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OBSERVATIONS ON ISOLATED LYMPHATIC CAPILLARIES IN THE LIVING MAMMAL

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SEVEN FIGURES

INTRODUCTION

In a former study carried out in the transparent tails of living amphibian larvae (Clark, '22) a description was given of the behavior of lymphatic capillaries which had been experimentally isolated from their connections. It was found that an individual severed lymphatic retained its vitality and power of growth for comparatively long periods of time and that it was eventually reincorporated in the lymphatic system through anastomosis, either with the vessel from which it had been severed or with a sprout from another adjacent lymphatic. The new lymphatic growth which took place in such cases was identical with the normal growth of lymphatics observed in living amphibian larvae (Clark, '09) and with their growth in regenerating tails (Dziurzynski, '11). The union of blood capillaries severed experimentally from their connections took place much more promptly since they were reincorporated in the blood vascular network by the same process of sprout formation within 3 days after the operation, in contrast to 10 to 24 days in the case of severed lymphatics (Clark, '18, '22). Throughout its period of isolation, the lymphatic capillary retained its specificity, showed no signs of degeneration and gave evidence, by widening and narrowing of its lumen on different days, of the passage of fluid in both directions through its endothelial wall.

In recent microscopic studies in transparent 'round table' chambers installed in rabbits' ears, the regeneration of lymphatic capillaries by the same process of sprout formation, accompanied by mitotic division of endothelial nuclei, from pre-existing lymphatic endothelium was demonstrated in the living mammal (Clark and Clark, '32). The new lymphatics were found to advance into the space over the table from the periphery in a manner similar to that described for regenerating blood vessels (Sandison, '28; Clark et al., '31) and to form a specific system of vessels distinct from blood vessels, connective tissue cells and tissue spaces (Clark and Clark, '33, '37).

In the course of prolonged microscopic studies of the blood vessels and lymphatics in twenty-five 'round table' chambers, a number of instances of isolated lymphatics have been encountered, in which it has been possible to obtain continuous records, with high microscopic magnifications, of the original ingrowth of the same vessels, of the time at which they became isolated, of the factors which produced their separation, and of their appearance, behavior, and subsequent fate.

FACTORS RESPONSIBLE FOR THE ISOLATION OF LYMPHATICS

In the 'round table' chambers, in which a central celluloid table, 6.5 mm. in diameter is fitted into a hole cut clear through the ear at the time of installation (Clark et al., '30) the regenerating blood vessels and lymphatics invade the thin observation space as outgrowths from preformed vessels surrounding the hole. The isolated lymphatics, which were observed intensively, were in all cases originally parts of new lymphatics which had been seen to invade the table space in this manner and to become separated secondarily from their connections. In no case was there any sign of their having originated in situ. From prolonged microscopic studies of regenerating lymphatic capillaries, in which frequent records were made with the Leitz drawing eye-piece, it was possible, in many instances, to see the process of isolation of a lymphatic capillary and to determine the factors responsible.

The main causes for the isolation of newly formed lymphatics from their former connections were found to be mechanical factors some of which were inherent in the construction of the chambers while others were due to secondary alterations of the observation space or of other tissues present therein. The restricted and semi-rigid character of the table space, which was maintained at a uniform thickness, ranging from 40 μ to 75 μ in the different chambers studied, although it proved to be so advantageous for the microscopic study of the growth and cellular details of the new vessels and tissues, was found to hamper the movement of fluid in the regenerated lymphatics. Thus the normal flow of lymph, which has been known to be very meager in peripheral mammalian lymphatics in the absence of massage (Starling, 1898; Henry, '33) was still further impeded in the thin and rigid chamber areas (Clark and Clark, '32, '37; Clark, '36). This lack of movement appeared to render the lymphatic capillaries more susceptible to blockage from external pressure.

It was previously shown (Clark and Clark, '32) that regenerating lymphatics which invaded the table space soon after the insertion of the chamber, at a time when the intervascular substance consisted of a soft gel, were capable of as rapid growth in a linear direction as the new blood capillaries but that, after the first few weeks, their further extention was frequently impeded by the formation of connective tissue fibers or by the increase in size of blood vessels across their path. In the presence of such obstacles the lymphatic capillaries ceased to grow, their pointed tips receded and the ends became bulbous. It was also noted that the formation of dense unyielding bands of connective tissue frequently constricted the new lymphatics at various points along their course, thereby still further impeding the movement of fluid inside.

After the first month, the formation of connective tissue fibers was especially marked in the region at the table edge. At this time some of the lymphatic capillaries which had grown onto the table at an earlier stage and had followed

courses through the then fiberless connective tissue substance between the blood vessels, now became constricted in this region, by the newly developed fibers, to such an extent that no movement of their contents from the table area to the connecting preformed lymphatics occurred for days or weeks. It was in this region near the table edge and at the time of formation of dense connective tissue that the majority of cases of isolated lymphatics were observed. In a number of instances a previously continuous lymphatic after being constricted at the table edge by bands of connective tissue which blocked its lumen for several days, was seen to separate entirely and to acquire a rounded bulb at its proximal end similar in appearance to the distal ends of lymphatics which had ceased to extend.

In most cases of lymphatics which accompanied larger blood vessels in their growth onto the table area blocking and severing of the vessel in the region at the table edge did not occur. The presence of a perivascular space and the support afforded by the accompanying blood vessel in preventing external pressure were the apparent factors responsible for preserving an open lumen in lymphatics so situated. Moreover, in cases in which the companion blood vessel was an artery which had acquired active contractility a massage effect on the lymphatic was observed (Clark and Clark, '37).

Blood capillaries in this region at the table edge were not appreciably affected by the development of dense connective tissue since they were observed repeatedly to send out new sprouts, to enlarge from capillaries to arteries and veins and to maintain a normal circulation in regions too dense or restricted for lymphatics to penetrate or to maintain open connections.

In several chambers recently inserted, an effort was made to prevent the compression of lymphatics by bevelling the table edge in such a manner as to leave a more gradual slope and a deeper space in the region where new vessels and tissue invade the thin observation area. This modification apparently accomplished the desired result since new lymphatics

which grew onto the table between the blood vessels in such chambers, as a rule, maintained open connections and isolation of lymphatics in this region was less frequent. It should also be mentioned that in chambers in which the space over the table was as great as 100 μ or in which the previously thinner space was enlarged secondarily, either accidentally by warping of the chamber base or intentionally by loosening the screws which hold the two parts of the chamber together, cases of isolation of lymphatics at the table edge were rarely seen.

Aside from the cases of severing lymphatics by compression due to increase of connective tissue fibers in a restricted space, the occasional isolation of lymphatics by pressure from blood vessels was also observed. Regenerating lymphatics in their growth across the table area frequently crossed blood capillaries. At times such a small blood vessel, following circulatory changes, subsequently enlarged and changed to a relatively large artery or vein which compressed the lumen of the lymphatic at the point of crossing to such a degree that no cells could pass between its distal and proximal portions. In some of these cases the lymphatic was completely severed at the crossing point after which the two ends of the formerly continuous lymphatic remained as rounded bulbs, one on each side of the intervening vessel.

Another way in which a lymphatic became isolated was observed, in two instances, of vessels which accompanied a large vein at the table edge. Following a change in the direction of blood flow, the size of the vein diminished and at the same time the base of the table warped slightly, causing the space in this region to decrease. Deprived of the support of the formerly wide blood vessel the surrounding tissue in the constricted space at the table edge compressed the lymphatic to such a degree that it was blocked. A few days later a separation appeared in this region, leaving the portion on the table completely isolated.

Still another cause for the isolation of lymphatic capillaries was the secondary squeezing of the new tissue over the table

following an inflammatory reaction. As previously described (Clark and Clark, '37), during the period of active blood flow, dilatation of blood vessels and accumulation of extravascular fluid, the slightly elastic mica cover of the chamber was raised and the space on the table temporarily increased. With return of a normal circulation the blood vessels became narrower and for a few days the accumulation of leukocytes and fluid under the coverslip persisted and exerted sufficient pres-

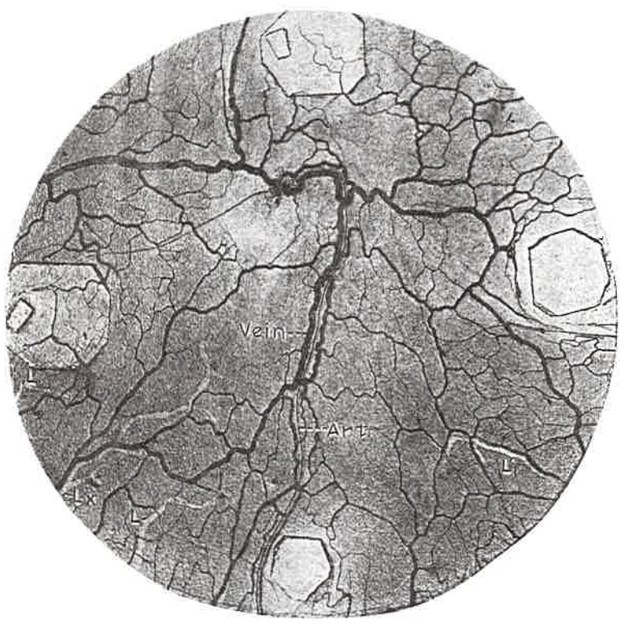


Fig. 1 Photomicrograph of a round table chamber 1½ months after installation. New lymphatics grew into table space at four separate points of lower part of table only and extended for varying distances toward center. L. X became separated from its preformed connections 1 week after its first appearance on the table. It continued to grow toward center of table for 3 weeks after becoming isolated. At time of photograph all the lymphatics, including *X*, had ceased to advance and acquired bulbous endings. They remained practically unchanged for 4 months. No lymphatics present in upper half of chamber. See text, page 66 and page 77. × 16.

sure on the tissue beneath it to force the blood out of the entire vascular plexus at times when the supplying arteries contracted actively. In most cases the excess fluid disappeared after a day or two, the tissue and vessels became adjusted to the thin space and normal circulation was resumed. In some instances, following such a period of recovery from inflammation, lymphatics which had previously been connected with preformed vessels and which had extended uninterruptedly for considerable distances across the table area, were interrupted at several points and remained thereafter as disconnected capillaries of varying lengths, rounded at both ends.

Apparently the pressing and holding together of the two opposing walls of a lymphatic capillary for a sufficient length of time may result in their sticking together and eventually growing together with loss or retraction of the affected portion and rounding up of the two ends of the severed vessel. There is some evidence that the stickiness of the lymphatic endothelium is increased during inflammation but this possibility requires further study.

All of the various factors responsible for the separation of lymphatic capillaries from their former connections may be grouped together as external pressure effects, which in the thin and rigid space of the transparent chambers were sufficient to constrict and obliterate the lumen. Just as it was previously shown that regenerating lymphatics, although displaying an inherent growth capacity similar to that of new blood capillaries, frequently ceased to advance upon encountering physical obstacles too slight to affect the growing blood vessels, so in the present study it was found that mechanical pressures too slight to interfere with normal circulation in the surrounding blood vessels with their higher blood pressure, might constrict and obliterate the lumen or interrupt the continuity of lymphatic capillaries.

BEHAVIOR OF ISOLATED LYMPHATICS

Following the separation of a lymphatic capillary from its former connections it was usually possible, due to two char-

acteristics of regenerated lymphatics, to make prolonged microscopic observations of the same isolated vessel. The first of these was the comparative sparsity of new lymphatics which invaded the chamber space in comparison with the greater abundance of the new blood vessels in the same area. Lymphatics usually grew onto the table at widely spaced points around the periphery, while the blood vessels advanced everywhere in great profusion. Moreover, even in chambers in which the new lymphatics appeared at an early stage and extended rapidly, simultaneously with the invading blood capillaries, toward the center of the table, they sent out comparatively few side sprouts and eventually formed a loosely meshed network in contrast to the richly anastomosing plexus of surrounding blood vessels (fig. 1), while in many cases the only new lymphatics in the entire table area were single vessels which accompanied a few of the larger blood vessels and possessed no connections with other regenerated vessels in the table area (Clark and Clark, '32, '37). Thus it happened that a lymphatic which became separated from its connection at one point was usually located at a considerable distance from other lymphatics on the table, while a blood capillary which became disconnected at one point in most cases remained connected with the richly anastomosing blood vessel network at its other end.

The other characteristic of living lymphatics which made possible prolonged study of isolated vessels was their greater stability as compared with blood vessels (Clark and Clark, '32). The growth of new blood vessels in the round table chambers was characterized by a great over-production of new capillary sprouts, the formation of numerous anastomoses, and by the subsequent remodelling of the original indifferent plexus into an 'adult' pattern by the transformation of certain of the new capillaries into arteries and veins and by the retraction of many others. After the complete vascularization of the table area, the blood vessels were still very labile and the sending out of new sprouts, retraction of others

and remodelling of extensive networks frequently occurred in response to changes in the circulation due to relatively slight stimuli (Clark et al., '31). The new blood vessels, considered as a plexus, showed greater growth response and a greater ability to penetrate dense tissue and to maintain connections and normal circulatory activity in a confined space with rigid walls than did the new lymphatics but, on the other hand, as individual vessels, the new lymphatics showed less tendency to retract or to undergo changes in size and form than did the individual blood capillaries.

Due to these two characteristics—the relative sparsity of regenerated lymphatics and their relative stability—it was possible to follow isolated lymphatics for long periods of time and to study minute changes in their form and behavior. In many instances, the same lymphatics were followed from their first appearance in the table space, as sprouts from preformed lymphatics, through their growth period across the table, to watch them become separated from their former connections and subsequently to follow them for weeks or months.

In the case of every isolated lymphatic studied, the specificity of the lymphatic endothelium was obvious. The severed vessel persisted with its wall intact, and resembled in all morphological characteristics the normally connected lymphatics in the same chamber. As was the case with normal lymphatics, the isolated vessels did not anastomose with neighboring blood vessels, even when they were in such close proximity that the two walls were in contact, and they also remained distinct from the connective tissue. In their persistence as endothelial-lined vessels, after complete isolation from the rest of the lymphatic system, the lymphatics of the rabbit behaved exactly as did the experimentally isolated lymphatics of Amphibia (Clark, '22).

Despite the absence of any outlet, the isolated lymphatics retained their fluid contents which, like those of normal lymphatics in the same area, could be distinguished from the gelatinous tissue substance by the bobbing of cells or particles inside (Clark and Clark, '33). The only exception to the fluid

character of the lymphatic contents occurred occasionally in vessels which were filled with erythrocytes following a haemorrhage from an adjacent blood vessel (p. 74).

In addition to retaining their specificity, the normal appearance of their endothelial wall and lumen and their fluid contents, the isolated mammalian lymphatics, like those of Amphibia, were found to retain their growth capacity. Many instances of the sending out of new sprouts, enlargement and extension of the lumen and formation of new anastomoses were observed in vessels completely isolated from the lymphatic system. Figures 2, 4 and 5 are records of lymphatics which continued to grow in a typical manner after being completely separated from their former connections. In the case of the vessel shown in figure 2 new sprouts formed at the distal end of the cut-off vessel, while in figure 5 new growth took place at the proximal end and in figure 4 new sprouts were sent out from both ends of the severed lymphatic. Retraction of isolated lymphatics was also observed (figs. 2 and 3).

Again, as was the case in Amphibia (Clark, '22), the endothelium of isolated lymphatics in the rabbit continued to exhibit its normal behavior as a membrane. When the same isolated vessel was observed from day to day it was seen to

Fig. 2 Selected records, Leitz drawing eye-piece, of the same isolated lymphatic (Lym.) and accompanying blood vessels (B.V.) followed for 18 months. Chamber inserted February 20, 1935. Lymphatic grew onto table March 18th, 26 days later, and separated from its connection at table edge April 2nd, when 14 days old. First sketch, April 4th, shows isolated lymphatic with rounded proximal end A and branching distal end with growing sprouts E, D and G. Sketches April 11th to August 1st show changes in extent and caliber of distal portion of lymphatic. New sprouts E and F grew out and new anastomosis formed between C and D after lymphatic became isolated. Sketch May 6th shows erythrocytes (ERY.) packed in bulbous end E following haemorrhage from blood vessel—sprout G retracted. August 1st shows separation of connection between B and D. Sketches August 20th to June 10, 1936 show whole extent of lymphatic with gradual retraction. Rabbit died October, 1936, 20 months after insertion of chamber and 18 months after isolation of lymphatic. At time of death lymphatic still present and had same appearance as shown in last sketch June 10, 1936. Dw.P., dwindled polymorphonuclear leukocytes which emigrated into lymphatic and remained imprisoned in lumen. × 28.4.

be wider on some days and narrower on others, thus demonstrating the passage of fluid in both directions through its wall (figs. 2 and 5). In this respect also the behavior of isolated lymphatics did not differ from that of normal, connected lymphatics in the same chamber. As a rule, the isolated lymphatics, like the connected ones, were wider during periods of active blood flow in the surrounding vascular network and

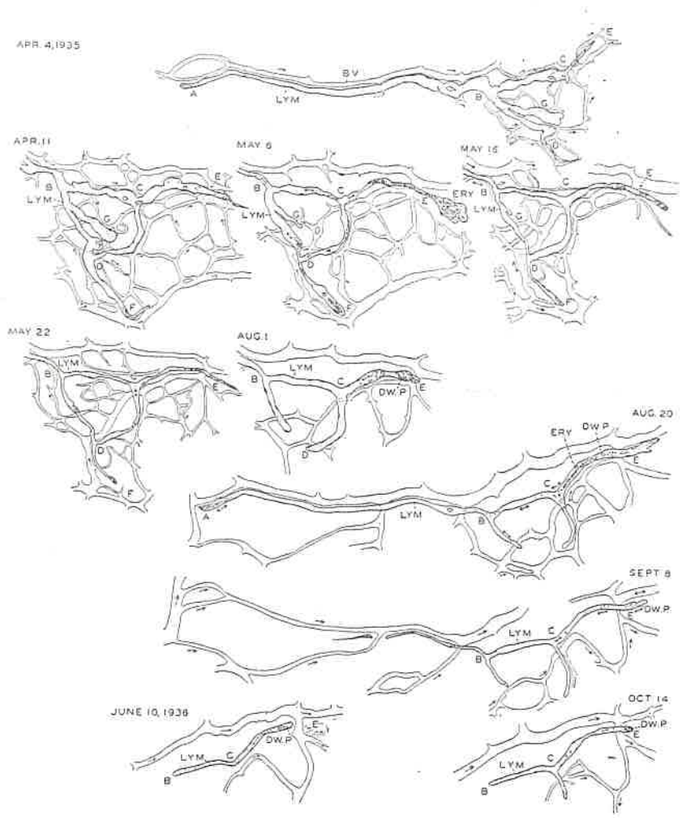


Figure 2

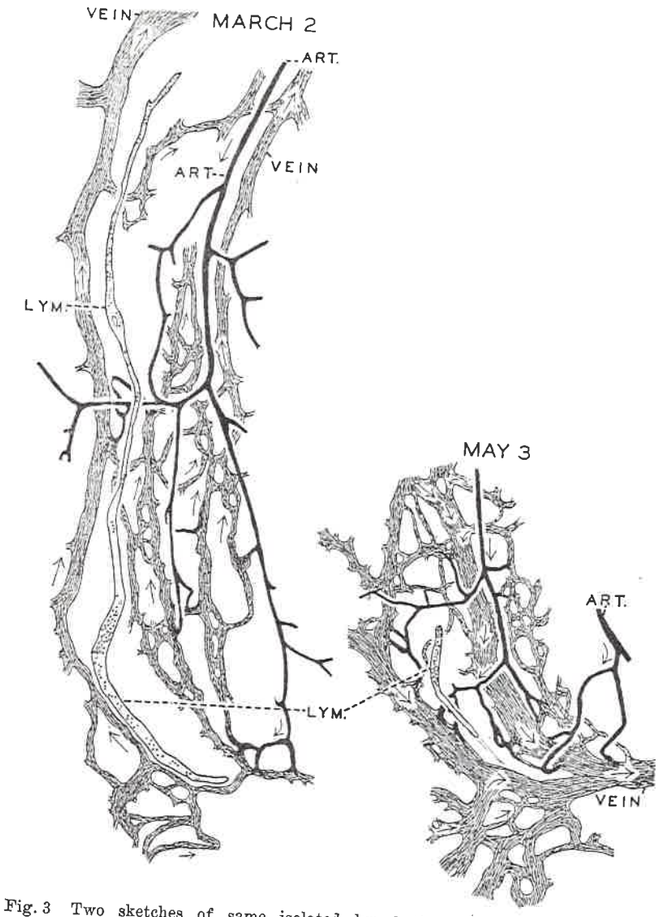


Fig. 3 Two sketches of same isolated lymphatic and accompanying blood vessels. Lymphatic separated from its connections 1 month before first sketch March 2nd. This vessel retracted more rapidly than lymphatic shown in figure 2. Blood vessels underwent marked changes in size and pattern during observation period. Leitz drawing eye-piece. × 58.

narrower at times of subdued circulation (Clark and Clark, '37). On days of moderate circulation in the blood vessels instances have been noted in which an isolated lymphatic was wider than on the day before while another isolated vessel in the same chamber had become narrower. In some cases the two isolated lymphatics, displaying this difference in behavior on the same day, had previously been continuous portions of the same lymphatic.

The appearance of isolated lymphatics, even after months, did not as a rule differ from that of connected lymphatics; they continued to show the alternation of spindle-shaped nuclear thickenings with delicate thin internuclear stretches. An unusual feature presented itself in one isolated lymphatic, which consisted of the appearance, after several months in the regions of the nuclear thickenings, of a few small yellowish brown granules which persisted for months. Their origin and significance were not determined. Another phenomenon which was observed in this same lymphatic at an early stage was a tendency for an occasional endothelial nuclear area to round up, bulge into the lumen and become completely separated from the wall. Such desquamated cells were watched for several hours. Their cytoplasm had a lacey, or vacuolated appearance and their nuclei became opaque. While the ultimate fate of individual cells was not followed, there were seen on several occasions at or near a place where the separation of a cell had occurred the preceding day, small, amorphous, degenerating-looking masses which we interpret as representing the remains of the desquamated cells—thus apparently suffering the same fate as desquamated lymphatic endothelial cells in the tails of amphibian larvae (Clark and Clark, '27).

All of these observations on isolated lymphatics showed that they retained the same properties as normal, connected lymphatics in the same areas, i.e., their specificity, ability to grow and retract, to maintain an open lumen with fluid contents and to change caliber with passage of fluid in both directions through their endothelial walls.

CELLULAR CONTENTS OF ISOLATED LYMPHATICS

The cellular contents of isolated lymphatics varied with the presence or absence of cells inside them at the time of separation, with the proximity of such severed vessels to thin-walled blood vessels, the subsequent occurrence of inflammation in the chamber areas and with the presence or absence of macrophages inside the isolated vessels. Usually isolated lymphatics, like the normal lymphatics were colorless vessels containing only a few widely separated cells floating in their fluid contents. In the absence of subsequent inflammation in the chamber such an isolated lymphatic retained its clear and almost non-cellular contents throughout the weeks or months in which it remained isolated and under observation.

However, in a number of instances of isolated lymphatics which were located in close proximity to a vein or capillary, following a period of increased circulation, caused by heat, pressure or by mild infection which resulted in a change in the endothelium of the accompanying blood vessel (Clark and Clark, '35), emigration of leukocytes from blood vessel to lymphatic and, at times, haemorrhage into the lymphatic lumen were observed (Clark and Clark, '37). Following such an occurrence, the emigrated leukocytes or extruded erythrocytes were imprisoned in the lumen of the lymphatic, which terminated blindly at both ends, and in cases in which no further disturbance to the chamber area took place the same collection of cells remained inside the lymphatic and could be studied for long periods of time. Descriptions have already been given of the emigration of groups of leukocytes, consisting chiefly of polymorphs, from venules or capillaries, of their migration into lymphatics and of the subsequent change of typical polymorphs inside insolated lymphatics into small round cells with round nuclei and diminished vitality which led us to call them 'dwindled polymorphs' (Clark, Clark and Rex, '36).

Migration of leukocytes and haemorrhage from blood vessels to lymphatics have been observed both before and after the isolation of the latter from their original connections. For example, the lymphatic shown in figure 4 became filled with

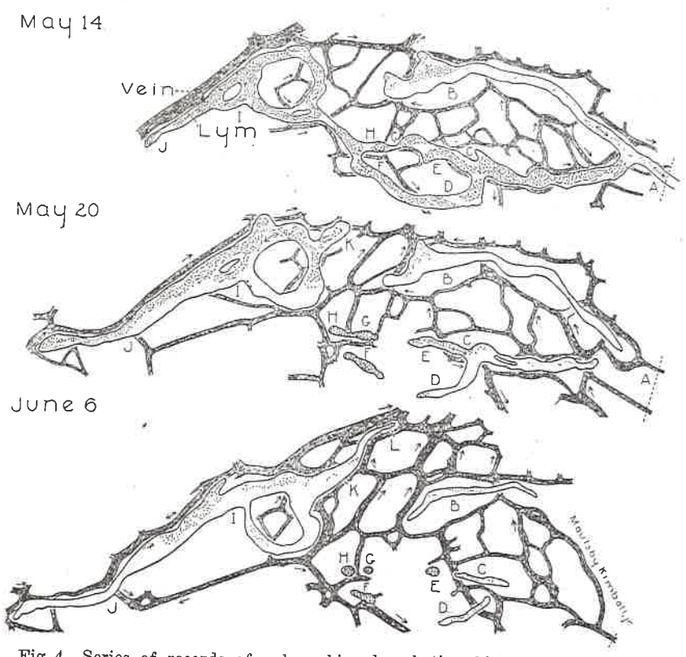


Fig. 4 Series of records of a branching lymphatic which separated from its connections due to pressure at the table edge and later broke up into a number of isolated segments following a period of mild inflammation (see text, p. 64). May 14th, sketch of continuous lymphatic connected at table edge A, with preformed lymphatics from which it had grown out 8 days previously. Lymphatic contains erythrocytes which entered it from neighboring vein. Two days later (May 16th) the lymphatic was cut off at the table edge A. May 20th sketch shows formerly continuous lymphatic separated into five isolated segments following mild inflammation. Distal isolated portion continued to grow at both ends (K and J). June 6th, lymphatic segment H-G separated into two small blood-filled 'balls' and segment ECD into three. Segment B retracted. Distal segment continued to extend (J and F). Thirteen days later the smallest lymphatic ball, G, burst and discharged contents into tissue. Other isolated segments persisted as long as chamber was under observation. Leitz drawing eye-piece. × 32.

blood from an accompanying vein during a period of inflammation in which the endothelium of the blood vessel underwent a change to a softer and weaker consistency (Clark and Clark, '35). During the period of recovery, in which the surrounding region was subjected to increased pressure (p. 65) the lymphatic became separated into five isolated segments, some of which were packed with blood cells at the time of separation (fig. 4, H, G).

In the specimen illustrated in figure 2 the lymphatic under observation, which became cut off at the table edge following the formation of dense connective tissue fibers (p. 61) at a time when no inflammation had occurred in the chamber area, contained only a few cells at the time of separation. A month later, following a period of mild inflammation, a haemorrhage into this lymphatic from an adjacent blood vessel was observed (fig. 2, May 6th).

As previously described (Clark and Clark, '37) the fate of blood cells which entered an isolated lymphatic from a neighboring blood vessel varied with the amount of blood in proportion to the size of the lymphatic and with the presence or absence of macrophages inside the lymphatic. In several instances of haemorrhage into an isolated lymphatic the blood cells were so closely packed together that the cell outlines were indistinguishable and the blood appeared to be laked. However, continuous observations of such a blood-filled lymphatic showed that this appearance was due to concentration of erythrocytes with loss of fluid from the vessel. In the lymphatic shown in figure 2 the blood was concentrated in a solid motionless mass in the terminal bulbous end for several days after its entrance from a nearby blood vessel. On one day the vessel became narrower and blood was squeezed out of the end into the tissue where it was seen to consist of typical discrete erythrocytes. The extruded blood cells were disposed of within 48 hours. In another chamber, in which a massive haemorrhage occurred into an isolated lymphatic, the blood was concentrated in two solid masses at both of its rounded ends and the intermediate, empty portion became so narrow

that the lymphatic had the form of an hour-glass. A few days later, the lumen of the lymphatic became wider and the blood masses at the two ends broke up into floating clumps in which the outlines of individual erythrocytes were visible.

When blood which entered an isolated lymphatic from a nearby vein consisted of a moderate number of erythrocytes, the blood cells could be seen to move freely back and forth, either singly or in rouleaux for the first day or two, after which they frequently clung together in clumps and at times were seen to adhere temporarily to the vessel wall (Clark and Clark, '37).

Phagocytosis of erythrocytes and dwindled polymorphs inside the lymphatic by macrophages has been observed repeatedly. The macrophages in some cases were formed from monocytes present in the original haemorrhage, while in others they migrated into the lymphatic from the outside tissue (Clark and Clark, '28, '30). However, in many cases no macrophages nor monocytes were present inside the isolated lymphatic at the time of the haemorrhage and frequently a considerable time elapsed before a macrophage from the tissue penetrated the lymphatic endothelium. Thus in the case shown in figure 2 erythrocytes from a single haemorrhage remained in the distal end of the isolated lymphatic and retained their red coloration for a month before a macrophage finally migrated through the wall from the outside tissue and ingested a number of the imprisoned cells.

Although the same groups of blood cells remained imprisoned inside isolated lymphatics for periods of weeks or months where it was possible to make daily microscopic studies of such cells and of the endothelium of the surrounding vessel, and although clumps of erythrocytes and individual blood cells were observed to cling to the wall of the lymphatic at times, no instance of the phagocytosis of the enclosed cells by the endothelial cells of the lymphatic was observed.

Extravasations of blood cells into the tissue, which usually occurred simultaneously with haemorrhages into lymphatics, were disposed of much more promptly—within a day to a

week, depending on the size of the haemorrhage. This was due in part to the greater number of macrophages present in the tissue than in the lumen of lymphatics. The phagocytosis of large numbers of extravasated blood cells by macrophages has been observed repeatedly. Our studies have also shown that erythrocytes may apparently dissolve without the agency of macrophages. In any case, the blood cells which were seen to enter isolated lymphatics simultaneously with the extrusion of erythrocytes into the tissue, invariably remained for long periods—after all trace of the haemorrhage in the tissue had disappeared. Moreover, blood cells which had been imprisoned inside an isolated lymphatic for weeks, have been seen repeatedly to be ingested by macrophages or to lose their red color and disappear 1 or 2 days after being pressed out of the end of the lymphatic into the tissue.

These phenomena just described of haemorrhage from blood vessel into isolated lymphatics and emigration of leukocytes from blood vessel to lymphatic were also observed in cases in which the lymphatics were still continuous with preformed lymphatics outside the table area. In the case of connected lymphatics the blood cells, although they frequently lingered for many days, gradually moved out of the observation area (Clark and Clark, '37).

FATE OF ISOLATED LYMPHATICS

In observing individual isolated lymphatics from day to day the most striking characteristic noted was their persistence. In many instances the same isolated vessels were followed for months, during which time although they might grow or retract, showing slight alterations in caliber from day to day and changes in cellular content in the ways just described, they persisted in the same locations and retained their characteristic shapes, so that they were easily recognizable as the same vessels. Repeatedly, lymphatics whose original growth onto the table space as extensions from preformed lymphatics had been observed, were watched as they became cut off and were then followed for as long as the

particular chamber was under observation. Individual isolated lymphatics, one or more in a chamber area, have been followed in this way in ten different chambers. In many of these chambers the pattern of the surrounding blood vessels underwent extensive remodelling during this period of study (Clark et al., '31).

The isolated lymphatic which was followed for the longest period of consecutive observation was present in a chamber which was studied microscopically for 20 months. The fate of this vessel is shown in figure 2. The lymphatic first invaded the table area as a sprout from a preformed lymphatic 26 days after the installation of the chamber. After the first week it became blocked at the table edge and 10 days later, when this new lymphatic was 17 days old, it became completely cut off (fig. 2, April 4, 1935). For the first few weeks after its separation the lymphatic continued to grow in a distal direction and two of its new sprouts anastomosed (fig. 2, D, C). During the succeeding months it gradually shortened and the loops formed by the anastomosing branches broke apart and retracted. At the end of 7 months the last of the new branches (shown in fig. 2, October 14th) was withdrawn and the remaining short stretch of the lymphatic persisted, unchanged except for slight alterations in caliber, in the form shown in the last sketch (June 6, 1936) until the death of the rabbit 4 months later. Thus this completely isolated lymphatic capillary persisted for 18 months.

In the case shown in figure 3, the isolated lymphatic ceased to extend and became shorter at a faster rate since the final record, made 3 months after the separation of the vessel from its former connections, showed only a short stretch of its distal end still present. On the other hand, lymphatics have been observed which continued to grow for a longer time after their separation than the vessel shown in figure 2 and to extend much farther. For example, the lymphatic marked X in figure 1 became separated from its former connections 1 week after its appearance on the table when it extended for only a short distance. During the next month this vessel continued

to grow steadily toward the center of the table and at as rapid a rate as other lymphatics in the same chamber which were still connected with preformed vessels outside the table space. The photograph (fig. 1) taken 3 weeks after the lymphatic X became isolated shows that this vessel had extended farther than any other of the regenerated vessels. During the second month all of the lymphatics in this chamber ceased to grow and remained with rounded terminal ends for 6 months, when observations were discontinued.

In some instances new growth and retraction took place simultaneously in different isolated lymphatics which had formerly been continuous portions of the same vessel. Figure 4 shows a lymphatic which was originally a continuous vessel with several branches. The whole vessel first became separated from its connections at the table edge (A) and a few days later, following a period of recovery from inflammation in which the whole region was subjected to increased pressure (p. 65) the lymphatic was further broken up into five isolated portions (May 20th). One stretch of the formerly continuous lymphatic sent out new sprouts and continued to extend both distally and proximally (fig. 4, sprouts J and K, L) during the 2 months in which the chamber was under observation. Meanwhile, other isolated stretches of this formerly continuous vessel gradually shortened (B, C) while two of them separated still farther (C, D, E and H, G) so that a month later (June 20th) there were eight isolated portions of the original continuous lymphatic.

In another chamber, following a period of inflammation, two regenerated lymphatics which extended, with several anastomoses, clear across the table area, became separated from their former connections in the preformed tissue and at the same time broke up into a number of short isolated stretches. During the succeeding months all traces of the inflammation disappeared except for the persistence of isolated lymphatics, some of which contained varying amounts of blood cells which had been present inside them at the time of their separation and others of which were practically free

from cells. The majority of these isolated lymphatics remained unchanged in length, a few retracted slightly, while two formerly connected portions, after remaining isolated for 4 months, both sent out new sprouts which advanced toward each other and reunited (fig. 5, D and B). After forming this new anastomosis the resulting lymphatic was still isolated

Fig. 5 Successive sketches of two isolated portions (A-B and D) of a formerly continuous lymphatic which reunited after being separated from each other and the rest of the lymphatic system for 4 months (see text, p. 78). Lymphatic D, a portion of which is shown, was also isolated at its proximal end from its former connections off the table area. After the formation of a new sprout on February 28th which anastomosed with lymphatic B on March 5th, the reunited lymphatic still remained isolated from the lymphatic system. December 7th, lymphatic A-B narrow and filled with concentrated blood which entered it from adjacent blood vessel. January 15th, lymphatic wider, blood cells moving. February 28th, some of the blood cells in lymphatic have been phagocytized by macrophages. Leitz drawing eye-piece. × 45.

from its former connections outside the table area and hence from the rest of the lymphatic system.

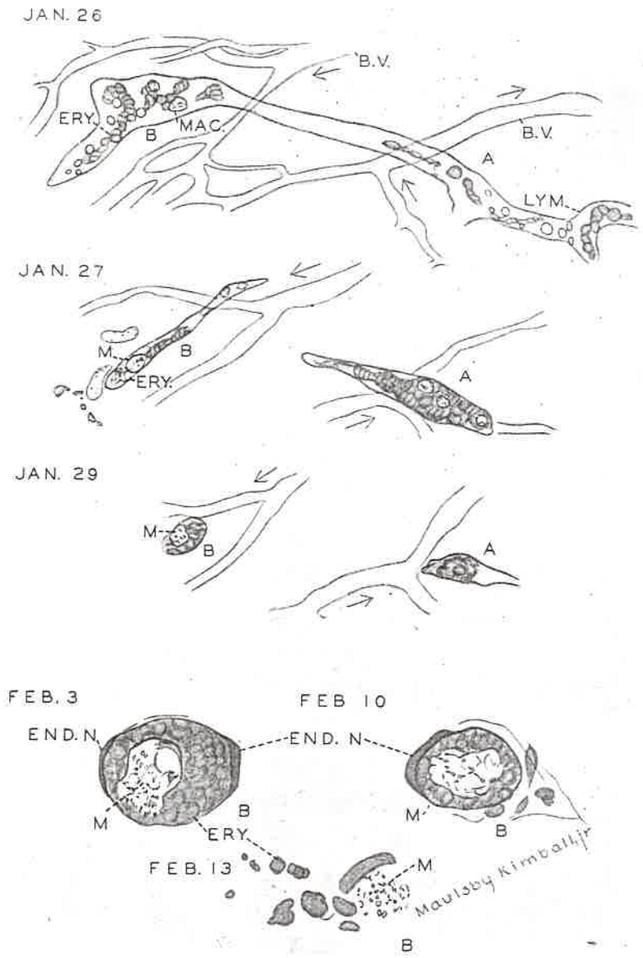
A similar case has recently been observed of two branches of a formerly continuous lymphatic which became isolated following the enlargement of a vein across their course (see p. 63). After remaining as two short isolated vessels lying

parallel to each other for 4 months, one of the vessels sent out a number of new sprouts from its two ends and also laterally, five of which anastomosed with the neighboring isolated lymphatic, thereby forming a small lymphatic plexus, which remained for several months still completely separated from the lymphatic system.

In a chamber which is still under observation, two regenerated lymphatics, each extending more than halfway across the table area, have been separated from their original connections at the table edge for over 5 months and during that time they have shown no change in extent or form, aside from a slight amount of retraction at their distal ends. During the same period of observation much of the blood vessel network of the same region has undergone extensive changes in pattern, involving formation of new capillaries, retraction of others, changes in size, shape and direction of flow in several of the larger veins, and formation of a new arteriole.

Although the majority of the lymphatics which became separated from their connections persisted indefinitely in the manner just described, in two of the chambers studied the disappearance of certain of the isolated lymphatics was observed. In every case of this kind the lymphatic either had the form of a small round ball, packed with blood cells, at the time of its separation, or it shortened from an oval to a spherical form within a few days. Figure 6 depicts the fate of a lymphatic the distal end of which (B), containing erythrocytes and a macrophage, became separated from its former connection A. During the first 24 hours of isolation its lumen narrowed and it sent out a short sprout from its proximal end, which was retracted on the following day. On

Fig. 6 Series of sketches with Leitz drawing eye-piece of a lymphatic capillary (Lym.) the end of which (B) became isolated from its connection (A). B.V., blood vessel; Ery., erythrocytes; M., macrophage. January 26th shows lymphatic 15 days after its appearance at table edge. January 2nd shows isolated portion of lymphatic B soon after separation from A. January 29th isolated lymphatic B retracted into rounded form filled with blood. × 135. Sketches February 3rd to February 13th, higher power drawings of lymphatic B showing disintegration of endothelium with discharge of contents. End.N., endothelial nucleus of lymphatic. Oil immersion. × 618.


JAN. 26
B.V.
ERY.
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MAC.
B.V.
A
LYM.
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M.
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ERY.
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M
B
A
FEB. 3
END. N.
M
B
ERY.
FEB 10
END. N
M
B
FEB. 13
M
B
Maulsby Kimball jr

Figure 6

the third day after its separation (fig. 6, January 29th) the isolated lymphatic had shortened and assumed the form of a small blood-filled sphere. It persisted in this form for 2 weeks longer and at 17 days after its separation the endothelium disintegrated, discharging its contents of erythrocytes into the tissue (fig. 6, February 13th) where they were subsequently ingested by macrophages. In this same specimen, two other lymphatics, which separated from their connections in the form of small, round, blood-filled balls, went to pieces in the same way, one of them after 5 days and the other after 7 days.

In another chamber, following a mild inflammatory reaction, two short stretches of a formerly continuous lymphatic packed with erythrocytes became isolated, assumed spherical forms, and disintegrated, one of them 7 days and one 13 days later. In this second chamber, two other short blood-filled stretches of the same lymphatic became separated from their former connections at the same time and although they also rounded up into small balls, indistinguishable in appearance from the two which disappeared, they both remained unchanged for 2 months and showed no evidence of disintegration when observations on the chamber were discontinued. A number of other isolated blood-filled lymphatic 'balls' were observed, in other chambers, which persisted unchanged for over 4 months after separation from the lymphatic system.

All of the preceding descriptions of the fate of isolated lymphatics have been concerned with vessels which in addition to being separated from their former connections outside the table were located at a distance from other regenerated lymphatics on the table. However, cases have been observed in which isolated lymphatics were more favorably situated with respect to other connected lymphatics present in the table area or to new vessels which subsequently invaded the table space, in which the fate of the isolated lymphatics was different. For example, in one chamber two new lymphatics invaded the table space at about the same time and advanced in an approximately parallel direction with no large blood

vessels in the intervening space. One of the new vessels became cut off from its connections at the table edge and 2 weeks later the neighboring vessel, which had remained connected, sent out a lateral sprout which anastomosed with the isolated lymphatic thereby reincorporating it in the lymphatic system.

In the case of the lymphatic illustrated in figure 7, the vessel separated from its connection at the table edge 1 week after invading the table area. At the time of isolation it contained a few freely movable leukocytes. Twenty days later

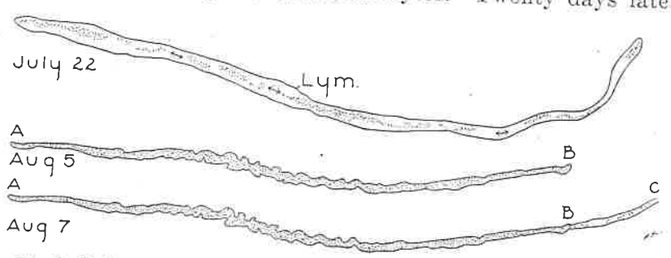

Fig. 7 Series of records of lymphatic capillary (Lym.) which was cut off at table edge 1 week after invading observation area and remained isolated for 49 days when it united at its distal end with a new sprout (C) from a lymphatic which grew across the table from the opposite side (see text, p. 84). July 22nd shows isolated lymphatic 2 months after it was cut off. August 5th, same isolated lymphatic filled with concentrated blood which entered it from adjacent vein following a period of inflammation. Shows wavy contour of wall. August 7th shows same lymphatic at time of anastomosis of its distal end, B, with new lymphatic sprout C. × 45.

during a period of inflammation, due to a small localized infection in the ear outside the chamber area, this lymphatic became filled with blood which entered it from an adjacent vein. The blood was packed so tightly inside the lymphatic that it formed a motionless mass in which cell outlines were indistinguishable and at the same time the outline of the vessel assumed a 'scalloped' appearance and its lumen became much narrower, indicating concentration of blood with loss of fluid. The isolated lymphatic persisted with little change in length, outline or contents for 3 weeks at the end

of which time a new lymphatic which in the interim had grown across the center of the table from the opposite side, came in contact with its distal end and united with it. After the formation of a continuous lumen between the two vessels, the mass of blood in the formerly isolated portion started to move back and forth. During the next few days the blood broke up at first into clumps and then into discrete erythrocytes. Twelve days after the formation of the new connection, the blood cells were washed out of the formerly isolated portion. This lymphatic had remained completely isolated for 49 days.

Two other lymphatics in the same chamber remained isolated for 50 and 72 days respectively before they too anastomosed with neighboring vessels in the same manner. In another chamber a lymphatic which became separated from its connections at the table edge remained isolated for 4 months at the end of which time a lymphatic in the preformed tissue outside the table area sent out a new sprout which anastomosed with the proximal end of the isolated lymphatic.

EFFECT OF ISOLATION OF LYMPHATICS ON THE TISSUE

In all of our studies on isolated lymphatics simultaneous observations of the surrounding tissue were carried out. In no case was there any visible microscopic change in the character of the intervascular tissue either at the time at which a lymphatic became blocked or cut off or in the weeks or months which followed. This was true of regions in the chambers in which completely detached lymphatics were the only vessels present as well as of areas in which continuous lymphatics were also located. In a number of chambers, such as the one illustrated in figure 1, new lymphatics invaded the table space from one side only and frequently failed to grow beyond the center of the table. In such cases the tissue on the side of the chamber in which a lymphatic supply was absent showed no difference, discernible with the microscope, in the amount or character of cells or fibers or intercellular substance from the areas in which lymphatics were present.

DISCUSSION

These studies, supplementing those previously reported on the growth and behavior of lymphatic capillaries (Clark and Clark, '32, '33, '37), shed light upon several aspects of the lymphatic problem in the adult and are extremely suggestive in relation to certain problems of lymphatic growth in embryonic stages.

The persistence of isolated lymphatics for as long as 18 months, during which they retained the characteristic morphological and physiological properties of lymphatics and did not become transformed into any other types of cells, indicates a high degree of specificity for this tissue. All of our studies indicate that new lymphatic endothelium forms from preexisting lymphatic endothelium by the process of sprouting, and that once formed it remains lymphatic endothelium.

Regarding the capacity of the endothelial cells of peripheral lymphatics in the mammal to phagocytize partially degenerated erythrocytes or other cells inside or outside the lumen, the answer, from these studies, is in the negative. We have seen in single isolated lymphatics hundreds of such imprisoned cells which remained with no apparent diminution in number for weeks and with no evidence of phagocytosis by the lymphatic endothelial cells. In other cases one or more macrophages have entered an isolated lymphatic which have sporadically phagocytized some of the partially degenerated cells, indicating that these cells are in suitable condition to be phagocytized (Clark and Clark, '37). There is also no evidence that the lymphatic endothelium gives off as a secretion any substance which might dissolve or digest semidegenerated cells contained in their lumen. On the whole, the evidence points to the remarkable situation that the endothelium of peripheral lymphatics may actually protect debris-like material from the action of macrophages and perhaps other chemical substances. This is indicated by the rapid cleaning up of extravasated erythrocytes from the tissue spaces, and has been demonstrated in most striking fashion by the disappearance from the tissue spaces, often within

24 hours, of partially degenerated erythrocytes or polymorphs which had been present in an isolated lymphatic for days or weeks, and which had been forced out into the tissue spaces by pressure over the lymphatic. Thus it appears that the peripheral lymphatics may actually harbor and protect debris and partially degenerated cells, just as Drinker et al. ('35) found that bacteria survive longer in lymphatics than in the blood stream, and that specific antiserum passes with difficulty, if at all, into lymphatics. In this discussion it should be emphasized that we are dealing with the endothelium of peripheral lymphatics, and not that lining the sinuses of the lymph node, where there is evidence from the observations of many investigators for pinocytosis and phagocytosis by lymphatic endothelial cells.

The failure to find any microscopic change in the surrounding intervascular tissue in the transparent chambers after the isolation of lymphatic capillaries from the lymphatic system or to detect any visible microscopic difference between regions supplied by lymphatics and those in which lymphatics were absent, as well as the previous observation (Clark, '36) that the removal of extravasated blood and other debris, present on the table following the insertion of the chamber, takes place at the same rate and in the same manner regardless of the presence or absence of new lymphatics, throw doubt on the theory of Drinker and Field ('33) that lymphatics are essential for the removal of protein from the subcutaneous tissue.

Regarding the problem of growth and regeneration of lymphatics in the adult, our recent microscopic studies of living lymphatics coupled with the observations reported here, which demonstrated the inherent growth capacity of lymphatic endothelium and at the same time the ease with which the extension of regenerating lymphatics may be prevented and their continuity interrupted by mechanical factors, afford a possible explanation for the failure of Meyer ('06) to demonstrate regeneration of lymphatics in his experiments on dogs. It is probable that either early formation of fibrous tissue in the wound obstructed the growth of new lymphatics or that later

formation of dense scar tissue blocked or severed lymphatics which had already regenerated thereby making impossible their demonstration by injection. Our findings thus supplement the results obtained by Reichert ('26) who repeated Meyer's experiments and found that regeneration of lymphatics occurred within 4 to 8 days in cases in which there was a minimum of tissue reaction and that bridging of the gap by new lymphatics was interfered with when dense scar tissue developed.

In relation to the still unsettled question of the mode of differentiation of lymphatics in early embryonic stages, these studies introduce possibilities which have hitherto been overlooked.

One of the possible modes of primary differentiation of lymphatics which was suggested by Lewis ('06) is that they may arise from transformed portions of veins. He based this hypothesis on the finding, in reconstructions from serial sections of rabbit embryos, of apparently isolated portions of endothelial-lined tubes which he interpreted as veins which had lost their connections with the blood-vascular system, in locations in which at a later stage there were connected lymphatics. Our studies indicate that the isolated structures described by Lewis may be lymphatics which have grown out from other lymphatics and become separated. Further doubt is cast upon this hypothesis by our observations of the behavior of isolated portions of blood vessels. According to our observations of living vessels in Amphibia and in the rabbit, blood capillaries, in contrast to lymphatics, are rarely completely severed from their connections and in the exceptional cases in which such a separation occurred, or when it was produced experimentally, the isolated blood vessel usually formed a new connection within 48 hours (Clark, '18, '22). In one instance, a case in which the circulation in the surrounding vessels diminished and growth of new sprouts ceased temporarily, an isolated blood capillary was seen to shorten and disappear within 3 days after its separation (Clark and Clark, '32).

The probability that 'veno-lymphatics' may be isolated lymphatics is further strengthened by the observations in embryos that lymphatics before the development of valves are normally filled with blood which backs up into them through their venous connections (Clark and Clark, '20) so that a lymphatic which became separated from its connections at an early stage would as a rule contain erythrocytes. Moreover, the studies reported here demonstrate that in many cases peripheral lymphatics contain blood cells which have entered them from adjacent blood vessels either before or after their isolation.

Another hypothesis regarding the mode of differentiation of lymphatics in the embryo which is affected by these studies is the one maintained by Huntington and McClure ('10) and their pupils and recently revived by Zimmerman ('35), namely, that lymphatics arise by the transformation of mesenchyme cells into endothelium, and which is based largely upon the finding of apparently isolated stretches of lymphatics in reconstructions from serial sections. Previous criticism of this hypothesis stressed the difficulty if not impossibility of making complete reconstructions, so that connections which were actually present may have been missed (compare E. R. Clark, '11 and E. L. Clark, '12). To this is now added the still more serious difficulty that there may be completely isolated portions of lymphatics which have formerly been connected but whose connections may have been eliminated.

The present studies are also suggestive as a possible partial explanation for the variations in distribution and richness of lymphatics, as compared with blood vessels in the adult mammalian body. Thus it is possible that, at an early stage of embryonic development, lymphatic capillaries may grow into many regions along with growing blood vessels, and that subsequently they may be blocked or eliminated following the formation of dense tissue or the development of mechanical conditions around them which may obliterate their lumen by outside pressure. This might, for example, explain the probable lack of lymphatics in adult bone marrow, muscle bundles and liver lobules.

In other organs, on the other hand, such as the lung, the absence of outside hindrances may present especially favorable mechanical conditions for growth and survival of lymphatics with a resultant wealth of lymphatics.

SUMMARY

In the course of microscopic studies of regenerating blood vessels, lymphatics and other new tissues in the transparent 'round table' chambers inserted in rabbits' ears, a number of cases were encountered of lymphatic capillaries which became completely separated from their former connections with the lymphatic system.

All of the new vessels which invaded the table space arose as endothelial sprouts from pre-existing vessels in the tissue around the table. The isolated lymphatics were in every case originally portions of continuous regenerated vessels and there was no evidence that such structures arose in situ.

The separation of lymphatics from their former connections was found to be due to mechanical factors which exerted external pressure on lymphatic capillaries in the restricted and rigid observation space. Regenerating blood vessels, owing to their higher internal pressure, were able to penetrate regions of dense tissue and to maintain connections and normal circulation in locations and under conditions in which the growth of new lymphatics was impeded, their lumens blocked and, at times, their continuity interrupted.

Isolated lymphatics remained specific and in most instances retained their vitality, their powers of growth and retraction, and their normal appearance. During their period of isolation there was evidence for the passage of fluid in both directions through their endothelial walls.

The cellular contents of isolated lymphatics varied with the amount of blood present inside them at the time of their separation, with the presence or absence of macrophages in the lymphatic, and with the occurrence of inflammation in the chamber area. Following changes in the endothelium of adjacent blood vessels, emigration of leukocytes and haemorrhage

from blood vessels into isolated lymphatics were observed. Blood cells imprisoned in the lumen of an isolated lymphatic remained for months in contrast to the rapid disappearance of extravasated blood in the tissue outside. Phagocytosis of partially degenerated leukocytes and erythrocytes inside isolated lymphatics by macrophages was observed. No instance of phagocytosis of imprisoned blood cells by the lymphatic endothelium was seen.

Many of the severed lymphatics remained isolated for as long as the chambers were under observation. In one case an isolated lymphatic persisted for 18 months—until the death of the animal. In a few instances short stretches of lymphatics, packed with blood cells at the time of separation, were seen to burst, discharge their contents, and disintegrate while other similar small blood-filled lymphatics persisted unchanged for months. In some cases two neighboring isolated lymphatics were seen to send out sprouts and form an anastomosis, while still remaining disconnected from the rest of the lymphatic system. In several instances, lymphatics, after remaining disconnected for months, were reincorporated in the lymphatic system by union with new sprouts which grew toward them from other regenerated lymphatics on the table area or from preformed lymphatics at the table edge.

No visible effect on the surrounding intervascular tissue, discernible with highest magnifications of the microscope, was produced by the separation of lymphatic capillaries from their previous connections.

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