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The Effect of Ischaemia on Lymph Nodes and the Lymphatic Circulation

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Summary

The changes in the superficial inguinal lymph node of the pig following either occlusion of all its blood supply, or occlusion of blood supply plus intra-arterial injection of Thrombin, or of a sclerosing agent (Ethanolamine), were studied by lymphography and histology.

After occlusion of blood vessels only, the nodes rapidly recovered to normal. After occlusion of the blood supply plus intra-arterial injection of Thrombin the changes were more marked but recovery was good though not quite normal. After occlusion of blood supply and intra-arterial injection of Ethanolamine damage to the lymph node was much more marked, and obstructive changes were seen on lymphography with only a little evidence of recovery.

Neither the effects of quiet thrombosis nor those of more violent inflammation appear to explain or parallel the changes occurring in clinical primary lymphoedema.

Introduction

Patients with primary lymphoedema, including Milroy's disease, have been extensively studied since the advent of lymphography and the findings have recently been described and discussed (1). In some patients deficiencies in the lymph pathways are present from birth and affect lymph vessels, nodes or both. In others the deficiences become manifest later and the question arises as to whether they are due to a failure in the lymph pathways themselves or whether the changes in the lymph system might be due to some failure in its blood supply, particularly that to the nodes.

The effects in lymph nodes of changes in the lymph or blood circulation through them have been studied experimentally by other workers.

Tilak and *Howard* (2) studied the effect of ligation and division of all the arteries and veins to the popliteal lymph node in dogs. They assessed the result with subcutaneous lymphangiography using 50% Hypaque (3) before operation and at regular intervals after operation, and by histological examination of the nodes after 5 months. They found that interruption of the blood supply was followed by maintenance of lymphatic circulation through the node and rapid restoration of a normal nodal pattern on lymphangiography and histology. However, auto-transplantation experiments on dogs (4) appeared to contradict this. A free lymphatic pedicle from the distal ileal mesentery of dogs was transplanted into the axilla; arterial anastomosis was obtained by a staple technique, venous anastomosis with microsuture technique and lymph node connection by anstomosing half of the proximal node in the graft to half of the in situ axillary node. In one group of dogs with satisfactory vascular anastomosis normal function of the transplanted node was demonstrated by lymphography and histological appearances were normal. In another group of grafts with vascular insufficiency the lymphatic circulation alone was not sufficient to sustain viability of the node. However, the amount of lymph that would flow from such a graft would presumably not be normal, particularly if the blood supply to the grafted tissue had failed.

Experiments on the popliteal lymph node of the rabbit (5) showed that complete occlusion of the blood supply caused no appreciable change in the flow and protein concentration in afferent and efferent lymph of the node. However, it did result in a fall in the cell count of the efferent lymph, which began to rise again after 5 days. They also studied the histological changes and found that occlusion of blood vessels caused degeneration of cells within the node except for small areas adjacent to the marginal and medullary sinuses. Regeneration of lymphatic tissue began from about the seventh day and was felt to be due to regeneration of blood vessels connected with the blood circulation outside the node. No lymphographic studies were performed in their series.

The purpose of the present study was to occlude both the arteries and veins to lymph nodes in a lasting and effective fashion. Thrombin solution was injected intra-arterially in one group of animals and a sclerosing agent in another group, after division of all the blood vessels leading to and away from the lymph node, to try to procure permanent occlusion of all blood vessels in the node. The effect of the ischaemia produced on the lymph node and lymphatic circulation was studied by lymphography and histology.

Methods

Experimental Animals

Pigs were used for this project because the lymph vessels are relatively large and easy to cannulate on repeated occasions. The lymph nodes may be clearly seen on lymphograms. The animals were of the "large white" variety. Five were females and two were males. At the time of operation they were 4-8 weeks old and weighed 10-20 kg.

Pigs have three to six afferent lymphatics from the hind limbs which drain to the superficial inguinal lymph nodes. The latter consist of one or two large and several small nodes which form a conglomerate mass (Fig. 2). These nodes also receive lymph vessels from the

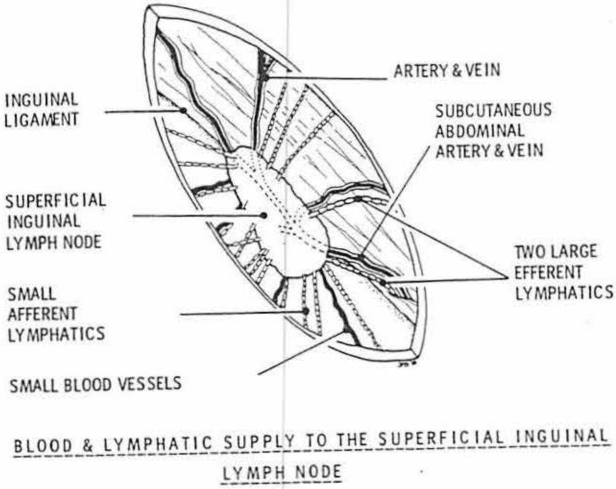

Fig. 1 The blood supply is mainly from the subcutaneous abdominal artery which runs deep to the node in close association with it, giving off several branches to the node and continuing with branches which supply the lower anterior abdominal wall. In addition several small blood vessels supply the node from the lower lateral side. Numerous afferent lymphatics leave in close relation with the subcutaneous abdominal artery and thence they travel up with the external iliac vessels.
After dissecting the lymphatic and blood vessels as shown in the diagram, the arteries and veins were occluded with Cushing's clips or diathermied, and divided.

anterior abdominal wall and lower trunk. From these nodes two large and a few small efferents usually pass to the external and internal iliac lymph nodes in the pelvis, and thence to the lumbar lymph nodes and trunks. It would appear that virtually all lymph from the lower limbs and from the lower part of the anterior abdominal wall and trunk drain through the superficial inguinal lymph nodes.

The blood supply to these inguinal nodes is from branches of the subcutaneous abdominal artery as shown in Fig. 1. This is a branch of the external pudic artery which arises from the deep femoral artery and emerges through the superficial inguinal ring.

Anaesthesia
Premedication was obtained using 0.5 to 2 ml of "Suicalm" (Azaperone, Janssen Pharmaceutica, Belgium) and anaesthesia was induced and maintained with Halothane, Nitrous Oxide and Oxygen. A cone shaped rubber mask was used which was strapped on the head, and this method of anaesthesia was found to be very satisfactory.

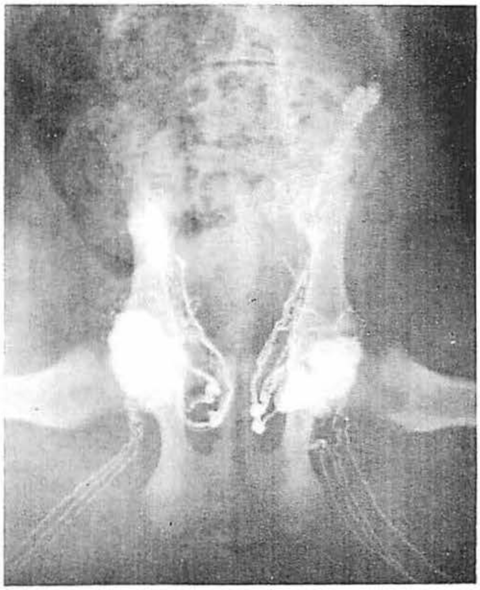

Fig. 2 Normal lymphangiogram before operation to occlude blood supply to lymph node.

Lymphography
The term lymphography is used to include both lymphangiograms, which show the lymph vessels, and lymphadenograms, which are performed at a later date and show the lymph nodes.

In each animal an initial lymphangiogram was performed. About a week later the operation to occlude the blood vessels to the superficial inguinal lymph node was carried out, and at the conclusion of the operation a lymphadenogram was taken. Further lymphangiograms were performed at one or two weeks after operation and thereafter at intervals of 3 to 4 weeks. Lymphadenograms were taken 2-4 days after each lymphangiogram, and in addition at weekly intervals for the first 6 weeks after operation, and at fortnightly intervals for the next month.

Patent blue violet 2.5% (Bengué Ltd) was injected subcutaneously into the dorsum of the hind foot. The lymph vessels were visible through the skin, and were easily exposed via a longitudinal incision about $\frac{1}{2}$" long on the dorsum of the foot. The lymph vessels were cannulated using a St. Thomas's Hospital pattern lymphangiogram set (6).

'Lipiodol' Ultra Fluid (May & Baker Ltd) in a dose of 0.25 ml/kg was injected (7) into lymphatics, half the dose being given on each side, using the Lund constant rate injector (8) at a rate of 1 ml every 15 minutes.

Technique of operations on the blood supply of the nodes
Before operation patent blue violet was injected into the medial aspect of the thigh, perineum and lower abdomen so as to surround the superficial inguinal lymph node on the side of the operation. An oblique incision was made over the node which was very carefully dissected out so that it could be completely freed from surrounding tissues and could be lifted out of its bed, remaining attached only by its arteries and veins and lymphatic vessels (Fig. 1). The larger blood vessels were occluded with Cushing's clips and divided and the smaller vessels were coagulated with the diathermy and divided.

The binocular operating microscope (Carl Zeiss) using a magnification X6 was useful in helping to differentiate small blood vessels from lymphatic vessels and by exercising great care, it was possible to leave the efferent and virtually all the small afferent lymphatic vessels intact.

This initial operation was performed in three groups of animals:-
1. In the first group occlusion and division of the blood vessels was all that was done.
2. In the second group 1 ml of "Topical Thrombin" (Bovine Origin; Park Davis) was injected into the largest supplying artery after all the blood vessels had been occluded.
3. In the third group 1 ml of 5% Ethanolamine oleate (Evans Medical) was injected into the artery after all the blood vessels had been occluded.

In each animal the superficial inguinal lymph node on the other side was left undisturbed to act as a control for comparison.

Histological Study
At the end of the studies of each animal the superficial inguinal lymph nodes on each side were removed at post-mortem examination and sections were cut for histological examination. This was difficult to interpret due to the gross distortion of architecture caused by repeated lymphangiograms. In addition to examination by the author the histological slides were studied by a consultant pathologist.

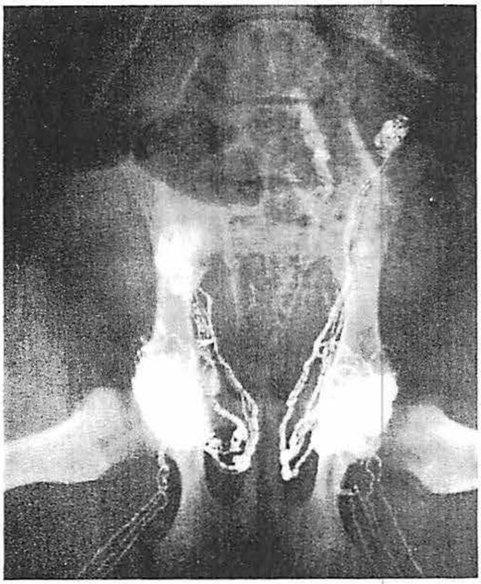

Fig. 3 Same animal as Fig. 2 a month after operation to ligate the arteries and veins to the right superficial inguinal lymph node, without any additional intra-arterial injection. As can be seen, there is no appreciable difference between this and the previous figure.

Results

1. *Ligation of blood vessels only*
There were three pigs in the first group in which all the arteries and veins supplying the inguinal lymph nodes in one groin were ligated. No additional intra-arterial injection was given. Operations were performed on the right side in two animals and the left in another. In each animal the devascularised lymph node became slightly smaller on lymphadenogram at 2 to 3 weeks, but by about a month the picture returned to normal. Figures 2 and 3 show lymphangiograms before and a month after ligation of blood vessels to the lymph node.

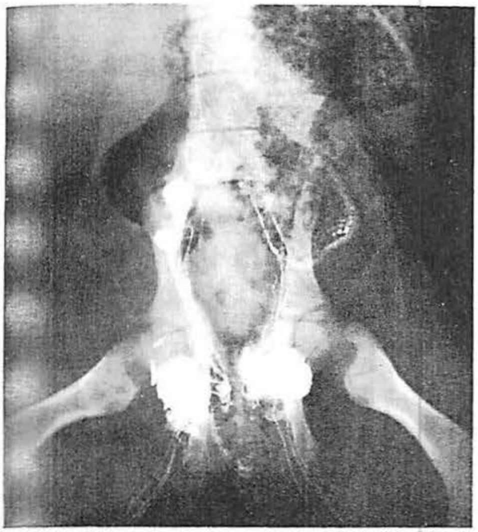

Fig. 4 Lymphangiogram 10 days after operation to ligate the blood vessels to the right superficial inguinal lymph node with additional intra-arterial injection of Topical Thrombin. The lymph node is seen to be about the same size as that on the normal left side. There is a little extravasation and a few collaterals have developed, but most of the dye is still passing through via the normal channels. (The Cushing's clips on the medial side are obscured by dye in the lymph node).

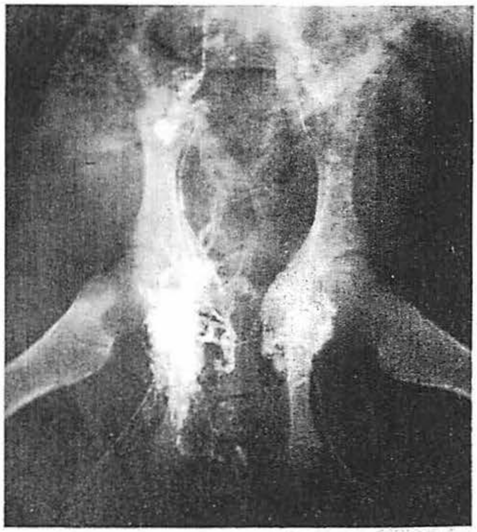

Fig. 5 The same animal as Fig. 4 but $3\frac{1}{2}$ months after occlusion of blood supply and intra-arterial injection of Topical Thrombin. There has been further extravasation and collateral vessels are better developed, but the main amount of dye has passed through the efferent lymphatics. (The Cushing's clips on the medial side are visible in this picture, as the dye is less dense).

At post-mortem examination there was naked eye evidence of re-vascularisation from multiple small blood vessels all round the node and this was confirmed on microscopy. Microscopy of the lymphoid tissue showed large areas which were apparently normal, between vacuoles created by the contrast medium. In respect of the lymphoid tissue the nodes on the operated side were indistinguishable from those on the control side.

2. *Ligation of blood vessels with intra-arterial injection of Thrombin*

There were two animals in this group. One operation was performed on the left and the other on the right side.

Lymphangiograms made at about 10 days after operation showed collaterals suggesting some obstruction and some extravasation, but the contrast material mainly passed through the node in the normal way (Fig. 4).

At three and a half months after operation the picture was basically the same but with more collaterals. Most of the dye passed through into the efferent channels (Fig. 5). The lymph node was smaller on the operated side.

Again at post-mortem there was evidence of revascularisation. Microscopy showed no significant difference in the lymphoid tissue between the operated and control side.

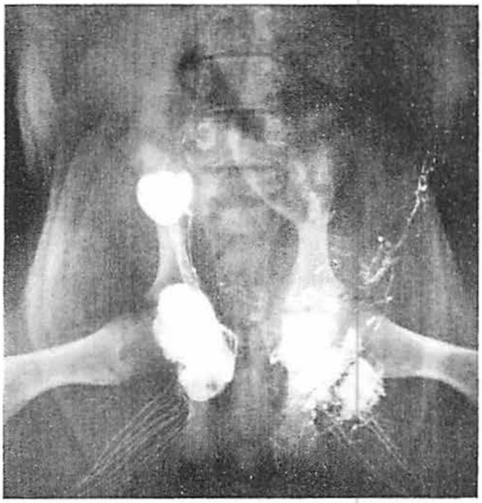

Fig. 6 Lymphangiogram one week after operation to ligate the blood vessels to the left superficial inguinal lymph node with additional intra-arterial injection of Ethanolamine. The lymph node has already become smaller than on the normal right side. There is marked extravasation and a collateral circulation is developing. There is no filling of efferent lymphatics indicating obstruction through the node.

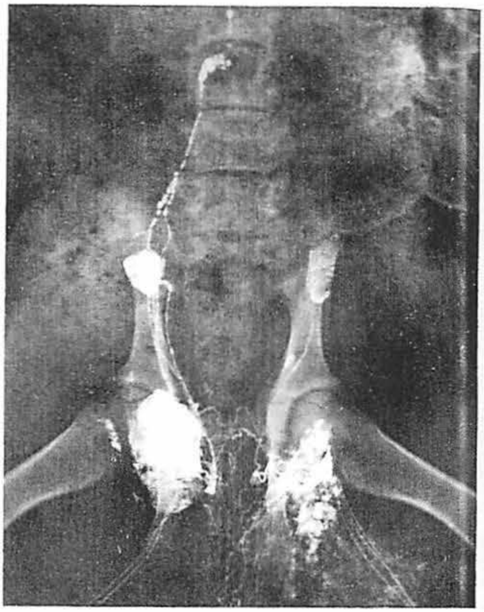

Fig. 7 Same animal as in Fig. 6 but $3\frac{1}{2}$ months after occlusion of blood supply and intra-arterial injection of Ethanolamine on the left. The lymph node is now much smaller than on the normal side. There is slight further extravasation and a well developed collateral circulation but a little dye is now going up via the efferent route. However, only one efferent lymphatic has filled compared with 5 on the right side.

3. *Ligation of blood vessels with intra-arterial injection of Ethanolamine*

There were two animals in this group. One had the operation performed on the left and one on the right. Both developed swelling and some redness at the site of the wound suggesting fairly marked inflammation. In one animal this settled down and a lymphangiogram at one week showed extravasation and collaterals indicating damage to the node with resultant lymphatic obstruction (Fig. 6). A month later there was a little fresh extravasation on lymphography and a well developed collateral circulation, but no dye was going through the lymph node. By this time the operated node was much smaller. There was probably some compensatory hypertrophy of the normal side, which had become well marked by three and a half months. There was still no clearly visible nodal architecture on lymphadenograms and the collateral circulation was well established. Some dye was reaching the efferent lymph vessels (Fig. 7).

Microscopy of this node still showed some areas of normal lymphoid tissue present. There was increased fibrosis on the operated side, but the appearances of the remaining lymphoid tissue were similar to the control side. This was surprising but the lymphogram showed that the total area of it must have been very small compared to the other side.

In the remaining animal, at about one week, the wound broke down and an abscess developed. This was drained and the swab grew a mixed growth of organisms suggesting that this was an infection secondary to necrosis of a node. After a few days a necrotic lymph node began to extrude and was excised and the wound re-sutured, following which it healed satisfactorily.

In this animal lymphography showed very marked obstructive changes with gross extravasation and a well developed collateral circulation, as is shown in Fig. 8. There was no evidence of any regeneration of lymphoid tissue in this area, even though a very small portion of lymph node had remained in the wound. Hypertrophy was noticeable on the opposite normal side.

Despite the marked obstructive change on lymphography there was never any evidence of oedema of this leg, presumably due to the extensive collateral circulation in the lymph system.

Histology at post-mortem showed no residual lymphoid tissue in the node area on the right.
This last experiment was really equivalent to ablation of the entire lymph node.

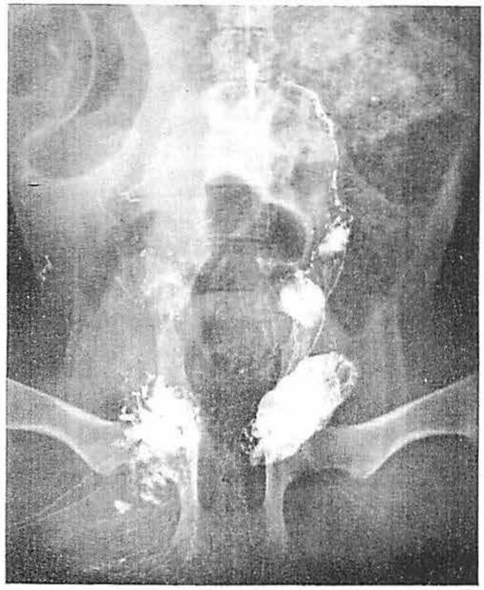

Fig. 8 Lymphangiogram at two weeks after operation to occlude the blood vessels to the right superficial inguinal lymph node with intra-arterial injection of Ethanolamine. This animal developed an abscess and this picture was taken after the wound had been drained but before the node had been extruded from the wound. The node is much smaller than on the left side. There is extravasation and extensive collaterals have developed indicating marked obstruction.

Discussion

The findings in the initial group of animals, where ligation alone was performed, confirm the results of other workers (2, 5) that lymph nodes are able to recover virtually to normality after complete occlusion of their blood supply.

If the lymph node were deprived of the blood supply but survived there are several possible explanations:-
i) there was sufficient nourishment from surrounding tissue and tissue fluid.
ii) it survived by nourishment from the lymph circulation through it
or iii) blood re-vascularisation was rapid.

It is unlikely that survival of the node is due to nourishment from surrounding tissue fluid as previous experiments have shown that slices of entire lymph node or of its medulla failed to survive as free autograft (9). It is most probable that lymph flow is essential for the initial steps of regeneration and that further regeneration depends on the re-establishment of the blood supply by the regeneration of new vessels (5).

Our findings of re-vascularisation suggest that this has been the sequence of events in our animals.

We had added the factor of intravascular thrombosis to the ligation of the vessels to see whether this might prevent a new collateral blood circulation from establishing itself. It appears that a relatively bland thrombosis is insufficient to do this. The collateral blood supply must have established itself into the blood vessels of the node which presumably had enough viability from persisting lymph circulation to allow the thrombosis to be cleared and recanalisation to take place.

It was only when a really violent inflammatory reaction was produced by injection of Ethanolamine that sufficient damage was done to the blood and presumably also to the lymph circulation to achieve permanent obliterative changes in the lymph node.

We were particularly interested in a blood vascular failure of lymph nodes as a possible first step in the degeneration of lymph pathways which occurs in some cases of primary hypoplastic lymphoedema, particularly in non-congenital cases and those occurring in later life. It might be postulated that lymph node degeneration might result from quiet thrombosis of the blood supply, analogous to 'quiet necrosis' in some bone diseases where a failure of blood supply results in bone change, e.g. in the hip or knee joint.

There is no direct evidence from these experiments about the rate of re-vascularisation. There is little doubt however that the circulation must have been very abnormal for at least several days, particularly after intravascular thrombosis with Thrombin. Yet these nodes showed nothing resembling the degree of change seen in the nodes of human patients with primary hypoplastic lymphoedema. It does not seem therefore that any process of quiet ischaemia of nodes can be postulated from the evidence of these experiments as a basis for the nodal changes in human primary lymphoedema. It was only when a violent inflammatory agent, sufficiently damaging in one case to cause necrosis was used that permanent substantial changes were achieved.

The development of primary lymphoedema of the praecox and tarda varieties in humans is not accompanied by signs of acute inflammation. Patients are occasionally seen with some little tenderness over the swollen area in the initial stages but it is exceptional. The occurrence of really acute inflammation is seen only as a secondary complication usually due to streptococcal infection in an already lymphoedematous area. There can be no suggestion that any really acute inflammatory process can be the initiating factor in the vast majority of patients with primary lymphoedema.

The clinical inference from these experiments is therefore that neither acute inflammatory blood vascular thrombosis, nor a quieter and more insidious one is likely as a pathologic basis or step in the development of primary lymphoedema in clinical practice.

Acknowledgment

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Types of Collateral Lymphatic Circulation

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Summary

Types of collateral lymphatic circulation depend mainly on the topographic localization of lymphatic obstruction. We describe the main types of collateral lymphatic circulation and demonstrate some of them on the lymphograms.

Obstruction of the lymphatic circulation may result in different types of collaterals. Roentgenological signs of collateral circulation and of retrograde flow of contrast agent as seen on the lymphogram are: Filling of lymphatics in unaffected areas, delayed filling on the affected side, localized dilatation of afferent lymphatics, filling of subcutaneous, interstitial and deep lymphatics, ones which are usually not visualized, dermal back flow, filling of the parietal lymphatics, filling of the visceral lymphatics (leading to the opposite side), direct communications with the caval or the portal system, filling of the lymphatics which by-pass the obstruction, and extravasation of contrast media.