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Are the Initial Lymphatics Normally Pulled Open by the Anchoring Filaments?

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Summary

The conformation of the walls of initial lymphatics was studied in oedema and during the filling- and emptying-phases of the inital lymphatic cylce in the mouse diaphragm and in the puppy jejunum. It was found that the anchoring filaments, attached to the endothelium, caused typical distortions of the vessels during oedema, but that these did not occur in normal tissue. This implies that these filaments do not pull them open during the filling-phase of the cycle, but that some other force causes them to fill.

Introduction

The forces which normally cause tissue fluid to enter the initial lymphatics are in some dispute (reviewed: 2, 6, 12, 16, 18, 35, 36, 42, 43).Everyone is agreed that in oedema the vessels are pulled open and a suction force is exerted by the anchoring filaments, which are attached to the ablumenal surfaces of the vessels and join them to the interstitial tissue (1, 11, 21, 22, 33); this, coupled with the increased tissue hydrostatic pressure (15) will result in a hydrostatic pressure difference directed into the vessels. The arguments arise in the normal condition. Even here, it is agreed that some organs (e.g. the kidney) have a positive tissue hydrostatic pressure and this may well be greater than the intralymphatic pressures (6).

In most normal tissues, however, it is very likely that the tissue hydrostatic pressure is negative (i.e. less than atmospheric – 15-18), while intralymphatic pressures – where these have been measured (12, 19, 23, 25, 26, 27, 29, 40, 41) – have all been approximately atmospheric. The one exception is in the work of *Hogan and Nicoll* (20, 27) which was performed in the wing of a species of bats which, uniquely, has contractile initial lymphatics and which had been made oedematous in order to visualise the vessels; here it was found that, during the filling-phase, tissue hydrostatic pressure was greater than intralymphatic pressure. The facts of oedema and of the contractile vessels completely vitiates *Nicoll* and *Taylor's* (28) attempt to extend this observation to explain the filling of all the non-contractile initial lymphatics in non-oedematous regions. In these the initial lymphatics are compressed by the tissues (which are themselves compressed by adjacent muscular activity, etc.); hence there is no extra-initial-lymphatic space – left by the contracting initial lymphatic – into which tissue fluid might be "sucked". However, it might be that a similar mechanism could function, if the anchoring filaments were tightened during compression of the tissue and served to forcibly dilate the initial lymphatics once the compressing force was removed. Then, the total tissue pressure (15), relative to the initial lymphatics, could be quite negative and yet could be applied to the endothelium (and to the lymph) via the filaments. Thus, the lymph pressure could be still more negative than the tissue hydrostatic pressure.

In the light of the almost uniformly atmospheric pressures measured in initial lymphatics, as mentioned above, such a suggestion might appear unlikely. However, except for those performed in the bat wing and mouse ear (and even these were restrained), these observations

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have all been made in exteriorised and motionless tissues. I have argued elsewhere (2, 3, 6, 7) against considering that such observations can tell us anything about a cycle which may well depend on variations in total tissue pressure to function. It seems only fair, then, to apply these arguments to the present case also. Unfortunately, it seems very difficult to use micropipettes to measure the pressures in the initial lymphatics at varying stages of the cycle, while applying varying total tissue pressures. This is quite a different problem from that solved so well by *Hogan* and *Nicoll* (20, 27), and requires external compression of the tissue – or local muscular contractions, while still measuring the pressures.

An alternative approach is to examine the state of the endothelial wall. It has been shown (1) that in oedema the filaments severely distort the wall, in an easily recognisable way. This is illustrated in Fig. 1. Also shown is the shape to be expected if the vessels are filled by an inflow of fluid (caused by other forces), with the intralymphatic hydrostatic pressure being greater than tissue hydrostatic pressure and thus simply flattening the endothelium against the more solid portions of the interstitial tissue. This shape would also be assumed by the vessels during the emptying-phase, when the increased total tissue pressure compresses their walls against the lymph. In a series of experiments (3, 8) we had obtained initial lymphatics, in the mouse diaphragm, which were known to be fixed in either the filling- or the emptying-phases. These were re-examined to investigate the conformation of their walls. To check that the alteration in the conformation of the initial lymphatic walls observed in dextran oedema of the rat foot-pad also occurred in this site, the initial lymphatics of mouse diaphragms which had been injured with histamine or mild heat (10) were also re-examined. The observations on initial lymphatics in both stages of the cycle were extended to include those of the jejuna of puppies, which have been fixed with the villi contracted or relaxed by drugs (5).

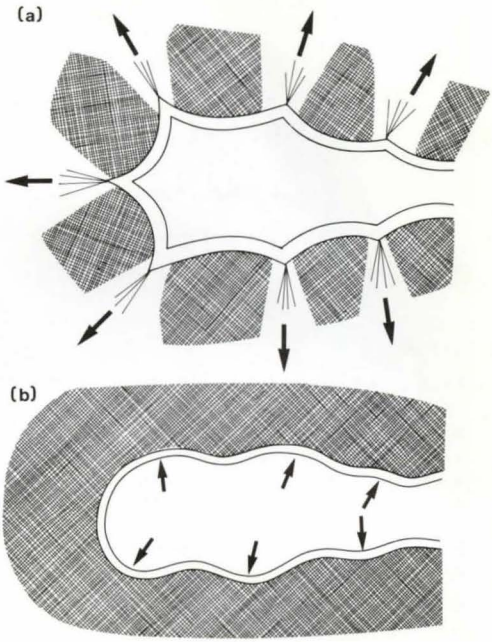

Fig. 1 A diagram showing (a) what is seen with an initial lymphatic in oedematous tissue. The anchoring filaments pull portions of the wall out into the tissues, while the remainder bulges in, due to the pressure against the tissues. Diagram (b) shows what would be expected if the intralymphatic hydrostatic pressure was greater than the tissue hydrostatic pressure – during either filling or emptying – pushing the endothelium out against the tissues

Materials and Methods

The animals and the experimental techniques have been described previously (3, 5, 8, 10). Essentially, the diaphragms were fixed (chemically or by freezing) when the lungs were expanded (inspiration, the emptying-phase), or collapsed (expiration, the filling-phase). Five animals per group were used in those whose diaphragms were chemically fixed: 2, 10, 30 or 60 seconds after the start of the filling-phase, or 2 or 10 seconds after the start of emptying-phase (3). There were four in each group of those immobilised by freezing, they were killed after 2, 10, or 30 seconds from the start of the filling-phase, or 2 or 10 seconds from the start of the emptying-phase (8).

Fig. 2 A lacune in a heat-inured mouse diaphragm, showing an endothelial darkening typical of one stage of injury, and an open junction (J). The wall is pulled out in two places, and bulges inwards around them. It was classified as very much "pulled out". 3250 x

The injured 15 animals had had histamine injected into the pleural cavities, a further 15 had had hot saline (54 °C) washed over their peritoneal surfaces; only those uninjected with benzo-pyrones were examined; these diaphragms were fixed randomly through the cycle (10). Eight puppies were used; adjacent regions of jejunum were either contracted with Carbacol (B.D.H.) or relaxed with atropine (5). One block was examined from each of the above animals, and five initial lymphatic cross-sections randomly selected and photographed at 1,000 to 8,000 x in a Siemens Elmiskop I.

The micrographs were examined blindly, i.e. without knowing from which group they had come. They were assigned to one of seven possible groups, depending on the conformation of their walls (Table). This method was the only one which seemed appropriate, since numerical tests of conformation are notoriously difficult to devise and apply. An attempt was made to improve accuracy by using

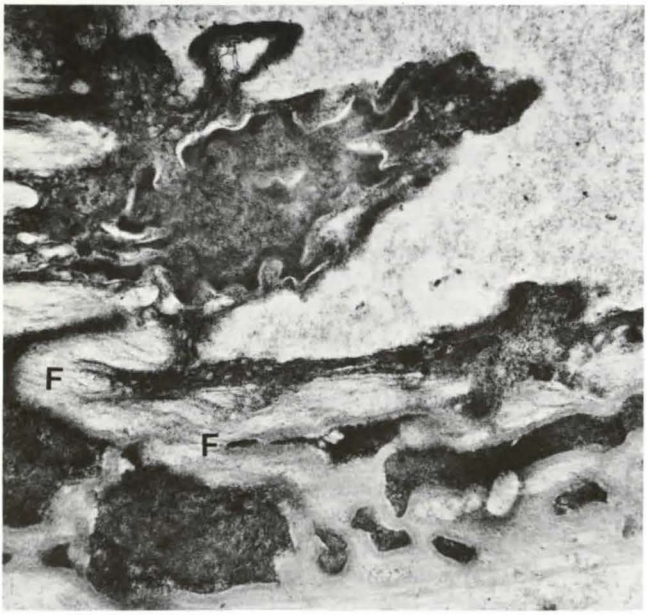

Fig. 3 A lacune in a histamine-injured diaphragm, showing many anchoring filaments pulling on a number of endothelial projections into the interstitial tissue (F). This was classified as moderately pulled out. 13000 x

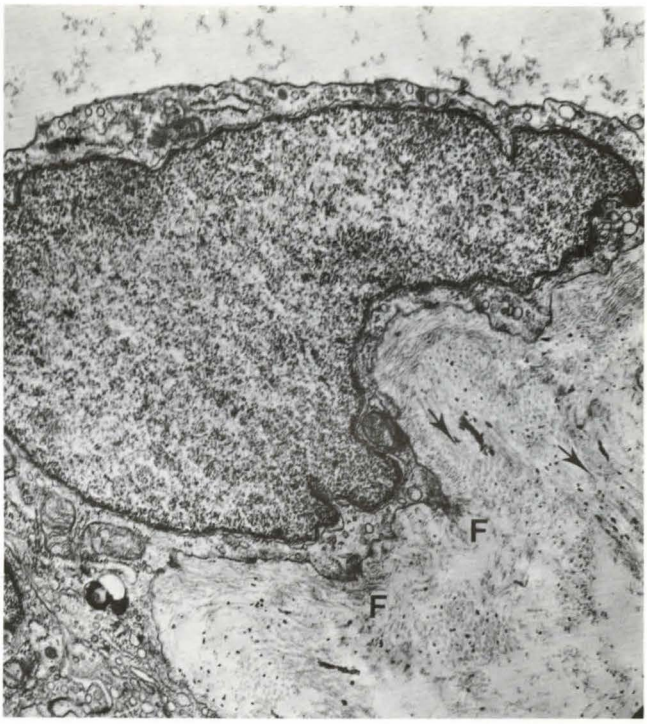

Fig. 4 As for Fig. 3, showing more filaments attached to projections (F) and some (arrows) pulling perpendicularly out from the wall. 26000x

a series of "typical" micrographs. The blind method at least eliminated bias.

Results

The general morphologies of the vessels have been described previously (op. cit.). It appeared that the walls of the diaphragmatic initial lymphatics (Figs. 5–8) were rather flatter than those of the jejunal lacteals (Fig. 9), except when the former tissue was injured (Figs. 2–4). This possible difference between the normal tissues was not substantiated statistically; that between the normal and the injured ones was (Table).

It could be seen that the diaphragmatic lacunes in the oedematous tissue (Figs. 2–4) were apparently as distended by the anchoring filaments as were those in the rat footpad (1), except that in the diaphragm they were more confined by the surrounding muscular tissue – instead of being able to be distorted for longer distances into the loose interstitial tissue. The pulling outwards of portions of the endothelium, and the bulging inwards of the remainder, was obvious. The fibrils could often be seen coming directly away from the wall (Figs. 3, 4) – obviously pulling on it. The endothelium itself often showed considerable divergences into the interstitial tissue (Fig. 2); between these it appeared to be pushed in towards the centre of the lumen – as would be expected if the filaments were pulling the portions of the wall to which they were attached outwards, while the rest was bulged inwards by the intervening tissue, with its high total tissue pressure.

It was apparent, by inspection, that the conformational characteristics of the initial lymphatics in oedematous tissue were not common in non-oedematous tissue – in either the diaphragm (Figs. 5–8) or the jejunum (Fig. 9). The walls of the vessels during the filling-phase were similar to those in the emptying-phase. They were characterised by gentler curves, with little suggestion of the endothelium being pulled into the tissues; it was obviously flattened over the tissues – as if held there.

Table The conformations of the walls of the initial lymphatics [a]

	"Pulled-out"[b]			Flat	"Pushed-out"[c]			
	Very	Mod.	Slight		Slight	Mod.	Very	Total numbers
Diaphragms – Chemical Fixation								
Filling phase								
2 secs.	0	2	1	8	8	4	2	25
10 secs	0	1	2	8	7	3	4	25
30 secs	0	1	1	6	8	4	5	25
60 secs	1	0	2	6	6	6	4	25
Emptying-phase								
2 secs	1	1	2	5	5	6	5	25
10 secs	0	2	1	7	6	4	5	25
Freeze Substitution								
Filling-phase								
2 secs	1	0	2	8	6	2	1	20
10 secs	0	2	1	7	5	3	2	20
30 secs	0	1	1	7	5	3	3	20
Emptying-phase								
2 secs	0	2	1	6	5	5	1	20
10 secs	2	1	1	5	4	4	3	20
Injury								
Heat	15	14	16	12	10	5	3	75
Histamine	17	15	13	14	9	3	4	75
Jejunum								
Filling-phase	2	3	3	7	10	9	6	40
Emptying-phase	1	3	2	8	11	10	5	40

a) χ^2-tests show no significant differences between any of the uninjured diaphragm groups, nor between these and the jejunum, but show a very significant [$p < 0.001$] difference between all of these and the injured-diaphragm groups, which do not differ between themselves

b) These vessels had sharp indentations in their endothelium, often with anchoring filaments attached to them, extending into the tissues.

c) These were essentially rounded, applied to the tissues, but with no indication that they were being pulled out into them

The anchoring filaments were still visible in both regions, although perhaps less frequent in the jejunum – as *Dobbins and Rollins* (11) observed.

The results of the classification of the vessels are shown in the Table. It can be seen that the visual impressions were confirmed by this more objective estimation. It is clear that during both the filling- and the emptying-phases the initial lymphatics are quite different in conformation than they are in oedematous tissue. No significant differences were found between any of the uninjured-diaphragm groups, irrespective of the phase of the cycle or the method of immobilisation, nor between these and the jejunal-groups, again irrespective of the phase. Similarly, there was no significant difference between the two forms of injury. On the other hand, there were very significant differences between the injured groups and the uninjured diaphragms or the jejunum.

Discussion

It is apparent that these results militate against the suggestion that normally, at least in these two tissues, the anchoring filaments pull the initial lymphatics open during the filling-phase. The results in the injured diaphragms clearly show that the filaments can do this; normally they do not. As *Pullinger and Florey* (33) pointed out, it is the possession of these filaments which ensures that the initial lymphatics remain patent in oedematous

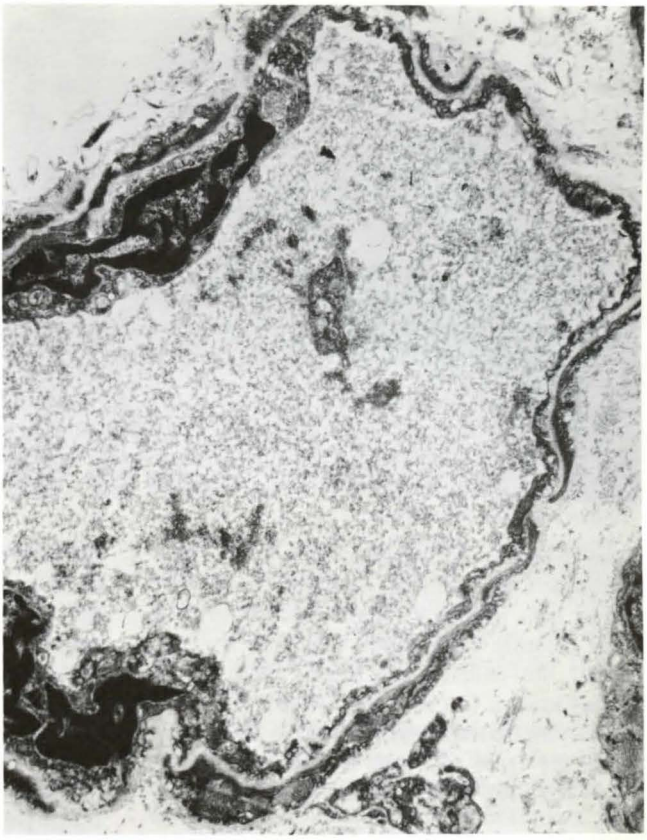

Fig. 5 A normal diaphragm, during the filling-phase, immobilised by freeze-substitution. It shows few signs of being pulled out, rather it was classified as being very "pushed out". 5200x

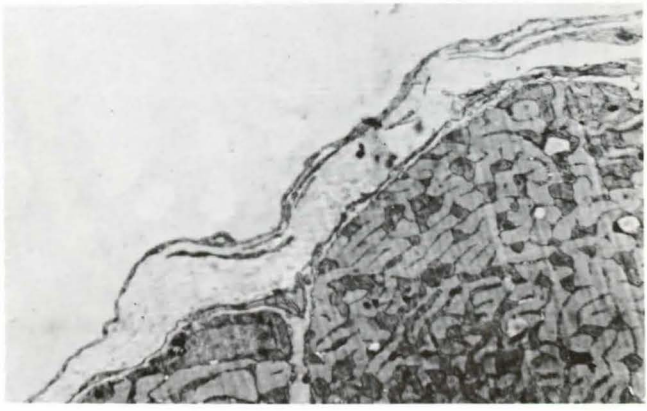

Fig. 6 As for Fig. 5, but with chemical fixation, showing a lacune which was classified as moderately pushed out. 1300x

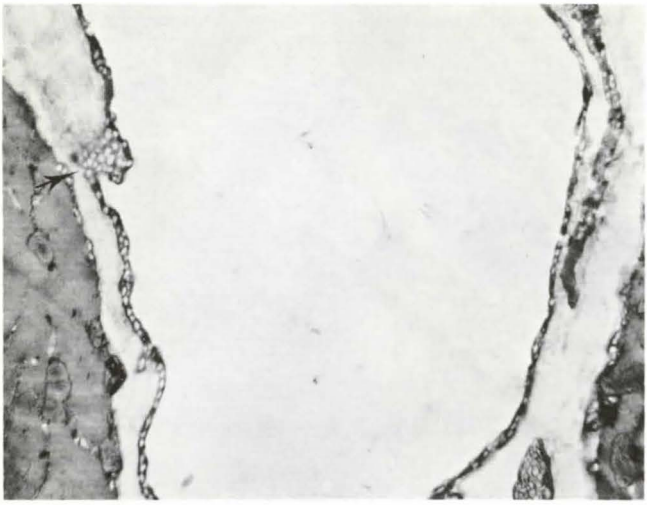

Fig. 7 As for Fig. 6, but it was classified as slightly pushed out. The region of wall at the arrow is not pulled out, but happens to be obliquely sectioned. 5200x

tissue, while blood vessels – with much higher hydrostatic pressures – collapse. It is well known how relatively minor increases in total tissue pressure cause blood vessels to shut in normal, non-oedematous, tissue. These are also well known to cause the collapse of the initial lymphatics in such tissues – indeed this is the cause of the emptying-phase of the cycle. Clearly the situation is very different between the initial lymphatics being held dilated (sucking fluid in) in oedematous tissues and these vessels in normal tissue, where they are allowed to collapse during increases of total tissue pressure. Indeed the conformation of these vessels in normal tissue, with the endothelium tightly applied to the tissues, suggests that the hydrostatic pressure in the vessels is greater than the tissue hydrostatic pressure.

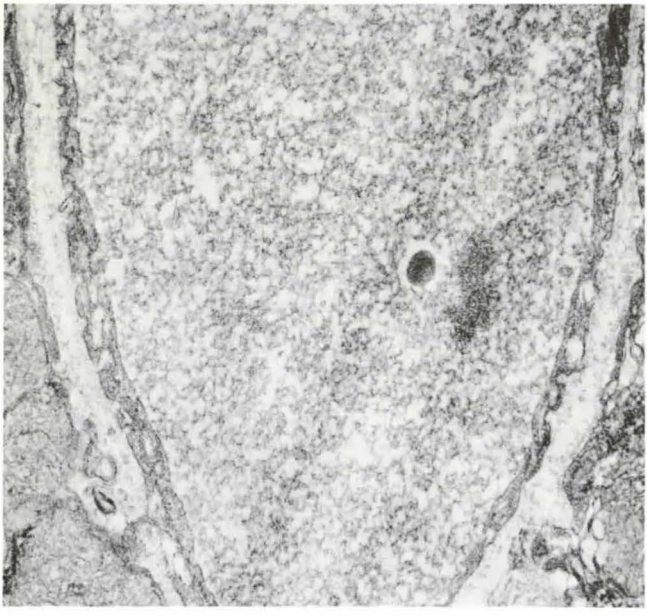

Fig. 8 An initial lymphatic in a diaphragm during the emptying phase; freeze-substitution. This was classified as flat. 7800x

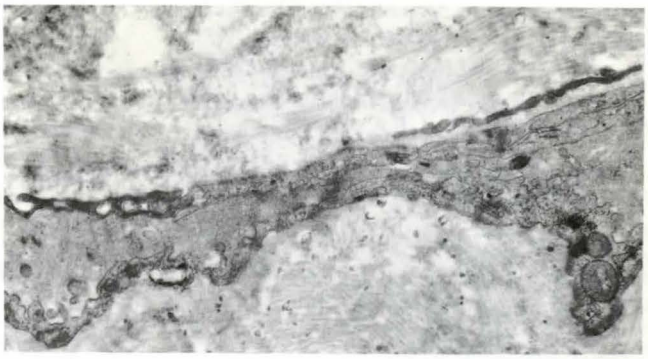

Fig. 9 A lacteal in a puppy jejunum, during the emptying-phase, was classified as moderately pushed out. 7800 x

Evidently the interstitial tissues are not greatly compressed during the increases of total tissue pressure which power the initial lymphatic cycle. This is understandable when one considers how much, notoriously incompressible, water they contain and the relatively incompressible nature of their other constituents.

Indeed, apart from the collapse of the blood vessels – if the increase of pressure is enough to cause this, the only other place where the tissues can obviously loose volume is by the expulsion of the lymph. It is obviously impossible for this to cause sufficient tension in the filaments upon release of the increased pressure (even assuming that they are perfectly elastic and perfectly placed to transmit the tension) to cause the initial lymphatics to refil – except asymptotically over an infinite time. Oedema obviously presents a quite different condition, when much fluid enters the tissues, distorting them and thus pulling on the anchoring filaments and hence the initial lymphatics.

What are the forces, then, which cause filling of the initial lymphatics in most tissues under normal conditions? The suggestion (11, 24, 31, 38, 39) that fluid is actively pumped across the endothelium ignores the facts that the endothelium does not in the least resemble tissues which engage in active transport, that the small vesicles transfer material randomly in the direction of the concentration gradients – without the need for cellular energy, and that much evidence shows that the initial lymphatics fill via their open junctions (reviewed: 2, 6). The suggestion that active contractions of the collecting lymphatics are transmitted to the initial lymphatics (34, 37) does not agree with the intralymphatic pressure measurements mentioned earlier (which do not show a fall during the relaxation of adjacent collecting lymphatic segments), nor does it accord with the absence of anchoring filaments on the walls of the larger vessels – which presumably would be necessary to cause such a suction effect.

We are left with the hypothesis that it is the colloidal osmotic pressure of the relatively concentrated lymph in the initial lymphatics which causes fluid (and macromolecules) to enter them (2, 6). It has been shown in vitro that macromolecules can exert nearly the whole of their colloidal osmotic presure across very wide pores and that this will cause an inflow of fluid and macromolecules from the more dilute solution (9).

In spite of earlier difficulties, this concept is now gaining support from theory (30, 32, 42). Also in spite of contrary evidence (usually obtained from experiments where the tissue had been immobilised and hence where it was impossible for the cycle to function), there is increasing reason to believe that the initial lymphatic lymph is more concentrated than interstitial fluid (reviewed: 2, 6, 7). There is also evidence that the lymph is diluted during the filling-phase (by the more dilute tissue fluid) and reconcentrated by ultrafiltration during the emptying-phase (3, 5, 8). Approximate calculations (4) indicate that much more lymph is formed in the initial lymphatics than is transmitted to the collect-

ing lymphatics, i.e. considerable ultrafiltration occurs. Finally, a mathematical model (14) shows that the whole cycle could function in this way and would be self-correcting, with negative-feedback.

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