that of the blood capillaries and the new-formed lymphatic vessels and their branches are much less numerous, the actual rate of growth 'in a straight line' of the individual lymphatic vessel, whose progress is not impeded by the density of the surrounding connective tissue or by other mechanical causes, apparently does not differ materially from that of the individual blood capillary. We noted that in cases in which there was no obstacle to lymphatic extension, the vessel grew out by this process of sprout formation, accompanied by migration and division of endothelial nuclei, at a rate of 0.22 mm. per diem, which does not differ materially from the average rate in a 'straight line' observed for the advancing network of blood capillaries (Clark et al., '31, p. 138). In several cases in which new lymphatic vessels invaded the central area of the 'table' in advance of the new connective tissue as described on page 60, this daily rate of lymphatic extension was exceeded.

However, the lymphatic was found to be much more susceptible to mechanical interference with its growth than was the blood capillary. For example, the lymphatic capillary whose growth is recorded in figure 9 grew out across the table at a fairly steady rate from December 7th to December 13th, when it came in contact with a blood vessel across its path of advance. This vessel then lost its pointed tip so characteristic of a growing lymphatic, enlarged at the end

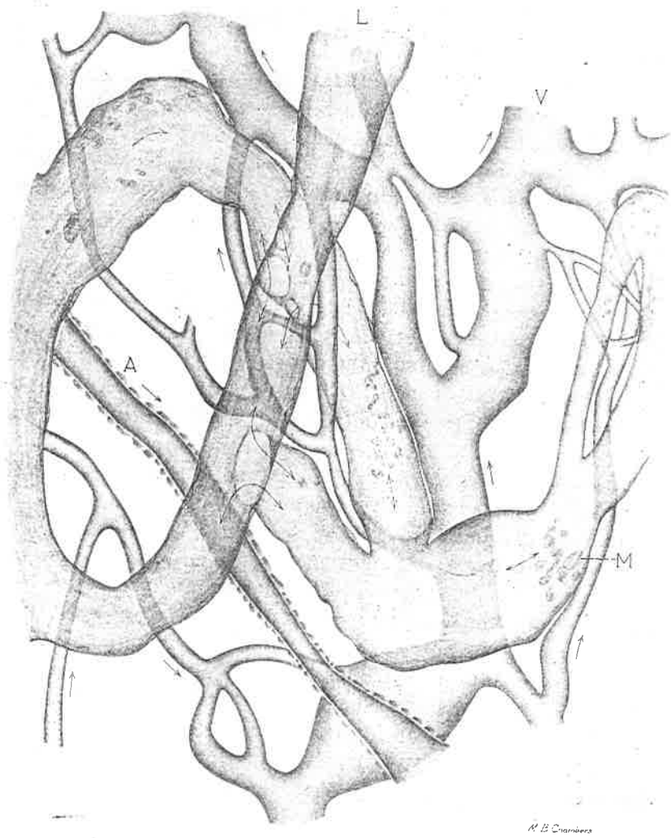


Fig. 11 Camera-lucida drawing of lymphatic and blood-vessel plexuses in a chamber in which the space over the table was secondarily increased to about 100 μ (thickness of space in other chambers shown—figs. 1 to 10—was from 40 μ to 75 μ). Shows two sets of vessels, intertwining but not anastomosing. Drawing made one month after operation and ten days after first ingrowth of lymphatics. *L.*, lymphatic; *A.*, artery; *V.*, vein; *M.*, macrophage. × 260. (Magnification of original drawing, × 700.)

and showed practically no change for several days. Finally, on December 19th, it sent out a solid protoplasmic sprout which curved around in a different direction from the original path of advance, and, following along beside the blood vessel which blocked its growth, it extended in the direction of a neighboring lymphatic. This small lateral sprout remained for weeks, but never formed an open connection with the other vessel. In this instance, the space over the table area was very thin and evidently there was not sufficient space between the obstructing blood vessel and the mica cover to permit the lymphatic to continue its advance. We have already mentioned the fact that growing lymphatic vessels avoid regions of dense connective tissue (p. 64). We repeatedly observed a lymphatic whose growth at the tip was impeded by the presence of a blood vessel across its path or by the thinness of the space, or by an increase in the density of the connective tissue in the region ahead of it, which ceased growing entirely, lost its pointed tip, and acquired a rounded bulbous end which persisted practically unchanged for months.[2]

On the other hand, the growth of blood capillaries was not interfered with so readily. We frequently observed the sending out of new blood-vessel sprouts into regions of comparatively dense connective tissue which the growing lymphatic sprouts were apparently unable to penetrate. And when the advance of new blood capillaries was definitely blocked by a line of epidermis, by one of the celluloid buffers, or by the thinness of the space (Clark et al., '31, pp. 143–145), neighboring blood capillaries, instead of ceasing to grow and acquiring the rounded blind ends displayed by lymphatics in such cases, were observed to send out lateral sprouts, which

[2] The present observations on the ease with which the new growth of mammalian lymphatics may be hindered mechanically afford a possible explanation for the failure of Meyer ('06) to find evidence of lymphatic regeneration in the case of his experiment on the resection of the lymphatic vessels of dogs, referred to on page 52. Quite possibly, a dense growth of connective-tissue fibers may have formed in the path of growth of new lymphatics and prevented their further extension in the manner just described.

united with similar outgrowths from adjacent capillaries and formed terminal loops which later frequently enlarged into a border vein.

That the lymphatic capillary, which ceases to grow and forms the bulbous end just described (p. 74), is still capable of renewed growth in case the mechanical interference is removed, has been shown in cases in which the space over the 'table' has been secondarily increased, either intentionally by loosening the screws which hold the mica cover in contact with the celluloid buffers or as a result of slight permanent bulgings in the cover following intervals of rapid circulation accompanied by increased transudation of fluids. In such cases, as already mentioned (p. 64), a secondary growth of new lymphatic capillaries, in a 'three dimensional' plane, occurred and a new plexus of vessels spread out on the surface immediately beneath the coverslip. Such an increase in the space over the table area also results in a secondary formation of new blood capillary sprouts, which, as was the case in the primary invasion of the table area, takes place more rapidly and is much more abundant than in the case of the lymphatics (Clark et al., '31, p. 147). A similar secondary formation of new blood-vessel sprouts was observed following prolonged periods of rapid circulation, such as that caused by overheating, in which no permanent increase in the space over the table occurred. In such cases, however, lymphatics failed to show a growth response.

Although the initial growth response of the lymphatic capillaries, following the operation for the installation of the transparent chamber, is always slower than that of the blood vessels, the number of new sprouts far less, and the resulting plexus less rich, the new lymphatics once formed are apparently more stable and persistent than are the new blood capillaries.

It was observed, both in the tails of amphibian larvae and in the chambers in the rabbit's ear (Clark, '18; Sandison, '28; Clark et al., '31), that the growth of new blood vessels was characterized by a great overproduction of capillary sprouts,

the formation of numerous anastomoses, and the subsequent remodeling of the original network in response to circulatory factors into an adult pattern, by the transformation of certain of the newly formed capillaries into arterioles and venules and the retraction or disappearance of many others. Our recent studies in two types of standard chamber—i.e., the 'round table' type, in which new vessels are allowed to grow into an artificially made space, and the 'preformed tissue' chamber, in which a window is installed for observation of the original vessels of the ear—have demonstrated the remarkable lability of the mammalian vascular system—its quickness to respond by growth or retraction to relatively minute changes in the environment (Clark et al., '31; Clark and Clark, '32). The present observations show that mammalian lymphatics are more sluggish in their growth reactions, as compared with blood vessels. They send out fewer new sprouts, form fewer anastomoses, and their advance is more easily blocked, but, on the other hand, they show fewer instances of retraction and disappearance.

One of the authors (E. R. Clark, '22) made a study of living blood vessels and lymphatics in the tadpole's tail which were isolated experimentally by making a cut across the tail fin. It was found that the severed blood vessels sent out new sprouts and reunited across the gap within two or three days after making the cut. In another experiment (E. R. Clark, '18) a single blood capillary was completely isolated by cutting through its connections. The isolated capillary sent out a sprout to meet a new sprout from the blood-vessel loop, from which it had been separated, and two days later it had been completely reincorporated in the vascular system. In the case of the experimentally severed lymphatic capillaries, the isolated vessel, or portion of one, was eventually reincorporated into the lymphatic system, but in this instance the process took place much more slowly, consuming twelve days to three weeks. During this period of isolation the cut-off lymphatic retained its vitality and was seen to send out a new sprout toward the approaching lymphatic outgrowth with which it eventually united.

In the process of new growth of mammalian blood vessels, plexus formation and remodeling into an adult pattern, which takes place in the space occupied by the observation table of this type of chamber, we have seen relatively few instances of complete isolation of single blood capillaries. The vascular plexus is so luxuriant and the connections so numerous that vessels which become separated at one point are usually still joined at another. The occurrence of retraction of blood vessels following a period of absent or scanty circulation, in which the capillary narrows down until its lumen becomes solid, and finally breaks, and the two separated endothelial parts of the capillary are withdrawn into the rest of the blood-vascular system, takes place constantly. Nevertheless, completely isolated blood capillaries, or portions thereof, have been seen and their reincorporation, by the sending out of new sprouts from nearby vessels, observed to take place just as it did in the tadpole's tail. In addition, we have observed one instance in which a portion of a vessel became separated from a capillary loop which was itself undergoing retraction, following a day of diminished blood flow through it, and, in this case, the isolated vessel dwindled and finally disappeared.

In the case of the new lymphatics which have invaded the table area, we have noticed several instances in which a vessel became isolated from its connections, when the space at the table edge became restricted due to warping of the kodaloid floors of the chamber or to an increase in the connective-tissue fibers. Then, too, in chambers of the splinted variety described on page 61, in which 'massage' of the area is prevented, lymphatics occasionally became cut off from their connections with the vessels of the preformed tissue. Since the new lymphatic vessels which grow into the space over the table are always separated from each other by relatively wide intervals, such a cut-off vessel is completely isolated. The isolated lymphatics were observed to retain their vitality —as did those of amphibia—and to remain comparatively unchanged for weeks and even months. In one such instance,

a vessel which had grown onto the table seven days previously was observed to become cut off at the table edge. It persisted as a completely isolated vessel, ending blindly at both ends, and with a fluid-containing lumen, for seventy days. During this period of isolation it was seen to extend in a peripheral direction, toward the center of the table, and, by the end of this time, it had formed a new connection with another lymphatic, which had grown out into the central area of the chamber and had eventually sent out a new sprout toward the isolated vessel. In the same chamber, we observed two other lymphatic vessels which had become cut off at the table edge, remained isolated for a period of two months and were then reincorporated in the lymphatic system, by the same method of forming connections peripherally with new lymphatics which had grown into the central area of the table.

Regarding the 'cut-off' lymphatics, it should be emphasized that all the evidence indicates that they are parts of preexisting lymphatics which have grown into the new area by sprouting, and that there is not even a suspicion that they have differentiated in situ, or that they form from 'cut-off' blood-vessel endothelium. In the majority of cases where isolated lymphatics were seen, either the actual blocking and elimination of the lost portion was observed directly, or the continuous lymphatic was first seen, and later a part of it was seen to be disconnected. Isolated portions invariably occurred in parts of the new growth in which there were other lymphatics, while there were many regions which for weeks had no lymphatics whatsoever, and where, in spite of the presence of new-forming, retracting, or retrogressing blood vessels, there was no trace of lymphatic capillary.

We have already mentioned that in these careful high-power studies of uniformly thin and transparent tissue we were able to see the endothelial wall of the growing lymphatic clearly and to distinguish it with certainty from the blood-vessel endothelium and from the surrounding connective cells and fibers. Hence, it has been possible to watch the process

of growth in individual living mammalian lymphatic capillaries and to be positive that the lymphatic endothelium remains specific and does not join with nor add to itself any other kind of cell. In many cases the lymphatic is in very close proximity to blood vessels. For example, as was the case in the vessels photographed in figure 14, the two vessels, a lymphatic and a venule, lay so close together that there was no intervening tissue between the two walls, and yet the two endothelial membranes remained absolutely distinct.

Similarly, the lymphatic in its growth usually makes its way through the midst of innumerable connective-tissue cells and fibers, often coming in such intimate contact with them that only by long-continued high-power studies was it possible to be sure that they remained distinct. However, in the case of chambers recently inserted with the detached splint collars, in which the development of connective tissue was retarded (p. 61), we have observed lymphatic capillaries which have extended beyond the region of connective-tissue fibers into a more primitive area occupied by blood cells, fibrin, and occasional macrophages and fibroblasts, in which the complete independence of the growing lymphatic endothelium from surrounding tissue cells was relatively easy to distinguish.

Figure 12 shows a lymphatic vessel whose growth had been watched for fifteen days in which connective-tissue bands and reticular tissue appear to run directly across its lumen. One method of formation of such strands was observed and found to occur as follows. The growing lymphatic, instead of advancing as a single vessel, may form a fork. The two branches thus formed may anastomose, enclosing a small island or bar in which there may be one or more fibroblasts or connective-tissue fibers. As the lymphatic continues to grow beyond this island, the size of the lymphatic may increase greatly as a result both of the increased transudation of fluid into it, and of the damming back of fluid from outside pressures. The increase in the size of the lymphatic occurs in all dimensions and may encroach on the island until it is

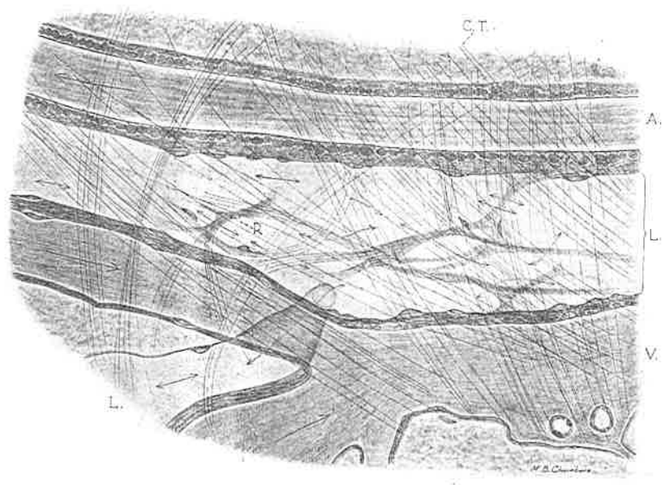


Fig. 12 Camera-lucida drawing, from a series of oil-immersion records of a lymphatic vessel whose growth into this region had been observed fifteen days previously, showing relation between lymphatic and connective tissue. Endothelium-covered reticulum-like fibers (*R*) form strands across the lumen of the lymphatic, and extend out among the fine connective-tissue fibers outside (*C.T.*). *A.*, artery; *V.*, vein. × 280. (Magnification of original drawing, × 700.)

Figs. 13 to 16 Oil-immersion photomicrographs of individual living lymphatic capillaries and blood vessels, which have grown into the table area.

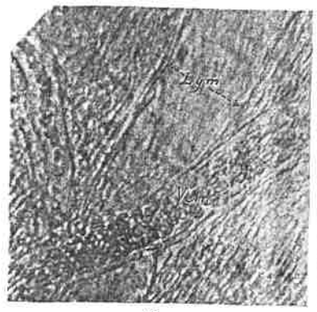
Fig. 13 Oil-immersion snapshot of lymphatic capillary crossing blood vessel (*Vein*). This chamber had been in ear for two months—new lymphatics grew onto table one month before photograph was taken. Slow circulation in vein and capillary—individual erythrocytes show. *Lym.*, lymphatic. × 330.

Fig. 14 Oil-immersion snapshot, taken with Leitz Mifilmca camera (1/10 second exposure), showing lymphatic capillary beside a venule. Chamber in ear two months. Lymphatic grew into this region three weeks previously. *Lym.*, lymphatic; *L.End.N.*, lymphatic endothelial nuclei; *V.End.N.*, venous endothelial nucleus. Arrow shows direction of flow in vein. × 530.

Fig. 15 Oil-immersion snapshot, Leitz Mifilmca (1/10 second exposure), showing branch of lymphatic running beside venule (*Vein*). This chamber in ear for three months, lymphatic present on 'table' for over two months at time of photograph. *Lym.*, lymphatic. × 530.

Fig. 16 Oil-immersion snapshot, Leitz Mifilmca (1/25 second exposure), showing lymphatic beside small vein. Three leucocytes sticking to wall of the venule. Chamber inserted three months and lymphatics grew onto table two and one-half months before this photograph was taken. *End. N.*, endothelial nucleus in wall of lymphatic capilary. × 530.

reduced to a reticulum-like strand. Such strands then remain as bands, covered by endothelium as indicated by the bulging endothelial nuclei, but giving the appearance of piercing and running through the lumen, while actually being outside the endothelium. Often the continuation of the fine fibers of the strands can be seen mingling with other connective-tissue fibers from which they are indistinguishable. The appearance of such reticulum-like strands reminds one of the reticu-



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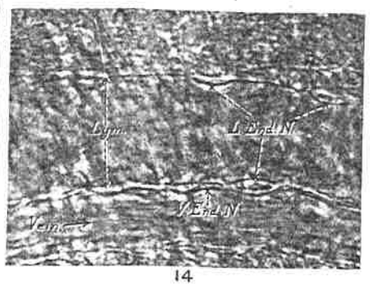

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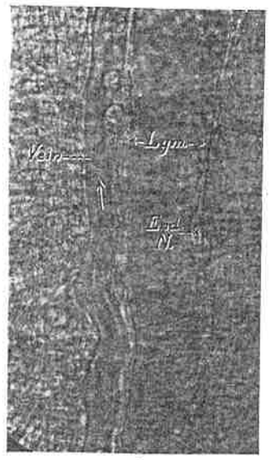

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lar arrangement in the medullary portion of a lymph gland. That the endothelial covering comes from endothelium and the reticulum-like part from fibroblasts we have been able to establish definitely. Again, after a lymphatic has grown out into the space over the table, it frequently increases in diameter, evidently in response to inside pressure, and, as it expands, its wall comes in contact at various points with unyielding strands of surrounding connective-tissue fibers. The lymphatic vessel then bulges on either side of such bands, which consequently take the form of constrictions around the lymphatic.

Throughout these studies—many of them involving continuous intensive observations with the oil-immersion lens of the same growing vessels for many hours daily over a period of months—we saw no instance of an endothelial nucleus becoming detached from the wall of a lymphatic, nor any suggestion of the formation of any form of wandering cell from lymphatic endothelium. Throughout this time the lymphatic endothelium remained completely specific.

Thus, it has been possible by such long-continued observations in the living animal to establish the facts that, in such a region, the mammalian lymphatics possess the capacity for new growth and regeneration, that they grow by the same method of endothelial sprouting accompanied by migration and division of endothelial nuclei, observed for the growing lymphatic vessels of the tadpole's tail, and that throughout their growth they remain specific and independent of surrounding blood vessels and connective-tissue cells. The lymphatics were seen to form as definite a set of vessels as the blood capillaries, with a distinct endothelial membrane which everywhere separated their lumen from the surrounding tissue.[3]

[3] An interesting exception to the well-nigh universal rule of closed lymphatics was encountered on a few occasions—one of them in an actively growing lymphatic—in which artificial openings occurred into lymphatic vessels, under the comparatively rare circumstances in which fluid was present in the outside tissue. Our observations on such accidental tears in the lymphatic endothelium will be reported in detail in a later article in connection with observations which have been made on the relation of living mammalian lymphatics to the outside tissue.

CONCLUSION

By the method of inserting transparent chambers into the ears of rabbits it has been possible to make long-continued observations on living mammalian vessels and other cells and tissues, with highest microscopic magnifications, similar to the studies which have been carried out in natural transparent regions of lower forms, such as the tail fins of amphibian larvae. Using standard chambers of the 'round table' variety, designed for the study of new growth of blood vessels and other tissues, it has been possible to see the finest details of structure of living lymphatic capillaries and to observe the growth of individual lymphatic vessels, by long-continued daily observations for periods of several weeks to several months. Camera-lucida sketches, photomicrographs, and cinematograph records of the living growing mammalian lymphatics—many of them with the oil-immersion lens—have been obtained. This is, to our knowledge, the first time that it has been possible to make studies of this kind upon lymphatic capillaries in the living mammal.

A description of the cytological characteristics of living lymphatics as seen in these transparent chambers in the rabbit's ear is given.

The growth of new lymphatics across the thin observation space was followed repeatedly by direct observations of the same living vessels over a period of several weeks, and it was possible to establish the following facts:

Lymphatic vessels of the adult mammal undoubtedly possess the capacity for regeneration or new growth, since all of the lymphatic vessels, as well as the blood vessels and other tissues present in the observation area, are newly formed structures which have been seen to invade a hole which was cut clear through the ear tissue at the time of installation of the chambers.

Lymphatic vessels in the rabbit's ear grow by sprouting. The pushing out of solid protoplasmic tips, from preexisting lymphatic endothelium, into which a lumen gradually extends, has been observed repeatedly with the oil-immersion lens.

Mitotic division of endothelial nuclei of growing lymphatic vessels has been observed and the times of the different phases recorded. Following a division, the daughter nuclei always remained attached to and a part of the vessel wall.

Ingrowth of new lymphatic vessels in the observation space is always subsequent to that of the blood vessels by days, weeks, or occasionally even months. The number of new lymphatic sprouts formed is always less than that of the blood vessels and the resulting network less dense.

The growth pattern of the new-forming lymphatics varies with differences in the consistency of the connective tissue present in the regions of invasion. In young chambers, or in those in which the connective-tissue growth and differentiation have been retarded—in which the cells are widely separated and the intervening spaces filled with fluid or a soft gel—the lymphatics tend to grow at random in the interstices of the blood-vessel network, and to form a loose-meshed plexus. In cases where the lymphatics invade an area after the formation of a denser connective tissue, in which cells and fibers are packed closely together, they advance as single vessels in a loose space present along the exterior wall of the arterioles or venules.

The rate of growth of lymphatic endothelium, in a linear direction, may equal that of the growing blood vessel. However, mechanical interference with growth is far more common in the case of lymphatics than of blood vessels.

Mammalian lymphatic capillaries are less labile than blood capillaries. They send out fewer sprouts, anastomose less frequently, and their growth is more easily interfered with than is the case with blood capillaries. On the other hand, once formed, they show much less tendency toward retraction or toward changes in size and form than do the blood capillaries. Isolated lymphatics retain their vitality and power of growth for weeks.

Throughout their growth period and later life in the transparent chambers, the lymphatics were found to form a definite specific system independent of the blood-vascular system and

of any form of connective-tissue or wandering cells. Connective-tissue fibers may be surrounded and encroached upon by the lymphatic until they form reticulum-like strands which appear to pierce the lymphatic. They remain, however, covered by endothelium and hence outside the lumen of the lymphatic. The differentiation of such strands from fibroblasts was observed. They play no part in the formation of endothelium.

The mode of growth of mammalian lymphatic capillaries, by a process of sprouting from preexisting endothelium, as observed in the living animal in these transparent chambers in the rabbit's ear, was found to be the same as that observed for similar vessels in the transparent tails of living amphibian larvae.

In our recent observations on the new growth of blood vessels, in sixty standard chambers of the 'round table' type, we found that the whole process of ingrowth of new blood capillaries, formation of anastomoses, and the subsequent remodeling of the indifferent plexus into an adult pattern, the retraction of many vessels and the enlargement of others to form arterial and venous channels in response to circulatory conditions, is a replica of the original growth process, as observed in the transparent tail fins of amphibian larvae and in the area vasculosa of chick embryos. In the present study, we have found that the same thing is true in the case of lymphatic vessels. The adult mammalian lymphatic system, like the blood-vascular system, evidently retains the same growth properties present in its embryonic development, after the stage of primary differentiation. However, in the course of these studies on living mammalian lymphatic vessels we have observed many interesting differences in their physiological properties as compared with those of Amphibia, which will be described in a subsequent paper.

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