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EDITORIAL

THE INTRINSIC LYMPH PUMP: PROGRESS AND PROBLEMS*

In the following essay, I briefly discuss some of the data generated in the last two years that is pertinent to the issue of the "lymph pump" and relate some of the major obstacles and problems associated with studies on lymphatic contractile activity. For a more comprehensive review of this subject, interested readers are referred to a monograph published in 1985 entitled *Experimental Biology of the Lymphatic Circulation* (1).

It seems to me that there are more investigators interested in the lymphatic circulatory system now than there were 10 years ago when I stumbled into this field. At that time, apart from one group in Belfast and one in Japan there appeared to be little interest in the mechanisms regulating lymphatic vessel contractions. One of the early problems I encountered was in convincing the Medical Research Council (MRC) of Canada to make a distinction between the terms lymphatic and lymphoid. Because the MRC reserved the right to have grants reviewed by the panel it judged most appropriate, and the terms lymphoid and lymphatic were synonymous in their view, my grant was reviewed by an "immunology panel." Needless to say, the grant did not get favorable reception from this group which was not surprising since the proposal had nothing to do with the lymphocyte. This situation has now improved but clearly, confusion still exists in some sectors of the biomedical community. As an example, a few months back, I was asked to present some of our work to one of the clinical departments at the University of Toronto. I spent 45 minutes discussing issues in lymphatic physiology that I thought were important and attempted to put the concept of an intrinsic lymph pump into a perspective that might make sense to a clinical audience. At the end of my talk, the Chairman of the department thanked me for my presentation but then asked a revealing question. Since (he claimed) all liquid that leaks from the terminal vascular bed was resorbed at the venular end of the microcirculation, he was unclear as to what liquid (lymph) I was talking about. He found it difficult to accept that one could actually put a catheter in a lymphatic vessel and collect volumes of liquid. At this point, I knew that I had failed to impress the audience of the importance of the lymphatic circulation (in fact perhaps, even of the existence of lymph!) and as a consequence, the concept of an intrinsic lymph pump would not make much sense.

Within physiological circles of course, the situation is quite different. The study of lymph and lymphatics if not fashionable, is still a respectable undertaking.

**Although lymphatic contraction has long been advocated as a prime mechanism for propelling lymph fluid forward, mounting evidence in recent years has firmly established the correctness of this physiologic concept (see p. 135). Along with burgeoning interest, however, remains the not to be underestimated difficulty and limitations of acquiring and recording lymph dynamic data and finally accurately interpreting and reporting the results obtained. In the above Editorial, these issues are addressed by one of lymphology's foremost investigators (Ed.).*

We are starting to see symposia at major meetings that include the intrinsic lymph pump as one of the discussion topics. However, I have found that the idea of a contracting lymphatic vessel playing a role in the movement of liquid and protein from the tissue spaces back to the bloodstream, is often met with hostility, particularly in North America. To be fair, it is no doubt true that our knowledge in this area is primitive and it is difficult to fit the concept of a flexible lymph pump into some physiological perspective. Nonetheless, the intrinsic lymph pump is an attractive concept. Increases in transmural pressures both in *in vitro* and *in vivo* preparations enhance lymphatic pumping activity (reviewed in 2) and it seems possible that the lymphatics increase or decrease contractile activity depending on the needs of the local tissues or perhaps the needs of the organism as a whole. It has been suggested that this pumping mechanism is also responsible for literally sucking tissue fluid into the initial lymphatics and therefore contributes not only to the propulsion of fluid within the vessels but also plays an important role in lymph formation itself (3).

Unfortunately, we cannot relate lymphatic pumping with events in the tissue spaces with the possible exception of the bat wing model where some progress along these lines has been made. For the most part, we are restricted to studies of the lymphatic vessel itself and can only speculate as to the implications at the interstitial level. Until techniques are developed to address this issue experimentally, this shortcoming will remain a serious obstacle to understanding the role of the lymph pump in fluid and protein regulation. Apart from this severe limitation, several other objections to the lymph pump concept have been raised. These can be most clearly demonstrated in the questions that followed a Lymphology symposium in Japan as part of the Microcirculation Congress of 1987. I direct any interested readers to the December 1987 issue of *Lymphology* (20:4, 235-238,271) for a synopsis of the spirited post-symposium discussion. I will address the most salient features here briefly.

At the outset, we need to define the term "intrinsic lymph pump." The term lymph pump has been loosely applied to all the forces that are responsible for moving liquid and protein from the tissue spaces into the initial lymphatics as well as those forces that are responsible for the propulsion of lymph once the liquid is inside these vessels. In the former case this could include osmotic gradients as has been proposed by Casley-Smith (4) or hydrostatic forces. The latter could include extrinsic compression forces or result from the contractions of the initial lymphatics (reviewed in 3). As the osmotic theory of lymph formation seems to have little support, the hydrostatic theory remains the most plausible. The question of which is the dominant force, i.e., the contracting lymphatic vessels (intrinsic lymph pump) or extrinsic forces acting on the lymphatics to cause compression/relaxation cycles, is still debated.

One frequent comment is that lymphatics in some species do not contract. This assertion, of course, may very well be true. However, the evidence for this contention seems to be based more on casual observation than on systematic experimentation. I have noticed non-contracting lymphatic vessels in a variety of circumstances. In fact, our early attempts to study contractile behavior in sheep and bovine lymphatics often resulted in failure. It wasn't until we refined our techniques after considerable effort, that we were able to study consistently the mechanical behavior of these lymphatic vessels. The key issue is that it is very easy to damage lymphatics; one must be exceedingly careful in preparation since handling of the ducts or changes in the local environment (such as temperature) can inhibit contractions. The lymphatic vessels often have to equilibrate for many hours after surgical and/or isolation procedures before intrinsic activity returns. In addition, many studies utilize anesthetics and these are known to have deleterious effects on lymphatic motion (5-7). For example, at concentrations above 10^{-4}M (levels likely to be achieved in anesthe-

sia), pentobarbitone or halothane characteristically abolish spontaneous contractions of bovine mesenteric lymphatics (7). To establish definitely whether a lymphatic in a given tissue is capable of contraction requires considerable experimental effort.

Whether a lymphatic vessel contracts or not has also been presumed to depend on its content of smooth muscle cells. In the larger vessels used for studying the mechanical properties of the lymphatics, it is clear that they contain multiple layers of smooth muscle cells and so the concept of contractile activity has been somewhat easier to accept. However, lack of smooth muscle in the walls of initial lymphatics has been invoked to suggest that they are incapable of exhibiting this type of activity. It is interesting, however, to note how the image of the endothelial cell has changed over the last several years. At one point, endothelia were considered to be passive lining cells but now are thought capable of dynamic interactions with blood elements and humoral factors (8). The notion that endothelial cells may contract is certainly not new. Endothelial cells are known to contain contractile elements and investigators who study the inflammatory response proposed many years ago that endothelial cells, especially in post-capillary venules, contract in response to certain inflammatory mediators and in the process, increased the permeability of the terminal vascular bed (9).

Initial lymphatics appear to be relatively simple endothelial tubes which lack the basal lamina characteristic of their blood vascular counterparts (10). As one might expect, the study of initial lymphatics poses experimental difficulties and only with the bat wing model have investigators been able to study this issue. In the bat, direct evidence is available that initial lymphatics are capable of contracting and measurements of micropressures in the initial lymphatics and surrounding interstitium suggest that this activity plays an important role in drawing liquid into the vessels (11). Unfortunately, the anatomy of these lymphatic vessels has not been extensively studied and therefore, it is not certain that they are completely devoid of smooth muscle cells. In any event, the question of whether initial lymphatics in other species are capable of contracting is an important area for further research.

Data from lymphatic vessels isolated *in vitro* or *in vivo* illustrate that pumping activity can be initiated by applying a transmural pressure to initial lymphatics (12) and to collecting ducts (13,14). As transmural pressures are raised, pumping increases up to a peak pressure, after which flow declines. Lymphatic function curves obtained in this manner suggest that the lymph pump is capable of adapting its activity to changes in interstitial fluid volume or is responding to a pressure load in such a way as to maintain lymph flow at a given level. However, work from Drake and his associates appears to challenge this concept. These investigators have monitored lymph flow rates from a variety of organs and studied the effects of raising the outflow pressure which results in a reduction in flow rates (15-19). The findings have led this group to propose a model of lymph flow that essentially ignores the concept of the intrinsic lymph pump. In their analysis, a constant driving pressure pushes lymph against a given resistance. A requirement for this model, however, is that the relationship between the fall in lymph flow and the outflow pressure must be linear. While Drake et al claim the results fulfill this requirement, others have been unable to generate similar data. The Belfast group, for example, has demonstrated that lymph flow rates decreased non-linearly as outflow pressures were raised in both afferent and efferent popliteal vessels in sheep (20). Measurements of lymphatic pressure revealed increases in contraction frequency (at the expense of stroke volume) as outflow pressures increased. Calculations of lymphatic power production were consistent with a lymph pump attempting to overcome the elevations in outflow pressures up to a maximum level, after which the level of power declined presumably as the pump failed. As it is easier in relative terms, to demonstrate a lack of pumping activity than it

is to show active contractions, it seems likely that the experiments from the Belfast group reflect the role of the lymphatic vessel in contributing to lymph propulsion, at least in the tissue bed they were studying. Whether this phenomenon is true of other tissues has yet to be determined.

In this regard, an intrinsic lymph pump may not be necessary in all tissues. Other mechanisms may be equally or more important. For example, in the bowel, experimental evidence suggests that lacteal pressures correlate with intestinal motility and that in some species, contractions of the mucosal villi exert a milking action that forces lymph into the submucosal lymphatics (21,22). In this case, the smooth muscle in the villi appears to provide a major part of the propulsive force at least until the lymph enters the larger collecting ducts. In other tissues as well, extrinsic compression forces may contribute significantly to the movement of liquid and protein from the tissue spaces into the initial lymphatic vessels and possibly in some cases, aid in the propulsion of lymph along the collecting ducts (23,24).

If the regulation of lymph propulsion was exclusively by extrinsic compression forces, the removal of liquid and protein from the tissue spaces would be predominantly if not purely a passive process. One of the attractive features of the intrinsic lymph pump hypothesis is that the pump is flexible with the ability to adjust its activity depending on levels of interstitial hydration. The regulation of pumping appears to be largely myogenic but in addition, lymphatics are innervated (25) and respond to a wide variety of humoral factors (2) suggesting that the host is able to exert finer control over lymph propulsion in some situations. Indeed, evidence from *in vivo* experiments in sheep, suggests that the modulation of lymphatic pumping can be independent of changes in filtration. Lymphatic vessels can be isolated *in vivo* from lymph input with their blood and nerve supplies left intact (14,26,27). The input to the duct is provided from a reservoir, and by arranging the heights of reservoir and the outflow catheters appropriately, a transmural pressure can be applied to the prepared lymphatics. This pressure effect in turn stimulates lymphatic pumping activity which can be monitored in the anesthetized or non-anesthetized animal. McHale and colleagues (26) have demonstrated that a fright stimulus to sheep can alter pumping in this preparation and further that the administration of intravenous noradrenalin stimulates pumping while isoprenaline depresses flow (26).

Using a similar model, we have observed that a bleed of 25% of total blood volume resulted in increased fluid propulsion over a 6 hour period (27). Pumping activity increased up to 6-fold above control levels. In another study, we observed that intravenous endotoxin suppressed pumping activity (14). Because this model system distinguished between lymph formation and lymph pumping, we concluded that these effects did not relate to changes in vascular parameters or filtration. These results suggested that lymph vessels *in vivo* responded not only to myogenic stimulation, but also could be modulated by host-derived neurohumoral or other factors. The host's ability to modulate the lymph pump opens many new possibilities in the regulation of interstitial hydration in physiological and pathophysiological states. For example, it will be of interest to determine whether the host is capable of mobilizing interstitial protein through stimulation of lymphatic pumping following hemorrhage? Does the depression of pumping that occurs with endotoxin contribute to edema formation? Whatever the answers to these questions turn out to be, it would appear that further investigation of the role and significance of the intrinsic lymph pump is warranted and may well provide interesting new insights into some unanswered physiological problems.

Since the publication of the monograph, *Experimental Biology of the Lymphatic Circulation* in 1985, progress has been made in understanding the regulation of lymphatic pumping by neurohumoral factors and extending these studies

to the smaller lymphatics and lymphatics from other tissues and species. Much of the information on the lymph pump has come from studies on bovine vessels *in vitro* and more recently on *in vitro* and *in vivo* preparations in sheep. Mesenteric vessels are the most commonly used because of relatively easy access and suitable size.

Investigators in Belfast have been among the most active in studying the mechanisms regulating lymphatic pumping using predominantly the bovine mesenteric preparation. Largely through the efforts of this group, we know that lymphatic vessels are innervated by noradrenergic nerves and recently they have expanded their studies of the electrophysiological consequences of α-adrenergic stimulation (28,29), demonstrated the phenomenon of rapid desensitization of lymphatic adrenoceptors of exogenous noradrenaline or field stimulation (30) and studied the effects of K^+ channel blockers on electrical activity of these vessels (31). In addition, McGeown and associates (32) have studied the effects of sympathetic stimulation on lymph flow in the anesthetized sheep. They demonstrated that the stimulation of the lumbar sympathetic chain enhanced flow rates and increased lymphatic contraction frequency suggesting that the intrinsic lymph pump had been directly affected. In terms of humoral regulation, to complement earlier studies using the ring preparation, we have used the isolated bovine mesenteric system to investigate the effects of chemical mediators on pumping activity. Prostaglandin E_2, a thromboxane analogue (compound U46619), and the cytokines Interleukin-1α and β, were all capable of inhibiting pumping (33,34) and their entry into lymph may have some impact on the ability of the lymphatic vessels to drain the interstitium during inflammatory responses.

The potential heterogeneity of lymphatics is a problem that will have to be addressed. Blood vessels from different parts of the body respond differently to some agonists and inhibitors and it is likely that the same is true of lymphatics. There are now several studies using human lymphatics which tend to support the earlier work on bovine vessels. Thus, segments of human lymphatics from the groin (35) and from unspecified tissue sources (36,37) have been isolated and their contractile properties studied *in vitro*. As is the case with bovine vessels, some inflammatory mediators and noradrenaline and especially prostaglandins and leukotrienes, induce phasic and tonic changes in these lymphatics. The fact that human lymphatics seem capable of synthesizing various arachidonic acid products (38,39) supports our original hypothesis that arachidonate metabolism within the vessel wall plays a role in modulating spontaneous and agonist-induced contractions. In addition, studies along these lines have also been carried out on rat mesenteric lymphatics (40) and on the initial lymphatics of the bat wing (41). Many of the classical inflammatory mediators effect the rat preparation, but only bradykinin appears to exert an effect on the initial lymphatics of the bat. The latter studies are significant in that they probably are the first to examine humoral control of the initial lymphatics and it is hoped that this work will continue in the future.

The initial lymphatics respond to increases in transmural pressure by increasing their contractile activity much like the larger collecting ducts (12). However, infusion of saline into the interstitium of the bat wing produces no transmural pressure changes in the initial lymphatics but the lymphatics still respond to the infusion by increasing contractile activity similar to that which occurs following intraluminal injection (42). The authors suggest that this response is due to radially directed tension applied to the lymphatic wall by anchoring filaments which tend to pull the lymphatics open as interstitial volume increases. One potentially serious limitation of the bat model is that it is necessary to induce edema in order to locate the initial lymphatics before the experiment can be performed. Whether results from these experiments can be applied to the non-edematous state is not

known.

In summary, our understanding of the regulation and significance of the intrinsic lymph pump is still in a rudimentary state. Progress has been made and more investigators appear to be interested in lymphatics now that it has been substantiated (in some systems at least) that these vessels are not simply passive conduits, but have the ability to participate actively in fluid and protein homeostasis. Although there are those who remain skeptical about the idea of an intrinsic lymph pump, it is my admittedly biased view, that in most tissues and organs and in most species, lymphatic vessels play an active role in controlling extravascular liquid and protein. I find it hard to accept that anything as important as the regulation of tissue fluid could be left totally to passive, non-adaptive, haphazard forces. It is hoped that some of the more controversial issues related to the intrinsic lymph pump will be resolved by developing the capability to manipulate the lymphatic vessel *in vivo* and study the consequences at the interstitial level.

REFERENCES

1. *Experimental Biology of the Lymphatic Circulation*, Johnston, MG (Ed.), Elsevier Science Publishers, B.V. (1985).
2. Johnston, MG: Involvement of lymphatic collecting ducts in the physiology and pathophysiology of lymph flow. In *Experimental Biology of the Lymphatic Circulation*, Johnston, MG. (Ed.), Elsevier Science Publishers, B.V. (1985), 81.
3. Adair, TH, AC Guyton: Lymph formation and its modification in the lymphatic system. *Experimental Biology of the Lymphatic Circulation*, Johnston, MG. (Ed.), Elsevier Science Publishers, B.V. (1985), 13-44.
4. Casley-Smith, JR: The structure and functioning of the blood vessels, interstitial tissues, and lymphatics. In: *Lymphangiology*, Földi, M and JR Casley-Smith (Eds.), F.K. Schattauer Verlag, Stuttgart (1983), 117-127.
5. Takeshita, T, M Morio, M Kawahara, et al: Halothane-induced changes in contractions of mesenteric lymphatics of the rat. Lymphology 21 (1988), 128-130.
6. Takeshita, T, M Kawahara, S Akita, et al: Effect of halothane anesthesia on mesenteric intralymphatic pressure in the rat. Lymphology 22 (1989), 97-99.
7. McHale, NG, KD Thornbury: The effect of anesthetics on lymphatic contractility. Microvasc. Res. 37 (1989), 70-76.
8. Cotran, RS: New roles for the endothelium in inflammation and immunity. Am. J. Pathol. 129 (1987), 407-413.
9. Grega, GJ, SW Adamski: The role of venular endothelial cells in the regulation of macromolecular permeability. Microcirculation, Endothelium and Lymphatics 4 (1988), 143-167.
10. Leak, LV: *Electron Microscopy in Human Medicine*, Johannessen, JV (Ed.), Vol. 5: *Cardiovascular System, Lymphoreticular and Hematopoietic System*, McGraw-Hill International Book Co., Toronto (1980), 157-212.
11. Hogan, RD: The initial lymphatics and interstitial fluid pressure. In: *Tissue Fluid Pressure and Composition*, Hargens, AR (Ed.), Williams and Wilkins, Baltimore (1981), 155-163.
12. Hogan, RD, JL Unthank: Mechanical control of initial lymphatic contractile behavior in bat's wing. Am. J. Physiol. 251 (1986), H357-H363.
13. McHale, NG, IC Roddie: The effect of transmural pressure on pumping activity in isolated bovine lymphatic vessels. J. Physiol. 261 (1976), 255-269.
14. Elias, R, MG Johnston, WR Nelson, et al: Decreased lymphatic pumping following intravenous endotoxin administration in sheep. Am. J. Physiol. 253 (1987), H1349-H1357.
15. Drake, RE, DK Adcock, RL Scott, et al: Effect of outflow pressure upon lymph flow from dog lungs. Circ. Res. 50 (1982), 865-869.
16. Drake, R, M Giesler, G Laine, et al: Effect of outflow pressure on lung lymph flow in unanesthetized sheep. J. Appl. Physiol. 58 (1985), 70-76.
17. Drake, RE, SJ Allen, JP Williams, et al: Lymph flow from edematous dog lungs. J. Appl. Physiol. 62 (1987), 2416-2420.
18. Laine, GA, RE Drake, FG Zavisca, et al: Effect of lymphatic cannula outflow height on lung microvascular permeability estimations. J. Appl. Physiol. 57 (1984), 1412-1416.
19. Laine, GA, SJ Allen, J Katz, et al: Outflow pressure reduces lymph flow rate from various tissues. Microvasc. Res. 33 (1987), 135-142.
20. McGeown, JG, NG McNale, IC Roddie, et al: Peripheral lymphatic responses to out-

flow pressure in anaesthetized sheep. J. Physiol. 383 (1987), 527-536.
21. Unthank, JL, HG Bohlen: Lymphatic pathways and role of valves in lymph propulsion from small intestine. Am. J. Physiol. 254 (1988), G389-G398.
22. Womack, WA, PK Tygart, D Mailman, et al: Villous motility: Relationship to lymph flow and blood flow in the dog jejunum. Gastroenterology 94 (1988), 977-983.
23. Skalak, TC, GW Schmid-Schonbein, BW Zweifach: New morphological evidence for a mechanism of lymph formation in skeletal muscle. Microvasc. Res. 28 (1984), 95-112.
24. McGeown, JG, NG McHale, KD Thornbury: Effects of varying patterns of external compression on lymph flow in the hindlimb of the anaesthetized sheep. J. Physiol. 397 (1988), 449-457.
25. McHale, NG: Innervation of the lymphatic circulation. In *Experimental Biology of the Lymphatic Circulation*, Johnston, MG (Ed.), Elsevier Science Publishers, B.V. (1985), 121-140.
26. McHale, NG, K Thornbury: A method for studying lymphatic pumping activity in conscious and anaesthetized sheep. J. Physiol. 378 (1986), 109-118.
27. Hayashi, A, MG Johnston, W Nelson, et al: Increased intrinsic pumping activity of intestinal lymphatics following hemorrhage in anesthetized sheep. Circ. Res. 60 (1987), 265-272.
28. McHale, NG, JM Allen, HLA Iggulden: Mechanism of α-adrenergic excitation in bovine lymphatic smooth muscle. Am. J. Physiol. 252 (1987), H873-H878.
29. Allen, JM, JG McCarron, NG McHale, et al: Release of [^{3}H]-noradrenaline from the sympathetic nerves to bovine mesenteric lymphatic vessels and its modification by α-agonists and antagonists. Br. J. Pharmacol. 94 (1988), 823-833.
30. McHale, NG, JM Allen, JG McCarron: Transient excitatory responses to sustained stimulation of intramural nerves in isolated bovine lymphatic vessels. Quart. J. Exp. Physiol. 73:175-182.
31. Allen, JM, NG McHale: The effect of known K^+-channel blockers on the electrical activity of bovine lymphatic smooth muscle. Pflugers Arch. 411 (1988), 167-172.
32. McGeown, JG, NG McHale, KD Thornbury: The effect of electrical stimulation of the sympathetic chain on peripheral lymph flow in the anaesthetized sheep. J. Physiol. 393 (1987), 123-133.
33. Elias, R, MG Johnston: Modulation of fluid pumping in isolated bovine mesenteric lymphatics by a thromboxane/endoperoxide analogue. Prostaglandins 36 (1988), 97-106.
34. Hanley, C, R Elias, HZ Movat, et al: Suppression of fluid pumping in isolated bovine mesenteric lymphatics by interleukin-1: Interaction with prostaglandin E_2. Microvasc. Res. 37 (1989), 218-229.
35. Sjoberg, T, P Alm, EE Andersson, et al: Contractile response in isolated human groin lymphatics. Lymphology 20 (1987), 152-160.
36. Sinzinger, H, J Kaliman, E Mannheimer: Regulation of human lymph contractility by prostaglandins and thromboxane. Lymphology 17 (1984), 43-45.
37. Sinzinger, H, J Kaliman, E Mannheimer: Effect of leukotrienes C_4 and D_4 on prostaglandin I_2 liberation from human lymphatics. Lymphology 19 (1986), 79-81.
38. Sinzinger, H, J Kaliman, E Mannheimer: Arachidonic acid metabolites of human lymphatics. Lymphology 17 (1984), 39-42.
39. Ito, Y, S Magari, M Sakanaka: Prostaglandin I_2 synthase in the lymphatic endothelium of rat liver as revealed by pre-embedding immunoelectron microscopy. Lymphology 22 (1989), 51-55.
40. Ferguson, MK, HK Shahinian, F Michelassi: Lymphatic smooth muscle responses to leukotrienes, histamine and platelet activating factor. J. Surg. Res. 44 (1988), 172-177.
41. Unthank, JL, RD Hogan: The effect of vasoactive agents on the contractions of the initial lymphatics of the bat's wing. Blood Vessels 24 (1987), 31-44.
42. Hogan, RD, JL Unthank: The initial lymphatics as sensors of interstitial fluid volume. Microvasc. Res. 31 (1986), 317-324.

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