STUDIES OF THE REGENERATION OF LYMPHATIC VESSELS

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INTRODUCTION

THE following paper describes in sections work which has been going on for three years on some of the problems arising in connexion with the regenerative growth of lymphatic vessels in human and animal tissues.

In the proximate analysis one may distinguish two groups of problems in this field, the first concerned with the stimuli adequate to cause regenerative growth of the vessels and with the nature of this initial growth, and the second with the factors responsible for the subsequent elaboration of the vessel walls, that is, orientation, modelling, and valvular development in any of the newly formed lymphatics. While following the phenomena of regeneration in the living animal it will be seen that these two main problems overlap to some extent, but, broadly speaking, attempts have been made to analyse first the factors concerned in regeneration in the adult of the smallest lymphatic capillaries, next the capacity for regenerative growth of the larger lymphatic trunks in the adult, and lastly the laws governing the reformation in the adult of a fully formed system of functioning vessels orientated and valved for the carriage of lymph in an appropriate manner.

In the analysis of this series of interrelated problems it is difficult to set out experiments in a perfectly simple succession following logically from one deduction to the next. Also in the course of three years of work the technique and methods at one's disposal develop appreciably, so that later work yields more precise information than the earlier. For these reasons it is proposed to set out the work in the order in which it was actually done irrespective of the logical sequence of conclusions which appears from the whole, the expression of which is left to the summary. Relevant literature also is considered separately in each of the three sections of the work.

The first section deals with observations made from human material, and is subject to the serious limitations imposed by lack of proper control tests and the difficulties of complete analysis. In spite of this it is felt that the observations are worth recording. The second and third sections of the work were done in the two succeeding years, the second consisting of experiments on animals and yielding information chiefly of a preliminary and elementary character, while the third, which also describes experiments on animals, goes further into the details of the problems outlined above.

PART I. REGENERATION OF LYMPHATIC VESSELS IN HUMAN TISSUE

Literature

Although it must have been evident for a very long time that at least in some circumstances re-establishment of lymphatic circulation after incisions into the tissues must occur, there is only a relatively small literature dealing with this subject. Taking the significant papers more or less chronologically, it will be seen that Billroth (1863) failed to find that any lymphatic vessels developed across scar tissue in the early stages up to 7 days. On a different aspect of this same fundamental problem of the initial causes of lymphatic regeneration, Krause (1863) reported the growth of new lymphatics into tumour tissue. Then came a series of papers by Bayer (1885), Delius (1888), Ribbert (1889), and Heuter (1904) towards the end of the century, describing the rather equivocal results of lymph-glandular regeneration following excision. Unfortunately, it is not clear from these papers what changes, if any, were observed in the lymphatic vessels. The next literature to be noticed consists of quotations by Bartels (1913) that Talke found new lymphatics in pleuritic membranes, and Poirier and Morau found new lymphatics in recto-uterine adhesions.

These general statements of the previous century were followed by Meyer's work (1906) on the extirpation of lymph glands. Among other experiments he tied and divided lymphatics around the saphenous vein in eight dogs, and observed the animals for periods of 14–91 days. He could find no evidence of regeneration of these vessels, and attributed the failure to insufficient lymph stasis.

T. H. Coffin (1906) found new lymphatics growing in granulation tissue 10 days after bringing a loop of intestine outside the abdominal wall. H. M. Evans (1908) showed the presence of new lymphatic vessels in relation to a sarcoma deposit. Eloesser (1923) experimented on obstruction to lymphatics due to scar tissue by making incisions across the dorsum of rabbits' ears down to the cartilage, and leaving them to heal by granulation. On injection of the lymphatics with India ink it was found that only a few regenerated vessels existed across the wound, and the scar obstruction to the vessels persisted in animals examined as long as 4 months after the operation. A more scientific analysis of the effects of severing lymphatic vessels was carried out by Reichert (1926), and more recently the problems have been touched upon by the researches of Rouvière & Valette (1937). These latter papers, and the work done by Pullinger & Florey (1937), will be considered more fully in other parts of this paper.

The human material examined, methods, and summary of results

The human material used came from a variety of sources. In fact, any human material which might shed light on the problems was used, and I have to thank the surgeons of St Bartholomew's Hospital, and particularly Sir

Harold Gillies, for the generous way in which they gave me every possible help and facility.

The tissues so examined were either living or freshly removed at operation and still warm when they reached the laboratory. In each case the lymphatic vessels of the tissue were injected with thorotrast by the method of Woollard & Gray, which was first reported to the Anatomical Society in 1935, and later described fully by Gray (1939). X-ray photographs of the tissues were taken, and where possible the specimens were subsequently dehydrated and cleared by the Spalteholz method. Thick sections, as well as thin stained sections, were examined microscopically.

It may be stated before describing the results that the only control which can be used in examining adult human lymphatic vessels in this way is supplied by the fact that all authoritative accounts of the normal human subcutaneous lymphatic trunks agree in claiming a relatively high constancy of topography, involving position, direction and even to some extent number of these vessels in the different regions of the body. Study of the works of such authors as Sappey (1874), Poirier *et al.* (1903) and Rouvière (1932) reveals this, and my own injections of the normal have confirmed it.

Human tissue type no. 1. This was a specimen of the skin of the leg distal to an epithelioma which was circumferential in growth about the ankle, and had involved the skin of the leg in front and at the sides of the ankle, leaving only a narrow wick of normal skin posteriorly to drain the lymph from the whole foot. The ulcer was of varicose origin, subsequently becoming malignant, and causing deposits in the popliteal and inguinal lymph glands. In the areas affected there was much destruction of underlying tissue, exposing even the periosteum in patches. This tissue, and the findings obtained, have already been described in another paper (Gray, 1939) as tissue no. 9. At the margins of the ulcer in certain regions there was the usual warty overgrowth of the dermal papillae. This was so extensive that some individual papillae had attained a length and breadth of several millimetres. In such papillae very extensive networks of lymphatic capillaries were found. This observation shows that severing of a lymphatic capillary is not the only and essential stimulus necessary for the growth of these vessels in the adult. Also from a study of these enlarged papillae some indirect evidence is obtained as to the method by which this new production of lymphatic vessels occurs. Theoretically, there are several possible ways in which such a network of vessels could be formed. That they might be produced out of non-lymphatic tissue independently of pre-existing lymphatic vessels can be excluded finally because of the fundamental researches on the subject by Sabin (1902) and E. L. & E. R. Clark (1932). But from pre-existing lymphatics in the dermis they might be produced either by interstitial elongation of the loops and segments of the network, or by a sprouting of new segments as blind diverticula which later join other segments to form new loops. Lastly, both of the latter phenomena may occur together. The density of the network in these enlarged papillae does not favour the first of these latter possibilities as the sole method. Also examination revealed that in addition to the closed network in the papillae there were a number of blindly ending diverticula projecting from the network particularly at its peripheral margins, and showing the apex or free end of the tube in most cases turned back like a hook. The picture was thus suggestive of a sprout not yet united completely to form a loop.

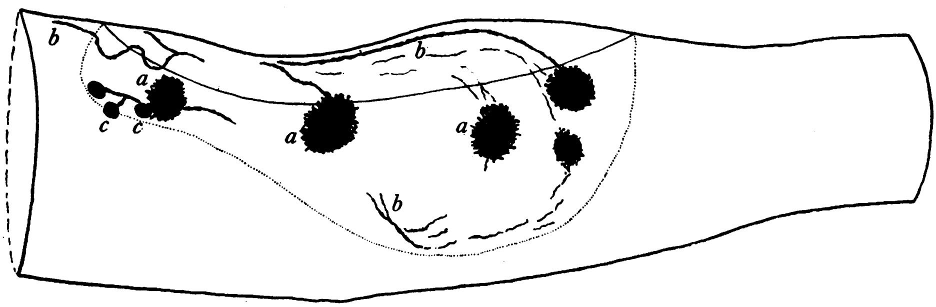
The above account describes the findings in the first type of human tissue examined in their relation to the first group of problems of lymphatic regeneration outlined in the introduction.

This tissue also provided some evidence in relation to the problems of vessel remodelling and reorientation as seen in the adult. The skin of the whole leg was removed in one piece, and later divided into many portions for detailed study. A plan of the whole skin was drawn, and the course which would be taken normally by the subcutaneous lymphatic trunks of the leg compared with that actually obtaining in the specimen. The findings have been recorded incidentally in a previous paper (Gray, 1939), to which reference must here be made. They show that in the pieces labelled "*E*" and "*G*" in the figures, representing skin overlying and postero-inferior to the lateral malleolus, the lymphatic trunks have changed their direction by about 90° to run backwards and upwards into the narrow area of normal skin overlying the tendo Achillis.

The presumption is that this change from the normal has occurred by the enlargement and further development of small existing anastomotic lymphatic vessels in response to the damming of the lymph flow by the extensive ulcer. Of course the findings provide no proof of this, but, as they stand, they are a definite contrast to the normal arrangement of lymphatics for this region. It may be added that at the time of operation there was considerable lymphoedema of the foot, and the specimen after injection of the lymphatics with thorotrast shows considerable dilatation of the lymphatic vessels below the level of the ulcer, but no noticeable dilatation above the ulcer. The very great number of medium-sized lymphatic trunks seen in the area of the narrow "wick" of normal skin overlying the tendo Achillis (piece *F*, Figs. 333, 336, Gray (1939)) is very different from the normal found for this region. Whether it is due to a dilatation of vessels or a growth of new channels cannot be determined from the specimen, though the former explanation seems the more probable.

Human tissue type no. 2. This tissue was studied in the living subject. Injections of the lymphatics with thorotrast were done in a large skin graft, which had been transplanted whole as a vascularized flap from the anterior abdominal wall to the volar surface of the right arm and forearm. The patient, Miss C. T., aged 28 years, was under the care of Sir Harold Gillies at St Bartholomew's Hospital, and was treated by plastic operations for the relief of lymphoedema of the legs. The history of the case and the successful operations devised have been published by Gillies & Fraser (1935). For the purposes of the present paper it may be stated that the transplanted skin came from the right side of the anterior abdominal wall, and included areas in which some lymphatics ascend towards the axillary glands while others descend towards the inguinal glands. The graft was transplanted on to the volar surface and medial side of the right arm and forearm in such a way that there could have been no possibility of accurate alignment of the lymphatic trunks already in the flap with those in the arm skin around the edges of the flap. The graft had been united for many weeks, and there was no oedema of the flap at the time of injection.

The findings are indicated in Text-fig. 1, from which it will be seen that they have a bearing on the second group of problems of this work, the final modelling of regenerated lymphatic vessels. The final vessels resulting in the transplanted skin do not in any way correspond in pattern or direction with those of abdominal skin. But they do follow roughly the lines which vessels would take in normal arm and forearm skin in this situation. Some of them



Text-fig. 1. Diagram constructed from radiographs of right upper extremity of a patient with abdominal skin transplanted to medial side of arm and forearm. Thorotrast injections (*a*) were made at five areas within the transplant, whose limits are indicated. Lymphatics are shown as wavy lines (*b*). Supratrochlear glands have been filled (*c*).

have succeeded in establishing connexion with the supratrochlear lymph glands.

As in the first type of human tissue examined it is seen that the final result of the remodelling and reorientation phenomena seems to accord with the particular functional requirements of the case, and one is inclined to attribute the changes to "hydrodynamic" forces which may act in a way analogous to Thoma's first and second laws for blood vessels.

Human tissue type no. 3. The third type of human tissue also has some bearing on the second group of problems. This tissue was a portion of a tubed pedicle made from the skin of the iliac region of the anterior abdominal wall for use in the cure of hypospadias. I was indebted for this specimen also to Sir Harold Gillies. The pedicle was constructed by the usual method of making two parallel incisions followed by turning under and suturing together the two edges of the strip of skin thus freed. The length of the pedicle given to me was 6·5 cm., and this portion was taken from the lower and medial part of the entire structure, which lay parallel to the inguinal ligament and was about 15–18 cm. in total length. The pedicle had been left for some 3 weeks before the specimen was removed.

Many injections of the specimen were done, and the findings, deduced from the radiograph and from thick cleared sections and thin microscopic sections, indicate that there have certainly been some changes leading to a rearrangement of the lymphatic trunks in the course of 3 weeks. The normal arrangement of the lymphatics in the skin used for construction of the pedicle is preserved as a series of loops of vessels circling round in the fat of the pedicle in a plane at right angles to its long axis (Pl. I, fig. 1). The chief modification which has occurred is that there is seen a tendency for these loops to become transformed into vessels running parallel to the long axis of the pedicle. The change is most noticeable among the smaller vessels visible in the radiograph. The larger trunks have retained the encircling disposition they were given when the pedicle was made.

It may be supposed that the small vessels tending to run parallel to the long axis of the pedicle represent pre-existing anastomotic channels, which, because of the necessity for conducting lymph one way or another out through an end of the pedicle, have become enlarged in the course of 3 weeks. Unfortunately, no specimen of a similarly placed pedicle left *in situ* for a longer period has been available. Although caution is necessary in making deductions from a single specimen, it may be said that 3 weeks is not sufficient time for very marked changes in the larger lymphatic trunks in response to special stimuli of altered flow and pressure, but is probably adequate for some readjustment sufficient in the circumstances of the present case to prevent lymphoedema.

The readjustment which has occurred may have taken place in one or more of several possible ways, which cannot be further elucidated from this single specimen. There may have been a dilatation of existing anastomotic channels as mentioned above; there may have been a growth of new capillary lymphatic vessels which became later elaborated into small trunks; or, lastly, some trunks themselves may have sprouted new vessels either along their length or at their ends severed when the pedicle was made.

PART II. REGENERATIVE CAPACITY OF LYMPHATIC VESSELS

Literature

Literature describing experimental work on this subject is not very extensive. The work of Meyer (1906), of Coffin (1906) and of Eloesser (1923) has been mentioned already. From such papers it appears that, although regenerative growth may occur from capillary lymphatics, very little, if any, can be expected from definitive lymphatic trunks. The reasons presumed by the authors for this limitation are either difficulty in producing sufficient lymph-stasis pressure in the severed trunks, or interference by scar tissue. Substantial evidence for the first of these suppositions is not obtainable from Meyer's work, and full histological analysis of the alleged interference by scar tissue is lacking in Eloesser's work.

In 1926 Reichert carried out an accurate analysis of the results of severing lymphatic trunks followed by replacement of the cut ends. After many trials he perfected a technique for making a circular incision through the middle third of the thigh leaving only the femur and femoral artery and vein. These too were stripped of all areolar tissue, and the incision went through the periosteum and the adventitia of the vessels. It was followed by very careful resuture of all the tissues. Healing by first intention with the minimum of scar resulted. Lymphatics were then injected with India ink in animals killed at intervals of 2 days to 14 months after the operation. The results are worth quoting in detail, as they not only represent a definite advance in knowledge but serve as the starting point for further work.

When the operation was perfectly done, Reichert found that the earliest regeneration appeared in the superficial set of lymphatics 4 days after the operation. At three or four areas along the line of skin division tiny lymphatics (presumably as seen with the naked eye) ran from the distal vessels across the cut, and then converged again to enter a single lymphatic on the proximal side. Elsewhere superficial lymphatics only filled with ink up to the cut. If much scar was formed, no such union of vessels was found to occur. Regeneration of deep vessels was seen first at 8 days, vessels which twined about in relation to the muscle septal sheaths crossing the wound as minute segments. From later injections, up to 20 weeks, it was seen that the main trunks were often united, sometimes with no evidence of the cut, sometimes with a kinking or irregularity in the vessel at the point of cutting. To be correlated with these findings there was oedema distal to the cut, beginning on the second day, reaching maximum on the fourth or beginning of the fifth day, and disappearing completely by the eighth day. Reichert summarized his results in the observation that there was some arterial regeneration by the third day, and some venous and lymphatic regeneration by the fourth day. He also tried to find out the relative importance of the veins and the lymphatics for the relief of the oedema by ligaturing the femoral vein at different times after severing the lymphatics. Ligature of the vein at the time of maximum oedema caused only slight delay in its subsidence. Ligature at 8 days caused reappearance of oedema only if the lymphatics were blocked by India ink. Ligature after longer intervals caused no oedema even if the lymphatics were also blocked. From this he concluded that the lymphatics are important in relieving oedema at the 8-day stage. Also from these experiments he reached the conclusion that lymphatico-venous stasis is a stimulus for the regeneration of both deep and superficial lymphatics. Reichert's experiments do not decide whether it was the severing alone, or the lymphatico-venous stasis alone, or both of these, which stimulated the regeneration. His account was confined, as far as can be determined, to naked-eye appearances. But his experiments do show that the cut ends of lymphatic trunks can rejoin to re-establish the circulation, if they are placed in apposition and the conditions as regards scar tissue are favourable. The exact methods and steps by which this occurs were not examined.

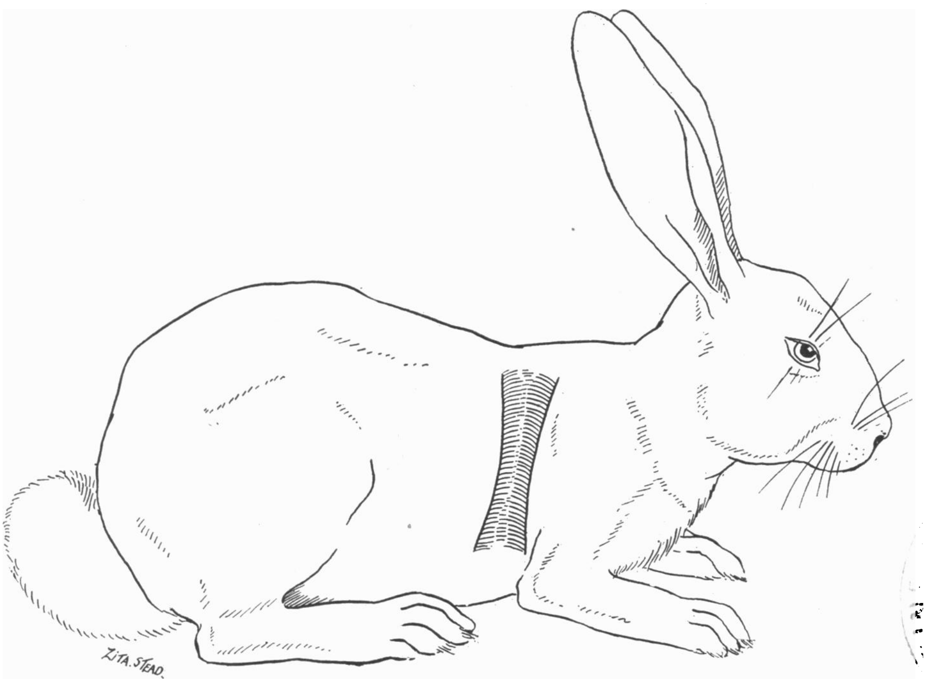
Analysis of the exact method of growth and regeneration of the lymphatic vessels has been done chiefly by American anatomists. Sabin (1902) proved that in the embryo all lymphatic vessels arise by sprouting from pre-existing capillary lymphatics, and E. L. & E. R. Clark (1932) followed the growth phenomena of lymphatic capillaries in the living adult rabbit's ear. They extended Sabin's statement to include new lymphatic vessel growth in the adult, and they were able to watch isolated capillary lymphatics for long periods of time. It is to be noted that they only recorded sprouting from the fine vessels. The large valved trunks with composite walls are not given such capacity by these authors. In fact in one of their papers they describe how the cut ends of large lymphatic trunks remain open in direct connexion with the tissue spaces for long intervals of time. It is to be noted that this observation was made on lymphatic trunks which had been torn or severed in the field of the transparent chambers used by these workers in the rabbit's ear. The environment of such vessels was therefore perhaps not absolutely identical with that normally existing.

The transparent chamber studies of E. L. & E. R. Clark have also shown in precise detail the manner in which fibrous connective tissue interferes with the growth and form of lymphatic capillaries. But the most striking illustrations of this mechanical influence of fibrous tissue were obtained by Pullinger & Florey (1937), who gave pictures of the rich network of minute lymphatics which invade an inflamed area at the early stages, only to be driven back by the subsequently elaborated scar tissue, until finally the central area is quite devoid of these vessels.

Material and methods

Attempts were made to determine the regenerative capacity of lymphatic capillaries and particularly of the trunks, and if possible to throw light on the second group of problems of this work by experiments on rabbits. The procedure was to watch certain selected lymphatic vessels over a period of time after application of the stimuli necessary for their growth. For purposes of identification it was thought necessary to isolate a group of lymphatics and subject them to the same treatment. This was achieved by constructing tubed pedicles in the skin of the flank of adult rabbits as shown in Text-fig. 2. The usual method of construction employed in surgery was adopted, anaesthesia was produced by ether, and full asepsis was adhered to throughout. The pedicles were left for some weeks before further operations were attempted.

The number, position, direction and size of the lymphatic trunks in the pedicles were estimated by radiographs taken after thorotrast injection into the skin of the flank just above the pedicle. With pedicles 2–3 inches long it was found very convenient to use dental X-ray films placed between the pedicle and the body of the animal. Normality of lymph flow in the pedicles was also determined before proceeding further by noting whether all thorotrast disappeared from the vessels with normal rapidity. Specimens in which the lymphatic trunks did not run parallel through the length of the pedicle, or in which the rate of flow of the lymph was for some reason or other not normal, were rejected as unsuitable for further work.



Text-fig. 2. Indicates position and extent of tubed pedicles constructed in the flank skin of rabbits.

A second operation was then performed. In this the pedicle was divided transversely across its whole width, and the cut surfaces then immediately resutured as carefully as possible. In some instances a small sample (3–4 mm. in length) of the pedicle was removed during this operation. The sample which was removed from one cut surface of the pedicle provided a picture of all the lymphatic trunks running along the pedicle, its width being the same as that of the pedicle. Transverse sections of the sample were cut and stained.

The fate of the severed lymphatic trunks in the reunited pedicle was then watched from radiographs after thorotrast injection at intervals. Animals were killed after varying intervals, and at the time of death a last thorotrast injection was made. The pedicle was cleared by the Spalteholz method for examination in its entirety under low magnification, and in thin sections cut from paraffin blocks for miscroscopic examination.

Modifications of these experiments were tried in which a cut end of one pedicle was implanted into the ordinary skin of the flank, or the cut end of a pedicle was sutured to a freshly incised free end of a previously constructed pedicle stump.

It was hoped that these modifications would not only serve as checks on the main experiments, but also throw light on the influence of lymph-flow pressure upon the phenomena of growth, anastomosis and remodelling of the vessels. Unfortunately there were not enough successful instances of these modified operations to allow of any deductions. The few in which union of the pedicle stumps occurred showed such large amounts of scar tissue that lymphatic growth or anastomosis was completely interfered with. It may be that further trials of these modified operations will later be more successful.

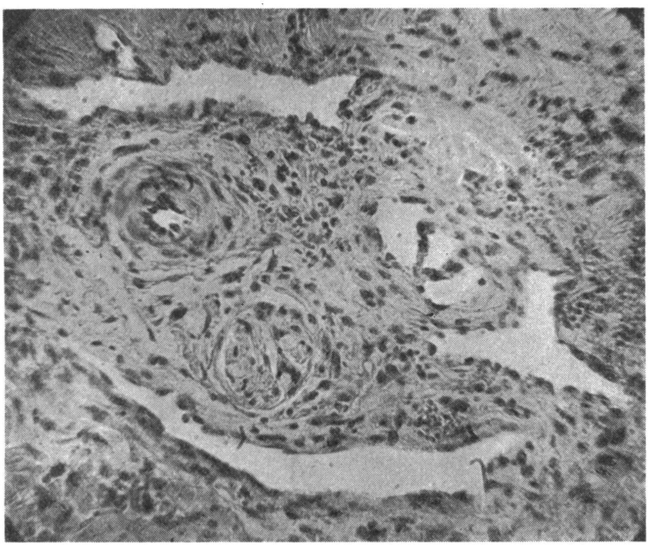
Results of Experiments

Suitable pedicles were made in twelve adult rabbits. Of these four were successfully severed and reunited under ether anaesthesia, and the events in the lymphatic trunks followed with thorotrast. The relatively high percentage of failures was due to the considerable technical difficulties involved. The successive operations and tests to which the animals were subjected can be seen from the protocol for one of the animals, which may be regarded as typical.

Rabbit 4	23. iii. 38	Pedicle made
	22. iv. 38	Thorotrast injection of flank skin above pedicle; X-ray of pedicle (30 days after construction)
	10. v. 38	Pedicle severed, and cut ends rejoined after removal of a sample.
	18. v. 38	Thorotrast injection and X-ray as before.
	27. v. 38	X-ray of pedicle without further injection.
	1. vi. 38	Thorotrast injection and X-ray as before.
	8. vi. 38	Animal killed; thorotrast injection and X-ray of pedicle taken at death. Pedicle then cleared for examination and section, 27 days after severing and reunion.

The findings in the successful operations were consistent with one another, and a number of points emerge which have not been demonstrated in the literature on the fate of severed lymphatic trunks.

First of all attention may be called to the appearance of the lymphatic trunks when injected with thorotrast in the pedicle as yet unsevered. The ordinary appearance is shown in Pl. I, fig. 2. Three trunks are seen definitely as fine lines running along the pedicle. The direction of flow of lymph in all these pedicles was from dorsal to ventral. As an important contrast with this appearance of normal undilated trunks in the pedicles, one may examine Pl. I, fig. 3, which shows the effect of compression of the lower end of a pedicle during thorotrast injection. The figures give an idea of the degree of dilatation of lymphatic trunks which can occur, and this should be borne in mind when considering the later figures in this section. A striking point also to be noted is the aggregation of thorotrast near the walls of the vessels giving the appearance of two parallel lines to be seen in the dilated vessels. This finding is no isolated one, but its explanation is not clear. It may be associated in some way with the well-recognized capacity of lymphatic endothelium for a kind of athrocytic absorptive activity towards particles of certain dimensions. The particles of thorotrast with a molecular size of 80 A. may come within the range of size of particles for which athrocytosis[1] has been observed. On the other hand, it may be that there are purely mechanical reasons for a segregation of heavy thorium particles towards the walls of the vessels.

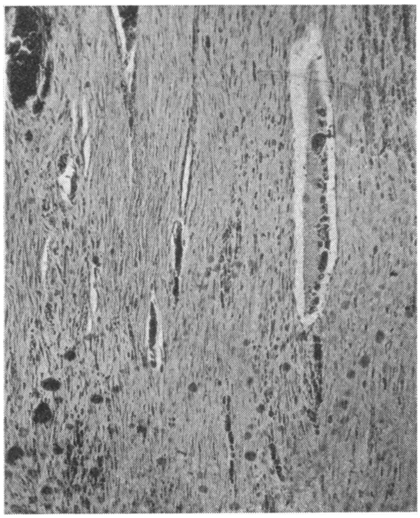


Text-fig. 3. Photomicrograph of 10μ transverse section of part of sample of a rabbit pedicle taken 2 days after the operation of severing and reunion. $\times 110$.

In regard to the events in the lymphatic trunks after severing, it may first of all be stated that the cut ends of the trunks become sealed off within a day or two. These experiments do not exclude the possibility that the sealing off occurs within a matter of hours. Although the ends of the trunks are thus closed, there is no extensive thrombosis along the vessels. A striking contrast is presented with the small blood vessels. None of the pathological changes which occur in the walls of the blood vessels at their cut ends are to be seen in the lymphatics. One may account for these effects by postulating that the lymphatic trunk is sealed by a simple growth forwards of the severed endothelium of the tube, the actual closure being perhaps brought about by the mechanical guiding influence of the surrounding fibrous connective tissue.

[1] The term "athrocytosis" is used to designate the intracellular flocculation of certain colloidal particles (see P. Gérard, 1935–6).

Whatever the mechanism of closure, its occurrence is definitely shown in Pl. I, figs. 7–10. Text-fig. 3, which is a photomicrograph of a portion of a sample from a pedicle in an animal which died of intercurrent pulmonary disease 2 days after the operation of severing and reunion, shows how thrombotic changes are absent from the wall of the lymphatics while present in those of an artery a little distance above the level of severing. A few sections below the one illustrated, the little artery showed the early signs of organization of the clot in its lumen and an absence of endothelium. Though the lymphatics in the specimen were not injected, one can be quite certain that some of the dilated endothelial-lined spaces in the picture are lymphatics, and their obvious dilatation is to be contrasted with the state of the blood vessel.



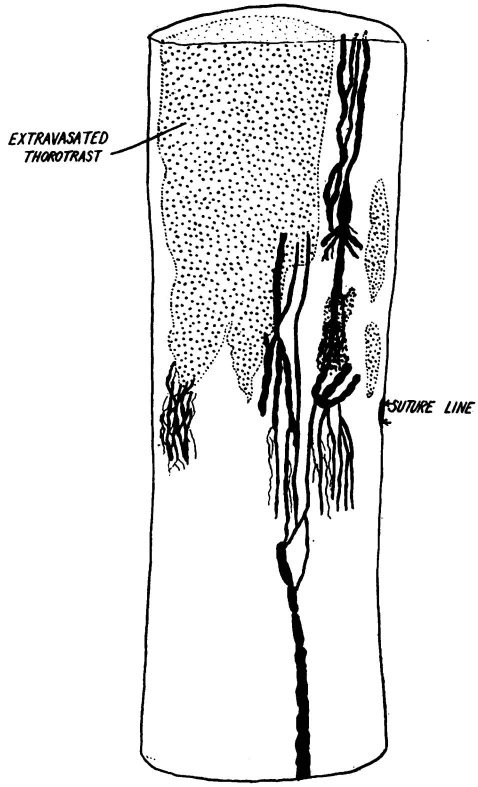
Text-fig. 4. Photomicrograph of 10μ longitudinal section of upper part of a rabbit pedicle which had been injected with thorotrast after the operation of severing and reunion, re-injected 11 days later during life, and then killed and given a final injection after a further 8 days. Fresh (unphagocytosed) and old (phagocytosed) thorotrast can be seen in the same lymphatic vessels. $\times 110$.

The lymphatic trunks on the distal side of the cut (in the dorsal part of the pedicle) become considerably dilated, and their contents stagnate within the lumen. This is shown in Pl. I, figs. 7–10. No coagulation of the lymph occurs in the lumen. The fluidity of the contents for weeks after the end of a lymphatic vessel is sealed has been demonstrated by the work of E. L. & E. R. Clark, and is here suggested for the pedicle lymphatics by varying amounts of thorotrast in them at different injections made at intervals after the severing (see Pl. I, figs. 7–10). It is finally established by examination of microscopic sections of pedicles made after "follow-up" injections of thorotrast up to the time of death. From these (Text-fig. 4) it is seen that thorotrast which has been injected into the lymphatics some time previously has been ingested by phagocytes, which have wandered into the lumen of the lymphatic from the surrounding tissues. This is a well-recognized phenomenon, which has actually been watched in the living subject by E. L. & E. R. Clark. Thorotrast which was injected at the time of death is of course not ingested in this manner. An examination of Text-fig. 4 shows the two lots of thorotrast, from earlier and later injections, lying side by side in some of the vessels. Coagulation of the contents is thus ruled out.

Next, in regard to the growth phenomena shown by the lymphatics in these pedicles, it must be stated that the radiographs show only macroscopic appearances (Pl. I, figs. 4–10), and can therefore give no direct indication of capillary lymphatic changes. At an interval of 8 days after the severing, radiographs showed no obvious connexion of lymphatic trunks on the two sides of the incision. In X-rays of one specimen (Pl. I, fig. 7) there are faint indications that thorotrast is being conducted across the scar, but it is not possible to say from the picture whether an anastomosis of trunks exists, or exactly what the nature of the connecting vessels is. By the time 3 weeks have elapsed after the operation, radiographs present no doubt that the trunks in the upper segments of the pedicle have become anastomosed with those in the lower segment below the incision. At this stage there may still be only a very narrow constricted connexion between the two portions of the vessels, and some trunks do not appear to have been successful in establishing connexion. But at least some have got connexions large enough to allow sufficient thorotrast to pass into the lower lymphatic trunk to render it opaque to the X-rays. The claim made in Reichert's work that lymphatic trunks can become reunited when severed is thus confirmed. The longer time taken for union in my experiments is not in conflict with Reichert's findings, because in the pedicle experiments there was no attempt to get exact apposition of particular severed trunks, and scar tissue formation was probably more abundant in my experiments.

It may be recorded that some oedema occurred in the upper segment of these pedicles when they were severed, and its duration corresponded roughly with that found by Reichert after incisions through the whole lower limb. But for obvious reasons my experiments, as carried out, were not suitable for studies of oedema in the same way as were Reichert's operations in which drainage from a whole limb was interfered with.

The work was carried further by the findings obtained from cleared pedicles, and from microscopic examination of these. Text-fig. 5 is a hand drawing of a cleared specimen obtained from the same pedicle as is illustrated in Pl. I, figs. 4–10. The cleared specimen was split in halves longitudinally, and the diagram shows the appearance of one of these halves when examined in oil of wintergreen under ordinary binocular magnification. The stumps of severed lymphatics are seen, and uniting these are a number of fine vessels which must have sprouted out from the trunks, grown along the pedicle parallel to its length, and finally established connexion with stumps of trunks on the other side of the incision. Particularly striking is the growth of a sort of spray of fine lymphatic vessels from the region of a valve in one trunk some distance away from the cut end of the vessel. To my knowledge this kind of sprouting has not been recorded hitherto. The sprouts from the cut ends of the vessels may have owed their origin to the single stimulus of the severing of the endothelium, or to the influence of the pressure of lymph stasis in the vessel, or to a combination of both forces. But the "spray of sprouts" from a region several millimetres above the stump of a severed trunk must be attributed to lymph pressure, and cannot be caused by any "setting free" of endothelial cells by cutting.



Text-fig. 5. Diagram of some of the lymphatics in a rabbit pedicle 27 days after severing and reunion operation. Stumps of lymphatic trunks and sprouts from these are shown effecting reconnexion.

The vessels uniting the trunks across the scar are narrow and very thin-walled. There has not been developed any assemblage of muscle and adventitial layers around the endothelial walls of these vessels in the course of the period of about one month for which they were observed.

From the above experiments it will be seen that the main results of Reichert's work are confirmed, and details of the steps in the phenomena he observed are now displayed. More definite evidence is presented that lymph stasis can cause sprouting from lymphatic trunks, although it is to be noted that the experiments do not exclude the possibility that the mere cutting of trunk endothelium is sufficient to start the growth of sprouts.

PART III. FURTHER EXPERIMENTS TESTING THE CAPACITY FOR LYMPHATIC VESSEL REGENERATION

Literature

It will be seen from the accounts of the literature and findings in the first and second parts of this paper, that besides the separate problems of the initial growth of regenerating lymphatics and the subsequent elaboration of newly formed vessels, one must bear in mind that these two aspects of the subject may be grouped as one, both being concerned with factors intrinsic in the vessels themselves. Thus they may be contrasted with factors extrinsic to the vessels and yet affecting regeneration.

Recent studies, particularly those of Clark & Clark (1937) and of Pullinger & Florey (1937), have cleared up very completely the subject of the effects of fibrous or scar tissue upon lymphatic vessel regeneration. The mechanical effect of this "extrinsic" factor is now fully understood, and from such studies Clark & Clark have been able to explain the topographical association of lymphatic vessels with arteries and veins. Also, Pullinger & Florey have shown how inflammatory foci exert a stimulating influence on lymphatic capillary growth in the stages of acute inflammation associated with hyperaemia and early fibroblast proliferation.

The present section is concerned chiefly with the analysis of factors intrinsic in the lymphatic vessels themselves.

Rouvière & Valette (1937) have described the results they obtained from experiments in which they interfered with the circulation of lymph in the rabbit's hindleg by removal of the popliteal lymph gland. These authors give the literature references to other similar experiments, such as removing an area of the lymphatics at the base of the ear to note whether any re-establishment of the lymphatic circulation occurs. The final new courses taken by the lymph after such operations were figured, and it appears from such work that these new pathways are provided most often by a dilatation and elaboration of pre-existing anastomotic vessels. In some cases the fresh growth of some capillary lymphatics for very short distances was apparently necessary in order to effect the establishment of lymphatic vascular anastomosis. No further details about the actual steps of these processes are available. The same may be said of the findings by Ssysganow (1931) and those by Vonwiller & Vannotti (1931).

Material and methods

It was apparent that the best way of making further advance would be to operate upon a single lymphatic trunk, severing it from its connexions or transplanting it in some way to test its capacity for growth. For this purpose the rabbit's ear is a suitable region, because a considerable number of relatively large lymphatic trunks run fairly straight courses from the distal towards the basal part of the ear under the dorsal skin, lying in the loose areolar tissue outside the perichondrium of the ear cartilage.

Operations were performed with full surgical aseptic precautions under nembutal or ether anaesthesia, and a suitable combination of operation table, microscope stage, and binocular microscope was used.

Ophthalmological instruments such as iridectomy scissors, paracentesis needles and finely pointed forceps, proved the best instruments. Thorotrast was not a suitable injection material for these operations as it is not visible in the living tissues apart from radiography. For this purpose it was found that ordinary blue-black ink, after removal of coarse particles by centrifuging, and approximate correction of its tonicity and acidity, was very suitable for interstitial injection of the lymphatics. At first the operations were all carried out on vessels so injected, and follow-up injections were done at intervals up to and including the time of killing the animal. But, in view of the possible irritating or toxic effect of the ink on the endothelium of the lymphatics, the same experiments were subsequently all repeated in animals in which no injection was done until the time of killing.

The individual vessels were reached by reflecting a rectangular flap of skin, and then working with knife and forceps under the binocular, the field of operation being kept moist by a saline drip. The skin flap was then replaced and its margins carefully sutured in place. Over twenty-five successful operations were carried out on ink-injected vessels besides many control and follow-up injections, and there were another ten operations on vessels which were not injected until the time of death. The operations may be classified as follows:

(1) Simple severing of a single lymphatic trunk. In some of these cases the trunk cut was one remote from other similar lymphatics or blood vessels. In others one member of the clump of trunks running with the main central blood vessels of the ear was severed. In yet other instances all members of this clump were severed. The severing was done usually by inserting the point of the fine sharp knife under the skin and cutting a particular vessel with the minimum of trauma. In some cases a particular vessel was severed after a skin flap had been reflected. The flap was then replaced and sutured. In one experiment done by chance, but which had interesting results, a trunk was severed in an area covered by a mica sheet fixed in position after the manner used in constructing a transparent chamber. The results were compared when the sheet of mica was left in place and when the sheet was later removed. This test was made on an uninjected trunk.

(2) Isolation of a portion of a lymphatic trunk 1–2 cm. long, by an enclosing rectangular incision made through the tissues under the skin. The vessel was thus left *in situ*, but cut off for a length from its connexions with other lymphatics. These experiments were devised in order to dissociate the influence of lymph pressure from that of severing upon lymphatic regeneration.

(3) and (4) Transplantation experiments in which a length of trunk was cut out from its environment and laid down in a new bed prepared for it in some other part of the ear. There were two types of this experiment. In one the transplant was orientated with its valves in the same direction as those of the trunks in apposition with its ends. In the other direction of the transplant was such that its valves were reversed relative to those of surrounding trunks. These experiments were devised to test the influence of the normal blood vessels of the lymphatic wall and its nerves upon regeneration.

(5) Removal experiments in which a centimetre or more of a lymphatic trunk was removed altogether. These experiments were planned in order to get a measure of the amount of growth possible from a severed lymphatic trunk.

At the end of the observation period for each animal an ink injection was made, the animal killed by overdose of anaesthetic, and the ears fixed and cleared by the Spalteholz method. The unoperated ear was a control for each animal and was likewise injected, although there is such a constancy of lymphatic trunk pattern in the ears of these rabbits, and the results of the operation were usually so definite, that this was scarcely necessary for every case.

The cleared specimens were examined after the cartilage and internal skin had been stripped off. In some cases a rough microdissection was carried out to determine the exact spatial relations of the vessels. When necessary, sections were cut from paraffin blocks and examined histologically.

Results of experiments

Before describing the results of the experiments it is right to notice the appearance of the vessels operated upon. A typical sample is shown in Pl. II, fig. 1. In this the valves and the interval between them can be readily appreciated. On histological examination with different stains such as haematoxylin and eosin, Passini's stain, Mallory's triple stain, and van Gieson, it was found that such a vessel, though possessing a relatively thin wall, yet showed a definite amount of smooth muscle tissue and a definite adventitia. The rich vascularization of the walls of such lymphatic trunks with blood capillaries has been pointed out by various authors, and was confirmed by Gray (1939, Fig. 316). They are also innervated by non-medullated nerves. In all the experiments the results were the same both when preliminary injections were made, and when no injections were made until the time of death.

(1) *Results of the simple severing of a lymphatic trunk.* Altogether twenty-six divisions of lymphatic trunks were completed. In twelve of these the vessels were definitely identified at all subsequent stages. Two of these twelve were operations performed with a mica window in place, and were not used for testing the uniting capacity of severed trunks. But these two cases do perhaps help to reconcile the conflicting claims of Clark & Clark (1937) that severed trunks remain open, and of Reichert and myself that they do not remain open, but first become sealed off and then later may join other vessels.

In one of these two cases a severed uninjected lymphatic was watched for a week through the mica covering. No sprouting could be seen under the binocular microscope, although when the right amount of transmitted and reflected light was used the vessel could be clearly seen. After a week the animal was anaesthetized and the mica sheet was removed. An appreciable amount of clear fluid lymph was found lying between the tissue and the mica. The vessel was then injected carefully with ink without causing any excessive pressure within it. The ink flowed freely out from the previously cut end of the vessel on to the surface of the connective tissue, which had been overlain by the mica. Further examination after killing the animal verified the open condition of the severed lymphatic.

It was thought that possibly the presence of the mica and a potential space between it and the cut end of the vessel had some effect upon the vessel, allowing perhaps a steady unrestricted flow of lymph through the open end, and thus preventing its closure. The operation was therefore repeated on a different animal and the mica sheet removed (under anaesthesia) at the end of 3 days. The same little pool of clear liquid lymph was found under the mica when it was removed. But 48 hr. after its removal the vessel end was found by injection to be closed, and a layer of fibrin containing leucocytes separated the stump of the vessel from the surface. Perhaps these tests need to be checked further, but at least they are suggestive.

Of the ten successful ordinary severings of trunks, four showed reunion of the cut ends, and union failed to occur in the rest (Pl. II, fig. 3). The minimum time for reunion in these experiments was 5 days. The sprouts from the cut ends were seen in one of the instances of union (Pl. II, fig. 2), and were also seen in three of the cases where union failed to occur. In one of the cases where union occurred it was found at the time of re-examination that a constriction was present at the point of incision. In another case, the vessel which united in 5 days, subsequent examination after an interval of 9 weeks showed that the original single reunited trunk had now split longitudinally into three small vessels at the area where the junction had been effected. The three parallel vessels then ran into one again a little below the point of the incision. This result is in keeping with the findings of Clark & Clark (1937) as an interference phenomenon due to coarse connective tissue fibres developing at the site of the original trauma.

These experiments leave no doubt that union can occur, and the steps by which it occurs are shown. But they also indicate the potency of fibrous tissue for interfering with the sprouts. It may be said that if the two ends of a severed trunk are more than about 1 mm. apart scar tissue will prevent their direct union.

(2) *Results of the isolation experiments.* Although this operation was tried in a number of animals, it was successfully followed up only in two. In one of these injections were made at intervals, in the other no injections were made at all until the animal was killed. The first animal was allowed to live for 10½ weeks after operation, but the second was killed after 6 days.

The results showed that the isolated trunk or trunks remain intact, sprouts occur from them, and these join with sprouts from other vessels which have been cut but not isolated. The sprouts grow from both ends of the isolated vessel. The results are illustrated in Text-figs. 6–9, and Pl. III, figs. 1, 2.

The appearance of sprouts from both ends of the isolated vessels shows that the pressure of lymph within the vessels cannot be the one and only essential stimulus to their growth. At the distal end of such an isolated trunk there can be very little if any lymph pressure exerted on the interior. The valves remain intact in such a vessel, and must automatically prevent the transmission of pressure to the distal end of the cut vessel.

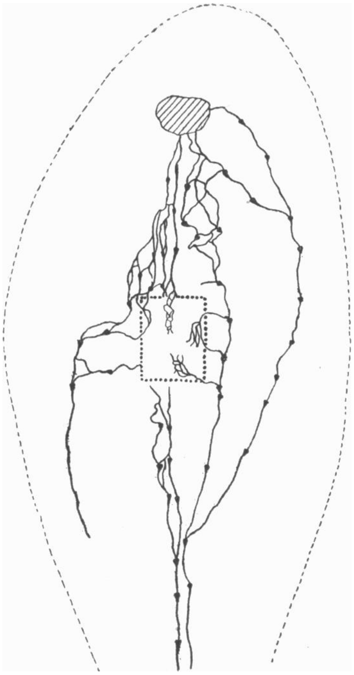


Fig. 6.

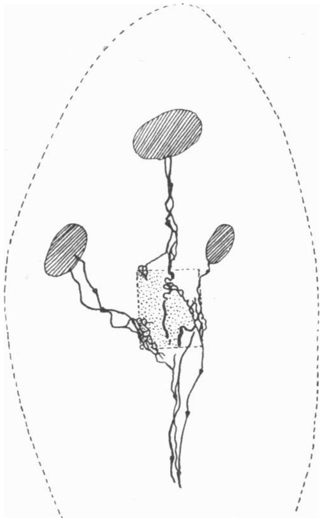


Fig. 7.

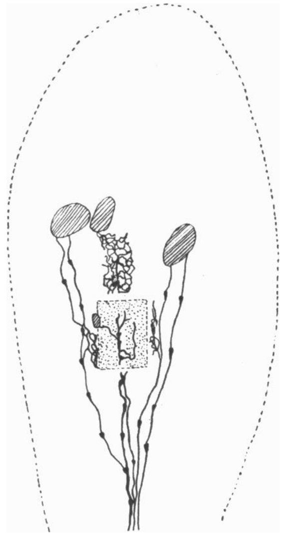


Fig. 8.

Text-fig. 6. Diagram of lymphatics in the ear of a rabbit in which isolation of the trunks was effected in the central area shown dotted. Injection 4 days after operation.

Text-fig. 7. As for Text-fig. 6. 4 weeks after operation.

Text-fig. 8. As for Text-fig. 6. 8 weeks after operation.

Text-fig. 9. As for Text-fig. 6. $10\frac{1}{2}$ weeks after operation. Animal killed.

From the illustrations the influence of scar tissue can again be seen. In the case watched for $10\frac{1}{2}$ weeks, Text-figs. 6–9, newly grown fine vessels are seen to have ranged themselves parallel to the strands of connective tissue, relatively few managing to cross the scar into the surrounding areas. It will be noticed also that the anastomoses established at the two ends of the isolated vessels were still only narrow vessels even after $10\frac{1}{2}$ weeks. It may be that this is chiefly a scar tissue effect, and not due to any intrinsic incapacity of such anastomosing lymphatics to enlarge and strengthen their walls. The operative procedure for this experiment necessarily involved an appreciable amount of trauma to the tissue, so that scar formation was encouraged somewhat.

(3) *and* (4) *Results of the transplantation experiments.* In these the disruption of the trunk from its normal environment was obviously more severe than in the isolation experiments. Also, it was not possible to extract a portion of a lymphatic vessel without taking with it some of the surrounding areolar tissue, and this may have introduced an unwanted nutritional question in connexion with the fate of the transplant.

At first eleven transplantation operations, in which the vessel to be moved was injected with ink before being extracted, were performed. The graft was subsequently always identifiable in these cases by the phagocytosed ink particles in its lumen. As an additional aid to identification the graft was usually placed in its new bed with a double curve in its course. In two of the eleven cases the transplant was not reversed, and in nine it was reversed. In some of the cases the transplant was cut from among the central lymphatics, turned through 180°, and laid down again in the bed from which it had been taken. In others a length of a paramedian vessel was shifted into the central region. The grafts were all from 1 to 2 cm. long. In some cases the bed prepared for the transplant was scraped quite free of all tissue down to the cartilage, and when the graft was fitted in place it was covered only by the very thin dermis and epidermis of the skin flap. In other cases the bed was not cleared so completely of all other lymphatic trunks. In all cases the graft was placed as accurately as possible in apposition with the cut ends of the trunk or trunks at the proximal and distal ends of the bed. The grafts were watched for periods up to 19 days. Besides these eleven experiments, four similar experiments were made in which there were no injections at all until the time of killing the animal. These grafting experiments were difficult to carry out and bring to a successful conclusion, but certain fairly definite results emerge from them.

There was little evidence of sprouting from the grafted vessels. A few sprouts were seen from the ends of two of the grafts, but they were much less abundant than in the other types of operation. The graft in most cases seemed to remain passive without much evidence of growth or other changes in its walls (Text-fig. 11).

In all cases there was much growth of lymphatic sprouts from surrounding vessels into the region of the graft. Most of these sprouts failed to establish connexion with the graft, but in some cases such anastomosis was definitely present. Text-figs. 10, 11 and Pl. III, fig. 3, illustrate this result.

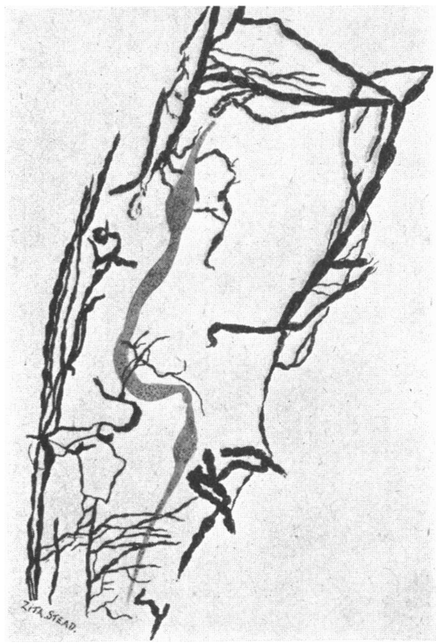
It may be concluded that transplantation of a lymphatic trunk does not preclude its subsequent participation in the lymphatic circulation, though it does appear to interfere somewhat with its growth capacity. Unfortunately successful operations were not followed long enough to show the fate of the valves of the grafts in the reversal experiments.

(5) *Results and findings from the trunk removal experiments.* Although many examples of removal of a length of lymphatic trunk (1–2 cm.) were

Text-fig. 10. Diagram of lymphatics in a rabbit's ear 13 days after transplantation of trunks (*a*) without reversal. No injections made until the time of death. Note impaired sprouting from the transplant and anastomosis of it with other trunks; (*b*) indicates areas where ink was extravasated at injection sites.

provided by the grafting experiments, only four operations were carried out specifically for the purpose of tracing the events following this procedure. In two of these four cases the removal may not have included all the lymphatic trunks in the area denuded. In the other two great care was taken to remove all trunks from the narrow rectangular field of operation. The animals were observed up to a maximum period of 16 weeks.

The follow-up and final injections in these cases showed only minute trunks just visible to the naked eye in the operation field some 14 days after operation. These were enlarged vessels which had not been removed at operation. The sprouts from the severed vessels had only grown about a millimetre after 18 days, and this length was no more than was found in as short a time as 8 days. After a month, small vessels growing from the surrounding areas had joined up with these sprouts from the severed trunks, so as to vascularize most of the area of operation. But even after 16 weeks the normal lymphatic trunk pattern was not restored. No doubt the effects seen in this group of experiments are much influenced by scar tissue. But the conditions of operation in this group probably correspond much more closely to what happens to lymphatic trunks in ordinary surgical procedures than do the conditions in the

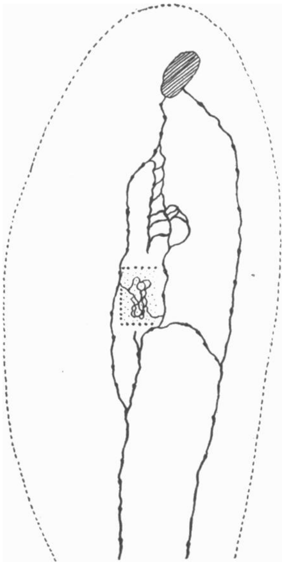


Text-fig. 11. Diagram of lymphatics in a rabbit's ear 12 days after transplantation with reversal of a single injected trunk. No sprouts from the transplant are seen, but connexion with sprouts from other vessels has occurred.

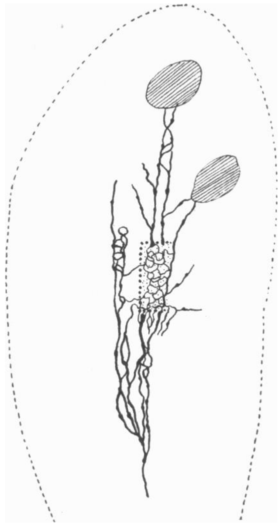
other groups of experiments. They show that in ordinary operative procedures growth from lymphatic trunks is much limited by scar tissue. But the great abundance of lymphatics in the regions where they are found at all will ensure re-establishment of the circulation in accordance with the descriptions given in this experimental work, even when relatively large areas have been interfered with. These findings are illustrated in Text-figs. 12–14.

Conclusions from operations on individual lymphatic trunks

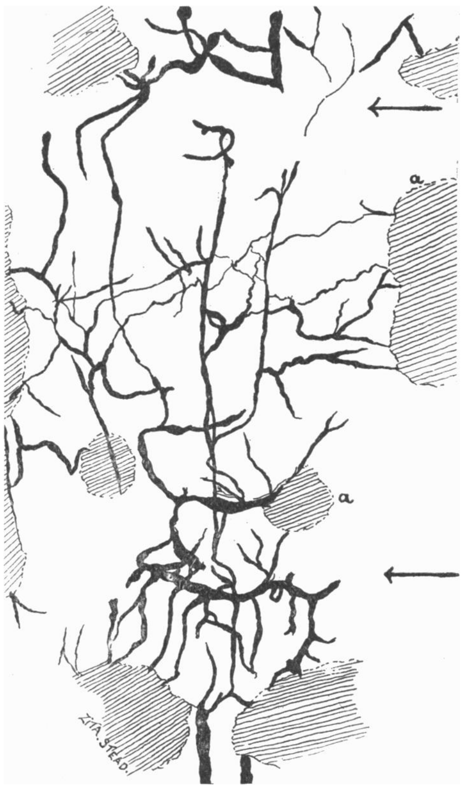
The main conclusions from the five groups of experiments which have been described above are epitomized in the diagram shown in Text-fig. 15.



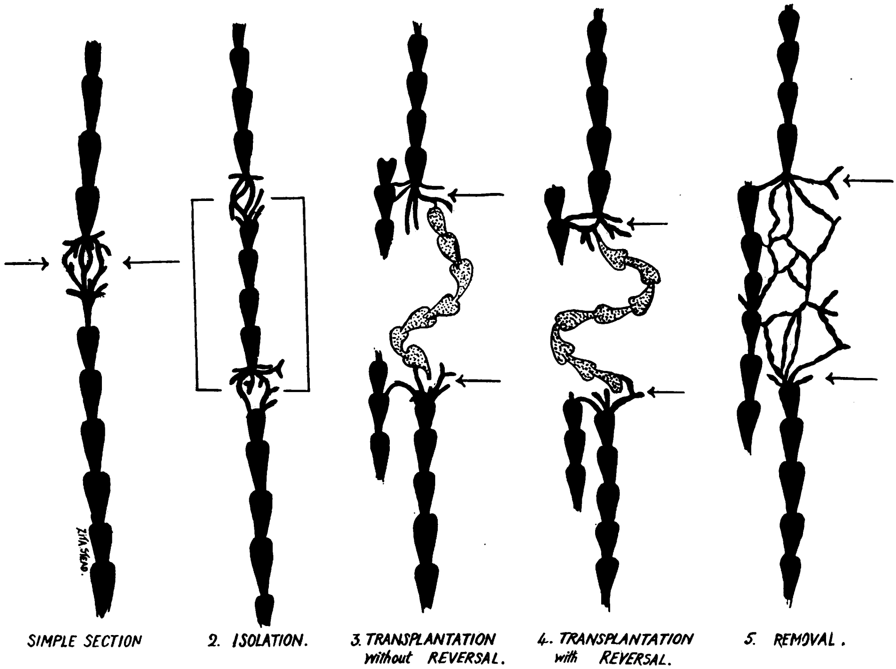
Text-fig. 12. Diagram of lymphatics in a rabbit's ear (R 17) 14 days after removal of about 1·5 cm. length of the central vessels. Fine vessels only are present in the area.



Text-fig. 13. As for Text-fig. 12 (R 17). 6 weeks after operation.



Text-fig. 14. As for Text-fig. 12 (R 17) at time of death, 16 weeks after operation. There is failure to restore the original lymphatic vessel pattern, although the flow of lymph is doubtless unimpeded. The limits of the trunk removal are shown by two arrows; (*a*) indicates extravasated ink.



Text-fig. 15. Diagram summarizing the results of the experiments on individual lymphatic trunks.

SUMMARY OF RESULTS

The following remarks summarize briefly the main findings from each of the three parts of this work.

Part I

Capillary lymphatic growth may be caused by stimuli other than cutting of the vessels. Hypertrophy of dermal papillae is such a stimulus. Very extensive remodelling and elaboration of pre-existing accessory or anastomotic lymphatic pathways may occur in response to altered pressure-flow conditions. Such remodelling is slow, being not very extensive in the course of 3 weeks.

Part II

Lymphatic trunks respond to severing in a peculiar manner quite different from that of blood vessels. There is sealing off of the cut but no thrombotic change. Sprouting and joining of trunks can occur after severing.

Sprouting from trunks caused by pressure-flow stimuli alone is seen.

Remodelling of newly formed vessels is influenced by fibrous scar tissue.

Part III

Severed trunks can unite, sprouting being the method, but scar tissue easily prevents this.

The sealing off of cut ends of trunks may require some stoppage or at least interference with lymph flow in the vessels.

A flow of lymph in a trunk is not necessary to the continued existence of such a vessel.

Sprouts from trunks may be caused by the stimulus of severing alone.

Sprouts from trunks may occur in response to the combined stimuli of severing and altered pressure-flow conditions.

Transplantation of a length of a lymphatic trunk does not preclude its subsequent usefulness as a conducting vessel for lymph.

Transplantation probably does impair the sprouting capacity of a trunk.

The total growth of sprouts from trunks is limited in practice by scar tissue, and by union with other vessels, to a few millimetres. This total is reached within 8 days.

The interference of fibrous scar tissue with the processes of remodelling and elaboration of new vessels in surgical procedures is very severe.

CONCLUSIONS

The work refers solely to adult mammalian lymphatic vessel regenerative phenomena. Human tissue subjected to pathological influences and to special surgical procedures has been examined. Experiments on lymphatics in tubed pedicles in rabbits and in rabbits' ears have been carried out.

Peculiar properties of lymphatic trunks. Thrombosis does not ordinarily follow trauma to trunks. A flow of lymph is not necessary to the continued existence of a trunk in the body. Tears or cuts in a trunk may remain open if the flow in the vessel is quite unrestricted. Tears or cuts in trunks do ordinarily become sealed up, and this may be associated with some damming up of the lymph flow. Transplantation of a trunk does not prevent its being used subsequently in the lymphatic circulation.

The initial regenerative growth of lymphatics in the adult. Such growth may occur from both capillaries and trunks. The method is by sprouts in both cases, and the sprouts may succeed in uniting trunk with trunk. The stimuli adequate to produce this growth may be cutting alone, altered pressure-flow conditions within the vessels alone, or a combination of these. Hypertrophy of dermal papillae is another adequate stimulus, but this has not been further analysed. Transplantation of trunks impairs their sprouting capacity. The total growth in length of sprouts as such is limited in life to 1–2 mm. by fibrous scar tissue or by union with other vessels.

Subsequent elaboration and modelling of lymphatic vessels. Remodelling and elaboration of pre-existing small vessels into large definitive trunks occur in response to altered conditions of pressure and flow of lymph. These changes are very seriously hampered by fibrous connective tissue. Modelling and elaboration of newly formed lymphatic vessels occurs, but is in practice usually much slower, and also is much interfered with by scar tissue. The rate of these processes is to be measured in terms of weeks.

ACKNOWLEDGEMENTS

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EXPLANATION OF PLATES I–III

Plate I

All ten figures are of normal size, unmagnified

Fig. 1. Radiograph of portion of human tubed pedicle after injection of lymphatics with thorotrast. Upper lateral end of the specimen is shown by the letter *U*.

Fig. 2. Radiograph of a rabbit pedicle 2 min. after thorotrast injection of its lymphatics. Note the size of the vessels.

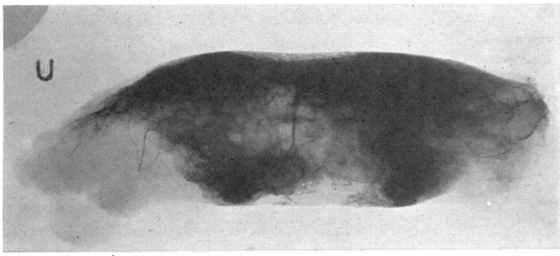


Fig. 1.

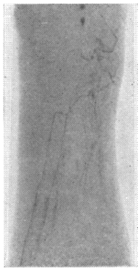


Fig. 2.

Fig. 3.

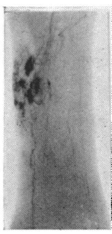
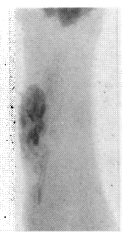


Fig. 4.

Fig. 5.



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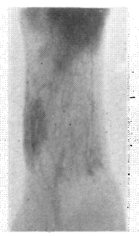

Fig. 7

Fig. 8.

Fig. 9.

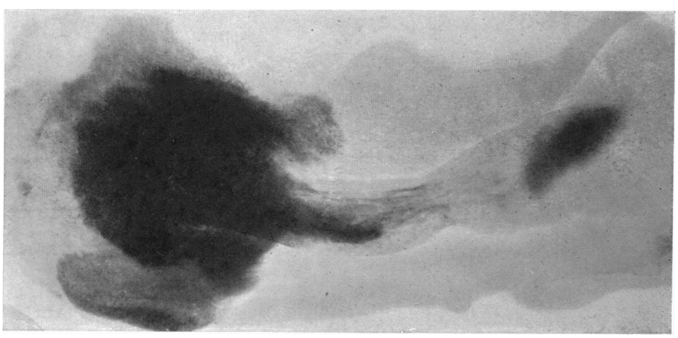

Fig. 10.

GRAY—Studies of the regeneration of lymphatic vessels

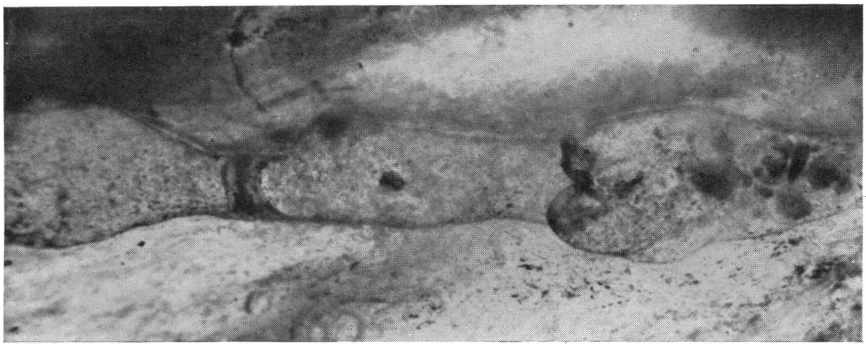

Fig. 1.

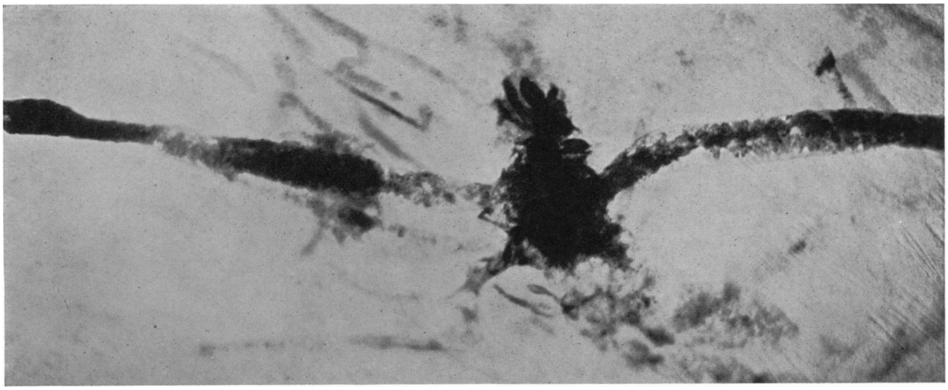

Fig. 2.

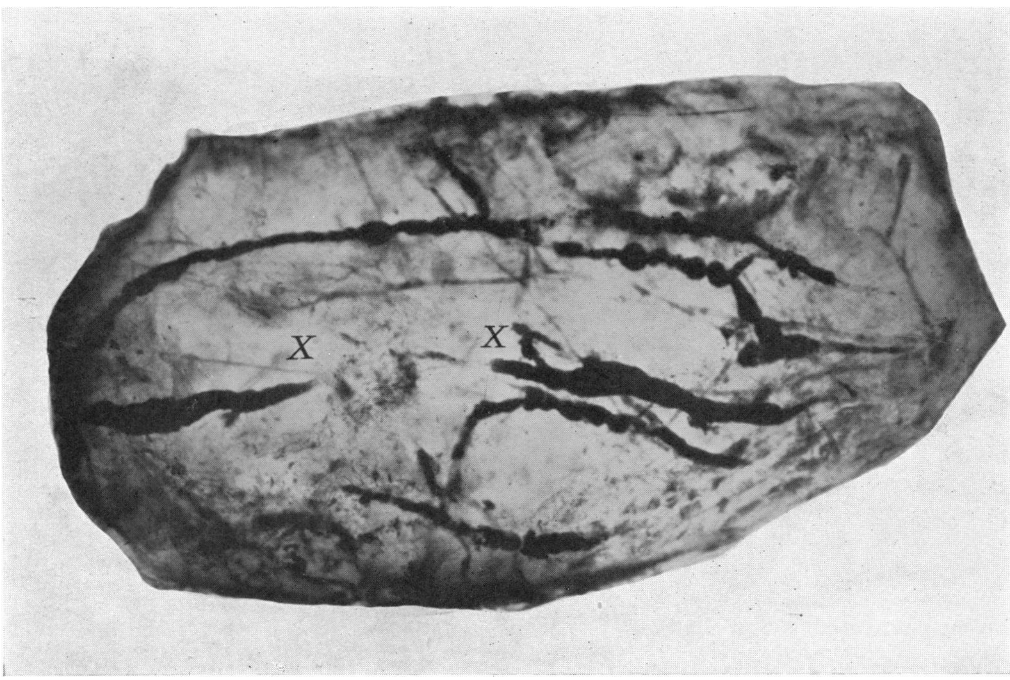

Fig. 3.

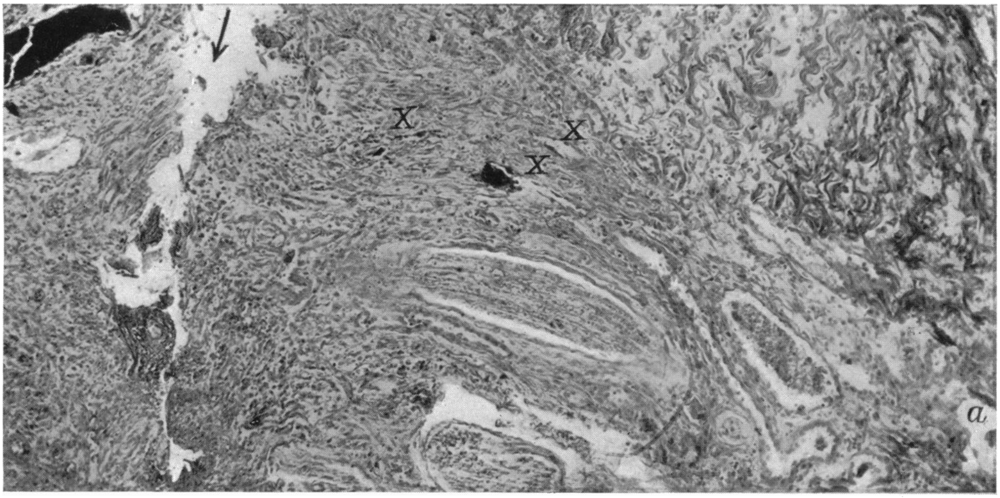

Fig. 1.

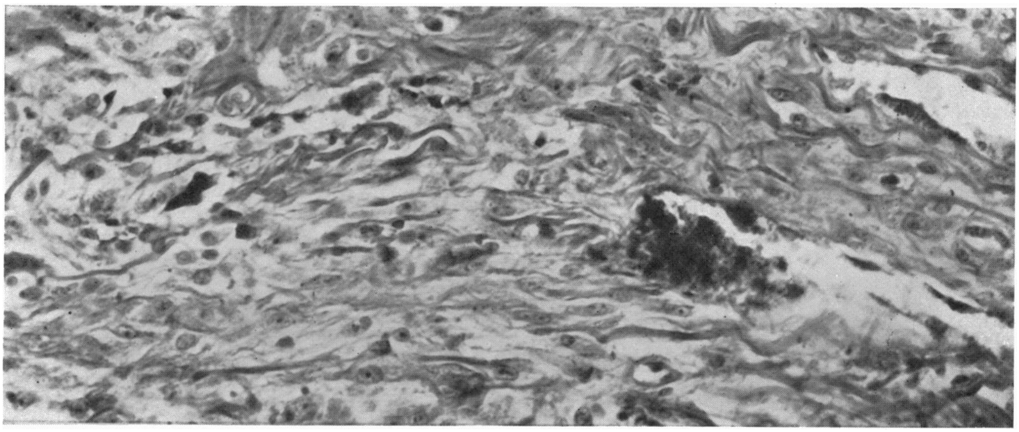

Fig. 2.

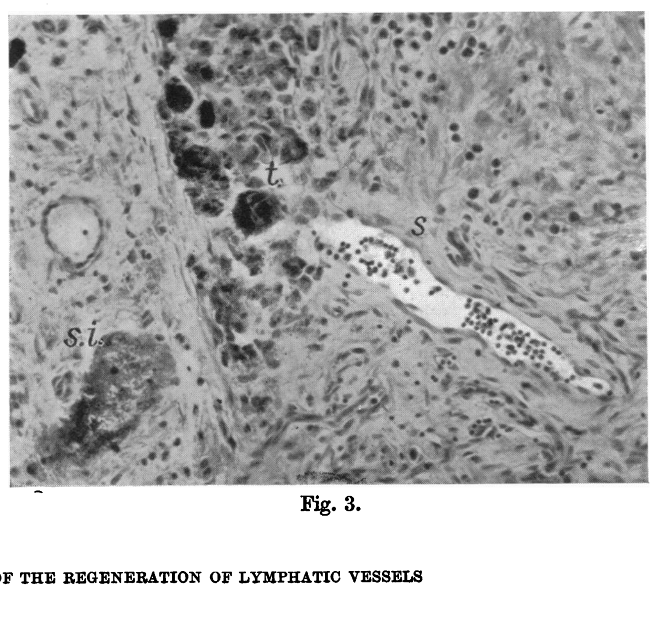

Fig. 3.

Fig. 3. Radiograph of a rabbit pedicle 2 min. after thorotrast injection of its lymphatics, the base of the pedicle being firmly compressed to obstruct the flow of lymph while massage was employed.

Fig. 4. Radiograph of a rabbit pedicle (R 4) 2 min. after injection with thorotrast.

Fig. 5. Radiograph of the same pedicle (R 4) after thorotrast injection followed by massage of the injected area.

Fig. 6. Radiograph of the same pedicle (R 4) 20 min. after thorotrast injection. Lymphatics now practically completely empty.

Fig. 7. Radiograph of the same pedicle (R 4) injected with thorotrast 8 days after the operation of severing and reunion.

Fig. 8. Radiograph of the same pedicle (R 4) 17 days after the operation, but without further injection.

Fig. 9. Radiograph of the same pedicle (R 4) reinjected 3 weeks after the severing operation.

Fig. 10. Radiograph of the same pedicle (R 4) at the time of killing and finally reinjecting the lymphatics 27 days after the severing operation.

Plate II

Fig. 1. Photomicrograph of an ink-injected normal lymphatic trunk in the rabbit's ear showing the type of vessel operated on. The ear was cleared by the Spalteholz method. The tissue was unstained. This segment of the vessel contains only a few particles of ink. ×25.

Fig. 2. Photomicrograph of an ink-injected lymphatic trunk in the rabbit's ear 6 days after severing. Reunion with an excess of sprouts has occurred. ×20.

Fig. 3. Photomicrograph of lymphatic trunks in a rabbit's ear showing failure of union of a vessel severed 14 days previously. The cut ends were left about 1·0 mm. apart, the vessels were uncovered during the operation by lifting up a flap of skin for inspection. Reunion has failed to occur. Histological examination showed that sprouts (not filled with ink) from the cut ends were present. (*X*) marks the cut ends. ×15.

Plate III

Fig. 1. Photomicrograph of a longitudinal section through area of isolated lymphatic trunks in a rabbit's ear; animal killed 6 days after operation. No injections until the time of death. Section shows the upper (distal) edge of the rectangular line of incision indicated by an arrow, and a small part of the normal distal tissue. Sprouts, *X*, are seen from the distal ends of the isolated trunk (*a*) growing distally. ×85.

Fig. 2. High power view of part of Pl. III, fig. 1. This shows several of the sprouts growing distally. Their endothelium and the contained ink can be recognized. ×350.

Fig. 3. Photomicrograph showing junction of a lymphatic capillary sprout (*s*) with the reversed transplanted trunk (*t*) shown also in Text-fig. 11. The sprout at this point contains chiefly lymphocytes. Ink was seen in it in other sections. The trunk contains phagocytosed ink. An ink-containing sprout is seen at *s.i.* ×80.