STUDIES ON THE LYMPHATIC VESSELS AND ON THE MOVEMENT OF LYMPH IN THE EAR OF THE RABBIT[1]

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FOUR FIGURES

Studies made in this laboratory on lymphatic vessels, as seen in transparent chambers introduced into the rabbit's ear (Clark and Clark, '30–'31, '31, '32 a, b, c) have brought out a number of new facts regarding the mode of growth of lymphatics, and the activity of the newly formed lymphatics. Since in all of these studies only the lymphatics seen in the chambers were involved, it seemed desirable to investigate the lymphatics of the normal ear—their distribution, relations, and activity, and to attempt to find out the extent to which they are modified by the introduction of the artificial chambers. This paper presents the results, some of which are given as preliminary reports.

It became apparent, soon after the work was started, that the normal ear of the rabbit offers a very favorable field in which to extend our knowledge of the behavior of lymphatics.

METHODS

The methods used included single and double injections of the lymphatics and blood vessels, injections of lymphatics, and observations of the movement of the injected granules in the living animal, the collection of lymph from the larger vessels of the intact ear, and the introduction of transparent chambers into the ear.

[1] The work in this laboratory on the study of living cells and tissues in the transparent chamber inserted in the rabbit's ear is being aided by a 5-year grant from the Rockefeller Foundation.

Injections of the lymphatics were made beneath the epidermis with an ordinary hypodermic syringe and a 30 gauge steel needle. In the intact ear, the substances injected were 50 per cent India ink in tap water, a saturated solution of Berlin blue in distilled water, and a saturated solution of methylene blue in 0.9 per cent sodium chloride solution. The specimens injected with India ink and Berlin blue were fixed in 10 per cent formalin, dehydrated in alcohol, and cleared in oil of wintergreen.

The methylene blue injections were made by the supravital technique as described by R. G. Williams (Bazett et al., '32) and as applied by him to the rabbit's ear (R. G. Williams, '31). They were carried out at Doctor Williams' suggestion, and their success was dependent upon his assistance and advice.

Blood vessels were injected with Ranvier's carmine-gelatin mass (Lee) immediately after killing the animal with chloroform and washing the vessels with Ringer's solution. In double injections, the lymphatic vessels were injected while the animal was alive. Then the blood vessels were injected as described.

In collecting lymph, the animal was placed on its back or side, the ear extended horizontally, or with its tip slightly dependent. The anaesthetic used was: 1) nerve block with 1 per cent cocaine solution; or, 2) nerve block with 2 per cent procaine solution; or 3) sodium amytal intraperitoneally or intravenously, 300 to 500 mg., depending upon the size of the rabbit and the duration of the experiment.

The cannulae used for collecting lymph were drawn out from ordinary glass tubing. They were calibrated in millimeters to facilitate observations. Their inside diameters (average, 1 mm.) were determined by an ocular micrometer in order to calculate the volume of lymph. Working under a binocular microscope, a transverse incision was made near the junction of the proximal and middle thirds of the ear, over a large blood vessel, preferably the median (main) artery. An accompanying lymphatic was isolated, distended

with lymph by massage toward that point, and a cannula introduced. An attempt to limit the flow of lymph in anastomosing vessels was made by adding a weight proximal to the site of operation—a weight heavy enough for this purpose, but light enough to have practically no effect upon the flow of blood.

The type of transparent chamber used was the 'preformed tissue' chamber (Clark et al., '30). In its insertion, the ventral (inner) skin and the cartilage of a portion of the ear are removed with a minimum of injury, the mica cover of the chamber being brought into contact with the subcutaneous tissue of the dorsal (outer) surface. Thus, the tissue and vessels observed are those already present ('preformed') in the ear at the time of operation. In the insertion of this type of chamber, a kodaloid disc or 'platform' 0.62 mm. thick, is used which occupies that part of the chamber away from the median artery, nerve, and lymphatic collecting vessels. The use of the 'platform' produces two distinct regions, namely, a thinner region over the disc, which will be called the 'platform' region, and a thicker region in which the large vessels and nerves are located, which will be called the 'groove.'

Injections used in conjunction with chambers were 33 per cent India ink in sterile tap water, sterile 1 per cent carmine in 3 per cent gelatin, and a sterile saturated solution of Berlin blue in 3 per cent gelatin.

THT DISTRIBUTION OF LYMPHATICS

Specimens of the intact ear, injected with India ink, show two rich lymphatic plexuses, deep and superficial, on each surface (fig. 2). Around the periphery, these are composed largely of numerous small capillaries with blunt ends. Nearer the center, the superficial layer contains many capillaries similar to those of the periphery. The deep layer, situated near the cartilage is composed of relatively large vessels, containing numerous anastomoses. These lymphatics empty into slightly larger collecting vessels, which for the most part follow the distribution of the larger blood vessels, form-

ing networks around them (fig. 1). Like the blood vessels (Hoyer, 1877), the lymphatics of the ventral surface penetrate the cartilage in many places, and empty into the collecting vessels on the dorsal surface (fig. 2).

In the general convergence of vessels at the base of the ear, the number of larger collecting lymphatics exceeds that of the large arteries and veins. Usually several lymphatics accompany each of these blood vessels. The total number of collecting lymphaticse at the level at which the cannulae were

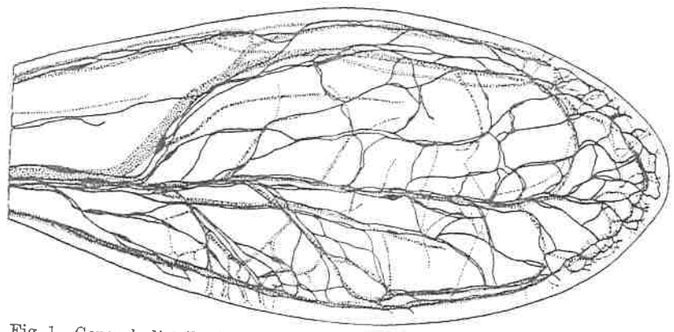

Fig. 1 General distribution of collecting lymphatic vessels (represented by solid black) of the rabbit's ear compared with that of the larger blood vessels (represented by dots). This illustration is a composite drawing made from a number of cleared specimens in which the lymphatics had been injected with India ink, and the blood vessels with carmine-gelatin mass. Natural size.

inserted varies from 10 to 14. In their further course, there is very little reduction in number, 8 to 12 running separately into the auricular nodes.

The lymphatic vessels of the rabbit's ear are endothelial tubes around the larger of which are small and varying amounts of connective tissue. In the specimens injected with methylene blue, occasional smooth muscle cells were seen on some of the larger lymphatics, but they were very few and irregularly spaced (fig. 3). The paucity of extra endothelial tissue is such as to indicate the likelihood that substances might diffuse readily into practically all of the

lymphatics of the ear, including even the larger vessels. Whether this does occur normally, however, has not been tested.

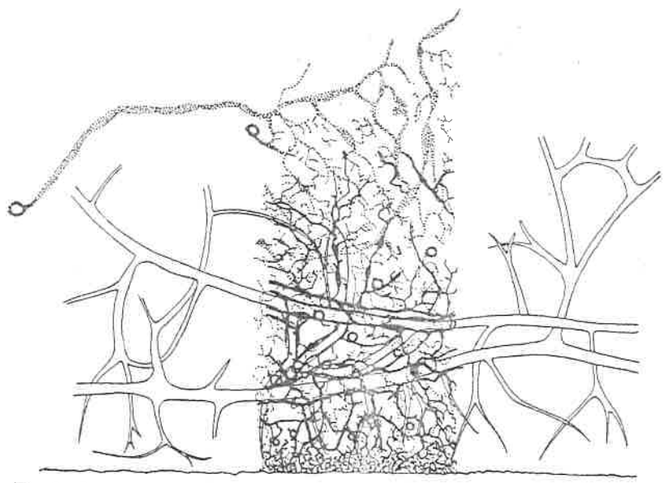

Fig. 2 Lymphatic capillaries at the periphery of the rabbit's ear. A number of lymphatics may be seen going through the cartilage (represented by circles). The blood vessels are shown in outline. Magnification, × 10. Lymphatics of dorsal side dotted; lymphatics of ventral side solid black.

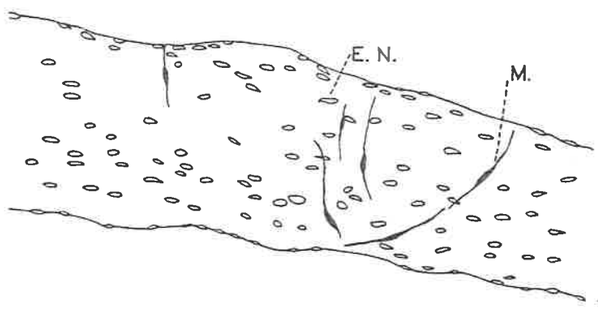

Fig. 3 The appearance of a large lymphatic collecting vessel in a rabbit's ear injected supravitally with methylene blue, showing endothelial nuclei (E.N.) and smooth muscle cells (M.). Magnification, × 130.

THE AREA OF LYMPHATICS

The relative surface area of lymphatics and blood vessels was determined upon injected specimens. The inaccuracies of this method are appreciable and the results are presented for their comparative rather than actual values.

The blood vessels in six fields and the lymphatics in another six fields were studied. Each field was selected for the completeness of the injection and was 0.75 mm. square. Drawings were made with a camera lucida at a magnification of 160. The areas (projections of the greatest dimensions of the vessels as seen from the surface) were computed with a planimeter. The average of these was taken, and reduced to actual size. From these results, determinations were made for 1 sq.mm. of surface area of ear. Assuming the vessels to be cylindrical, these figures multiplied by π give their surface areas. The results are:

Surface area of lymphatic vessels, 1.43 sq.mm. per sq.mm. surface of ear
Surface area of blood vessels, 1.63 sq.mm. per sq.mm. surface of ear

Calculations on living vessels were made in a similar manner in a 'preformed tissue' chamber. In this case only the vessels of the dorsal surface were measured, and only two fields of each were taken. The lymphatics were injected with India ink. The blood vessels contained an average amount of blood. The results are:

Surface area of lymphatic vessels, 1.36 sq.mm. per sq.mm. dorsal surface of ear
Surface area of blood vessels, 1.18 sq.mm. per sq.mm. dorsal surface of ear

From the results obtained from measuring several ears, 5000 sq.mm. (nearly 8 sq.in.) may be taken as the average area for one surface. For such an ear, the total surface area of lymphatics would be 5000×1.43 or 7150 sq.mm.

THE MOVEMENT OF LYMPH

The movement of injected particles (India ink, carmine, Berlin blue) in the smaller lymphatics of the intact ear occurs in both directions. In the larger vessels it is limited to proximal flow by numerous competent valves. Retrograde

injection, after the insertion of cannulae was found impossible. Similarly, once injected material enters the larger vessels, there is little or no return to the smaller.

With little force from behind, injected particles dart rapidly along. The many anastomoses permit particles injected at one site to follow a number of routes. Material injected on the ventral side of the cartilage readily runs through to the other. This is particularly noticeable after the injection of India ink on one side and Berlin blue on the other. Although the black ink is dominant, the appearance of contrasting colors in confluent vessels is sometimes quite striking.

Even when the larger vessels are ligated proximally, only slight pressure is necessary to carry particles injected in the smaller vessels into them. They become irregularly distended due to fusiform supravalvular dilatations. These occasionally rupture, even after injection has been completed. Very soon after the injection is stopped, rapid movement of the particles ceases. They are then seen to move along when helped by massage or by active movement of the ear. There is seen also an intermittent movement, apparently spontaneous.

In the 'preformed tissue' chamber, the following factors have been observed to produce flow of lymph, as indicated by the movement of particles in it:

1. Changes secondary to blood vascular phenomena; a) A slight back and forth movement, synchronous with arterial pulsation, and apparently caused by its transmitted force (compare Clark and Clark, '32 b). b) A longer excursion due to changes in caliber of the blood vessels. Thus, when the median artery contracts, particles in lymphatics in the 'platform' region of the chamber move toward it, and when it dilates, they move away from it (compare Clark and Clark, '32 b).

2. Active movements of the ear by the animal.

3. Massage. This produces rapid movement toward collecting vessels and along them centripetally.

4. Force from behind, as in injections.

5. The position of the ear. Movement is sometimes caused when the ear is held vertically, but the effect produced in this way has always appeared small.

Immediately after the insertion of a 'preformed tissue' chamber, some lymphatics are seen filled with masses of blood. Several days later these masses begin to move back and forth through short excursions, apparently caused by the transmitted force of arterial pulsation. They begon to break up, and after a number of days, the blood moves on. Then the lymphatic vessels appear as long, narrow, clear spaces, due to the colorless lymph contained. They vary in width from day to day. A lymphatic which yesterday was prominent may be invisible today, and one whose existence was unknown may appear. Injections show that relatively few of the total number are visible at any one time. It should be emphasized that this applies only to the 'preformed tissue' chamber, and not to the much thinner 'round-table' chamber, where all lymphatics are visible at all times (Clark and Clark, '32 b, '33).

Ten to fourteen days after the insertion of a chamber when recovery seems complete, particles of debris are occasionally observed in the small lymphatics of the 'platform' region. They may remain there for varying lengths of time—a few seconds to several days. That they are free is shown by their to and fro motion, and by their ready movement when the mica cover of the chamber is depressed, or when massage of the tip of the ear produces a rapid current of lymph.

While injected particles, once having reached the larger vessels accompanying the median artery, tend to move proximally, not infrequently some move peripherally into the vessels in the 'platform' region of the chamber. Thus, material inserted at the tip of the ear has been seen to appear in the chamber, 5 cm. away. Under ordinary conditions, following the injection of as much as 0.1 cc. this appearance is almost instantaneous. In small injections and in inflamed conditions the appearance is delayed.

Little or no obstruction is offered to the flow of blood by the 'preformed tissue' chamber. But there seems to be in the 'platform' area pressure sufficient to cut off lymphatic communication peripherally. With one exception, all material, no matter how close to the chamber it was injected, flowed around and entered the chamber via the collecting vessels in the 'groove,' accompanying the median artery.

OPENINGS IN LYMPHATICS

In the first few days after the insertion of a 'preformed tissue' chamber, places have been observed where fluid and erythrocytes seemed to come out from the lymphatic vessels to the fluid layer present between the surface of the tissue and the mica cover. In the same places, flow in the reverse direction, also, was observed. At times distinct openings were discerned (compare Clark and Clark, '33).

In order to make certain that these were actual openings between the lymphatics and the extravascular space, India ink, carmine, or Berlin blue was injected into the lymphatics distal to the chamber. The granules so injected passed along lymphatics to the chamber area, and in a number of cases streamed out into the layer of fluid over the top of the tissue through small but definite openings. The number of such was always small, varying from two to four per specimen.

A typical study of such openings, with the aid of injected granules in one specimen is as follows:

Three days after the insertion of the chamber, erythrocytes were seen emerging from and re-entering a lymphatic through an opening at the base of the 'platform' area, near the 'groove' (designated opening 1; see fig. 4). The next day this opening was obscured by extravasated erythrocytes between the tissue and mica cover.

Eight days after the insertion of the chamber, these erythrocytes had largely disappeared. India ink was then injected near the tip of the ear. The ink granules were at once seen passing through collecting lymphatics in the 'groove.' Some entered lymphatics on the 'platform' of the chamber,

and from one of these vessels, particles could be seen emerging and becoming free in the fluid space between the tissue and the mica cover. With the compound microscope (magnification of 100) the course of the lymphatic and the opening itself could be distinctly seen (designated opening 2). On this day, no particles were seen re-entering. The movements of the particles within the lymphatic vessels were of the

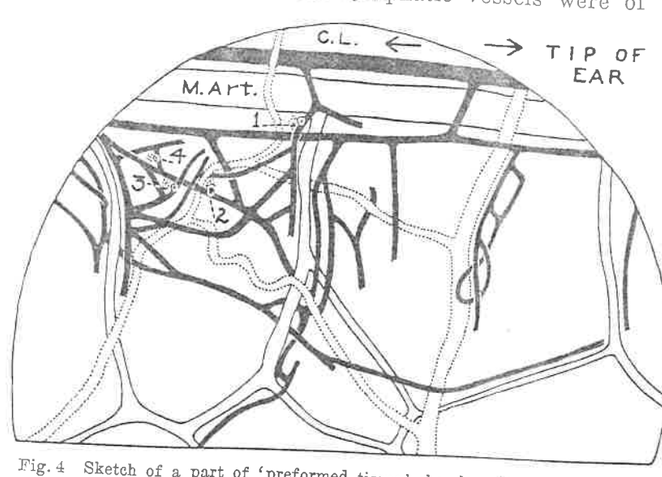

Fig. 4 Sketch of a part of 'preformed tissue' chamber (3-9-32L) illustrating the location and appearance of the four collections of India ink granules outside of the lymphatic vessels. Made on the ninth day after the installation of chamber, and 24 hours after the injection of India ink into the tip of the ear, distal to the chamber. The numbers in the figure corresponded to the designations given the sites in the text. Magnification. × 7.

kinds already mentioned as due to forces produced by changes in the blood vessels (v. s.), but movement forward exceeded that backward. Very gentle massage produced a rapid movement forward, and an outburst of particles from the opening.

On the ninth day, four collections of carbon granules were seen. Two were around openings 1 and 2. At the other two, no openings were seen. An occasional erythrocyte or ink granule came from opening 2.

On the following day, the collections of granules were unchanged. Erythrocytes were emerging and a few were reentering at opening 2. While watching one of the collections of granules at which no opening had been seen, erythrocytes appeared and a small opening was identified (opening 3). At the fourth site, erythrocytes appeared at a definite location, but no distinct opening was identified.

On the eleventh day, an occasional erythrocyte came out or went in through opening 2. None appeared at the other sites. Massage of the tip of the ear increased the flow from opening 2, but produced no perceptible effect at the other sites. During the next 2 days, the condition remained unchanged.

On the thirteenth day, an injection of carmine was made beside the previous injection of ink. Carmine granules appeared at and emerged from opening 2 in about 30 seconds. Other granules were seen traversing uneventfully the vessels formerly containing openings 3 and 4, passing the sites as through normal vessels, and none emerging.

The following morning, many more granules were around opening 2, concealing it. A few were around opening 1. None were at or near sites 3 or 4. On the 5 following days, no change was noted, and nothing was seen emerging or entering at 1 or 2. India ink was injected again. Particles passed uneventfully through all the lymphatics formerly containing openings, none emerging.

Thus, three distinct openings, whose existence was confirmed or discovered by the injection of granules into the lymphatics, were watched from day to day. One persisted for 10 days, and two for 13 days after the insertion of the chamber.

In a later chamber, two similar openings were observed from the second through the eighth day.

THE AMOUNT OF LYMPH

The results of the collection of lymph by the introduction of cannulae into the larger vessels are given in table 1.

When the collection was begun, the temperature of the ear was usually just a little above room temperature, and varied upon different occasions from 25°C. to 29°C. After an hour or so, the ear was heated by electric lights to 38°C., and the temperature maintained at that point for as long as an hour. This increase in temperature was followed by dilatation of the blood vessels, and a speeding up of the flow of blood. But no increase in the rate or amount of flow of lymph was noted.

Since the lymph collected was in each case from only one of the eight to twelve parallel vessels draining the ear, it was but a fraction of the total lymph flowing from the ear.

TABLE 1

The collection of lymph by cannulae introduced into lymphatic vessels of the rabbit's ear

EXPERIMENT	ANAESTHETIC	SPONTANEOUS FLOW	TIME	MASSAGE FLOW	TIME	TOTAL FLOW	TOTAL TIME
		Cu.mm.		*Cu.mm.*		*Cu.mm.*	
1	Cocaine	9.75	4° 15′	69.81	25′	79.56	4° 40′
2	Cocaine	15.07	2° 15′	1.94	1′	17.01	2° 16′
3	Sodium amytal	c.2.00	1°	0.50	10″	c.2.50	1° 10″
4	Sodium amytal	0	2°	25.57	3°	25.57	5°
5	Sodium amytal	44.0	4° 55′	19.50	20′	63.50	5° 15′

This fraction is variable, and its value can be only roughly estimated. From the effect of massage in various parts of the ear while the lymph was being collected, and from injections made at the completion into different parts of the ear to determine the area drained by the vessel in which the cannula was inserted, it is estimated that the collected fluid represents about 10 to 20 per cent of the total.

There are other sources of error such as possible obstruction caused by the cannula, injury at operation, the effect of capillarity, etc. The results given in the table were obtained with the cannulae horizontal, and no allowance was made for capillarity. Cannulae were usually left in until dislodged by movement of the animals.

Taking the total amount of lymph collected (189 cu.mm.) whether obtained by spontaneous flow or massage, and dividing by the total time (18.18 hours), a mean flow of approximately 10 cu.mm. per hour is obtained. Assuming that this is but 10 per cent of the whole, there would be 100 cu.mm. per hour from all the vessels of a whole ear.

These figures may be correlated with those for the area of lymphatics (v. s.), remembering that the possible errors of both still remain. The total mean flow (100 cu.mm. per hour) divided by the total surface area of lymphatics of our assumed average ear (7150 sq.mm.) gives 0.014 cu.mm. of lymph per square millimeter of lymphatic surface per hour.

By similar calculations, the spontaneous flow in the separate experiments varied from 0 to 0.0125 cu.mm. of lymph per square millimeter of lymphatic surface per hour. The greatest continuous massage flow was 0.084 cu.mm. of lymph per square millimeter of lymphatic surface per hour.

DISCUSSION

It is quite likely that the calculations of surface areas presented are understatements of the actual areas. Especially is this probable in computations made on the fixed specimens. In them the values for both surfaces of the ear are but a little higher than for the one surface studied in the living. In the numerical expression of the surface area of the lymphatics, the smaller figure was used, so that the given ratios of rate of flow per unit area are probably high.

For such an extensive endothelial surface, the amount of lymph flow in the lymphatics seems exceedingly small, especially when the size of the blood vessels and the amount of blood vessels and the amount of blood flowing through them are compared. It is, however, in general agreement with studies of lymph flow in peripheral parts of the body, such as the extremities, where investigators have found little or no movement of lymph in the resting animal (compare Starling, '09; White, Field and Drinker, '33; Drinker and Field, '33). Attention is again called to the possibilities of error in the quantitative expression of the lymph flow.

It is interesting that my finding regarding the relative sizes of blood vessels and lymphatics in a given area of the ear agrees closely with somewhat similar measurements made by Drinker and Field ('32) in the web of the frog's foot. They also found the lymphatics and blood vessels approximately equal in area.

The factors which have been observed to cause movement of lymph are listed. It will be noted that all of them are passive, in so far as the lymph vascular system itself is concerned. However, movement of particles was frequently observed which could not be assigned definitely to any of the accessory factors mentioned. The muscular factor in the propulsion of lymph must be negligible in peripheral vessels, because of the extreme scantiness of smooth muscle cells on such vessels. The questions of filtration, osmosis, diffusion, and secretion, and the elasticity of the endothelium as factors concerned with the movement of lymph in lymphatic vessels, have not been directly studied in this investigation.

Since these studies were carried out, the 'preformed tissue' chamber as used in them has been modified by the addition of a splint and shield which greatly reduces trauma to the tissue in the chamber. It seems that, unlike the old, the improved chamber offers little or no obstruction to the lymphatics at the periphery of the 'platform.' In a personal communication, Dr. E. R. Clark informs me that in the improved chamber he has observed erythrocytes within the lymphatics move onto the 'platform' from the periphery and in the reverse direction with no apparent impediment. Since the chief difference in the two consists of the greater amount of new tissue formed in the older type of chamber in reaction to minute injuries, it is suggested that this factor alone may be sufficient, in a confined space, to interfere with the movement of lymph in the lymphatic.

With regard to the openings in lymphatics, the injection experiments reported have proven that, following the insertion of a chamber, which involves injury to lymphatics, definite openings may be present which connect their fluid content

with the fluid outside. These may persist for as long as 13 days. This time may have been prolonged somewhat by the force of the injection or by the presence of the foreign material.

It should be emphasized that all of the openings seen were between the lymphatics and the layer of fluid over the top of the tissue, between the tissue and the mica cover. The significance of these openings has already been discussed in detail (Clark and Clark, '33).

SUMMARY

1. The rabbit's ear possesses a rich lymphatic supply of both superficial and deep vessels, with eight to twelve collecting vessels, which follow the course of the main blood vessels.

2. The vessels of the inner side of the ear pass through the cartilage to join those of the outer side.

3. The total area of the lymphatics of the ear as shown by surface projection is approximately equal to that of the blood vessels.

4. By the introduction of cannulae into lymphatic vessels draining the ear, it was possible to secure data regarding the amount of lymph flow. This was found to be variable, but always extremely small, even when massage was freely used.

Calculations indicated figures ranging from 0 to 0.0125 cu.mm. per square millimeter of lymphatic surface per hour, spontaneous flow, and to a maximum of 0.084 cu.mm. per square millimeter of lymphatic surface per hour after massage.

5. Studies of injected granules indicate that there was some obstruction of lymph flow in the thinner but none in the thicker parts of the older type of 'preformed tissue' chamber.

6. By injected granules it was possible to prove definitely that the suspected openings between lymphatics and the supernatant fluid, seen following the installation of the 'preformed tissue' chambers, are real openings into lymphatics, and that they may persist for as long as 13 days.

These studies were made at the suggestion and under the direction of Dr. Eliot R. Clark, whom I wish to thank for his interest and assistance. I wish to thank Dr. R. G. Williams also, for his many helpful suggestions, especially in matters of technique.

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