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PANEL DISCUSSION: HOW LYMPH IS PROPELLED

DR. M. WITTE: During this discussion, since we've identified a property in which the lymphatics might truly differ from the blood vessels, perhaps we should focus for the next 10 minutes or so on the mechanism whereby lymph flows and is propelled. We did already talk about another important function, permeability, and I think we can get back to that perhaps in the last discussion period. Let's discuss here the mechanisms by which lymph is propelled, perhaps differences from organ to organ and differences of opinion.

DR. SCHMID-SCHONBEIN: I have personal experience on lymph transport mechanisms only in skeletal muscle and recently learned some more details on lymph transport in the intestine. Dr. Simionescu yesterday made a very good statement which Dr. Ohhashi repeated in other words today: generalization is your greatest enemy. The lymph transport mechanism in each organ is very specific, and I'd like to illustrate that briefly for skeletal muscle. In contrast to what we learn from Drs. Roddie and Johnston, the lymphatics in skeletal muscle have no smooth muscle of their own. This entire organ is completely free of lymphatic smooth muscle and consists only of highly attenuated lymphatic endothelium. There's recent evidence suggesting that the entire human heart has no lymphatic smooth muscle of its own. In fact, the intestinal lymphatics have been recently shown to be lined only by endothelium. The vessels that Dr. Roddie, for example, described are really at the downstream end. They are the conduits carrying fluid away. The key question in lymphatic research that we view is how does fluid enter from the interstitial space into the lymphatic terminals? There are some organs like the mesentery that Dr. Hauck has studied which have terminal endings which consisting only of lymphatic endothelium. But, within the organ if you go further downstream, the lymphatic channel is a lymphangion with its own smooth muscle contractility.

DR. RODDIE: I must say I would have worried quite a bit about this absence of smooth muscle until 3 years ago when I was in Professor Tsuchiya's department where he had a lovely picture of mesenteric lymphatics. There were little tiny ones contracting rhythmically and well but the problem was that they just didn't have any smooth muscle. This phenomenon at first seemed to be a passive downstream consequence of a contraction upstream. But since that time, so much actin and myosin has been found in endothelial cells that if someone were to make a proposition to me that endothelial cells could do this on their own, I wouldn't object to it. In Professor Tsuchiya's little lymphatics, there was an occasional smooth muscle but there wasn't a complete sheet, and yet they were contracting and relaxing prettily.

DR. M. WITTE: I think we've touched a nerve, or should I say an endothelial cell, here. I'd like to give a chance to anyone on the panel who wants to respond to this specific issue before we go to the audience.

DR. O'MORCHOE: Well, I have the same sort of comment. I was concerned that Dr. Roddie and Dr. Johnston were talking about their lymphatics as though it were true of all lymphatics. I believe that it's not. There are lymphatics that don't show that pulsatile expression and pulsatile flow that you demonstrated. For example, the one I'm most familiar with are the renal lymphatics, which in the dog *in vivo* never exhibit contractions. If you look at the renal lymphatics in a rat, you will see beautiful peristaltic contractions just like the ureter. The rat has much more smooth muscle in its renal hilar lymphatics than the dog where you never see pulsations.

DR. CASLEY-SMITH: I've got two points. First, endothelium of lymphatics does contract. It's been known for about the past 20 years from the batwing study, but only a few species of bats. People have looked at mouse ears, at rabbit ear chambers, where we know very well that the initial lymphatics do not contract and have never been seen to contract over hundreds of years of observations. So, they differ in muscle because when you chaps were talking about leg lymphatics, I remembered some obvious clinical observations about paralyzed limbs getting lymphedema. They get lymphedema in spite of having perfectly good collecting lymphatics presumably contracting away like mad. Why do they get lymphedema?

DR. LEAK: I just wanted to agree with what was said earlier. But, we've done cinephotographic studies on liver lymphatics and the guinea pig's ear, and we've also done this in mice and rats. Those lymphatic capillaries we've shown from the EM studies do contain actin filaments, and they do rhythmically contract.

DR. M. WITTE: I think also that some of the precapillary sphincters in the spleen have been clearly shown not to be muscle at all but rather endothelial cells assuming different shapes because of changes in actin.

DR. MIURA: We observed mesenteric lymphatics as Dr. Roddie indicated. In the lobular portions, there is considerable smooth muscle but in between, endothelium only is present. We think that both smooth muscle and endothelium are important for lymphatic contractility. Also, absorption of dietary fat depends on lymphatic function in the intestine and is unique and distinctive for this organ compared to skeletal muscle and other organs. There are also species differences as between bovine lymphatics and rat lymphatics. Dr. Ohhashi showed that bovine mesenteric lymphatics have considerable smooth muscle but rat mesenteric lymphatics have much less.

DR. MORRIS: I'd like to make an observation that people don't recall. It's really only in the last 20 years or so that the dogma has been challenged about lymph flow and propulsion through passive movements of muscles surrounding lymphatics. This was in spite of the fact that Florey back in the 1930's showed that the lymphatics in the mesentery of guinea pigs contracted rhythmically. It was only when lymph flow could be examined in unanesthesized animals like the sheep that it became obvious that the lymphatics were in fact contracting and squeezing lymph out in a regular fashion. Even in the sheep under anesthesia, you tend to diminish the contractility of the system. So while I've no doubt that all species of animals display this contractile lymph propulsion, it isn't displayed to its maximum extent except in the unanesthesized animal. One thing we haven't discussed today is the propagation of contractions along the lymphatic vessels and the realization that at certain points along the vessel one arrives at lymph nodes which themselves may have contractile capability. This feature of lymph propulsion has never been examined. The intrinsic contractility of the capsule and the vessels associated with the lymph node might be very important not only in modulating the return of fluid from the periphery but also in regulating the expulsion and transmission of cells from within the node to the efferent lymphatic. I'm a bit of a purist so I have to tell Miles Johnston that the mesenteric lymph nodes of a sheep are not discrete but rather a band that runs around the whole mesentery so he'll have to redo that diagram. Also, Dr. Roddie's got to get his valves pointing in the right direction when he puts a diagram on the board. Those are the sorts of things that we haven't addressed yet in relation to the propagation of fluid from the periphery to the jugular venous confluence. There's any number of intermediary stations along this transport line that have their own intrinsic contribution to make to the propulsion of lymph.

DR. OHHASHI: In my presentation, I've already shown that lymph vessels may be divided in two groups. One is the type that has a lot of smooth muscle cells in the wall and is capable of spontaneous activity. The other type has little or no smooth muscle in the wall. For example, the canine thoracic duct exhibits no spontaneous contraction in an isolated preparation. And finally, I think there is marked variation among species regarding spontaneous contractions depending on the content of smooth muscle cells in the wall. Another point is important. Some drugs, for example, prostaglandin-f2-alpha, produce spontaneous contractions only in some lymph vessels. Also, the content of smooth muscle cells in the vessel wall may change under physiologic or pathologic conditions. For example, venous and lymph vessels are very sensitive to the pressure level in the lumen. For example, after a coronary bypass operation, the saphenous vein develops more smooth muscle cells in its wall.

DR. SCHMID-SCHONBEIN: On the issue of endothelial contractility in skeletal muscle, the shape of the lymphatic channels and intervital observations done over virtually 40 years give clear evidence that there's no such thing. We studied the cytoskeleton of individual endothelial cells and leukocytes and are familiar with shape changes, pseudopod formation and actin myosin interaction. There's no organization with the endothelial cell. Neither is the shape of the lumen due to the irregularity of the lymphatic channels of such a nature that you can generate a hoop stress that resembles smooth muscle contraction for lymphatic propagation inside the skeletal muscle. Second, you don't need it. You have in skeletal muscle wonderful propulsion mechanisms provided by the anatomical arrangements, namely the fact that the lymphatic channels only follow the arterioles. They are closely associated with them in every skeletal muscle we've investigated. Therefore, vasomotion, pressure pulsation, and muscle contraction are the mechanisms for lymph generation and propulsion in that particular organ.

DR. M. WITTE: But in that particular bed at rest there is essentially almost no lymph formation. I think the real question is what happens in that bed when lymph formation is going on at a great rate under vigorous exercise.

DR. SCHMID-SCHONBEIN: That is the beauty of having multiple mechanisms. They adjust themselves according to physiological needs.

DR. C. WITTE: I arise with some degree of circumspection because the several studies presented are absolutely beautiful. On the other hand, I just want to interject a word of caution. Dr. Johnston alluded to dissociating contractility from lymph flow, but as I listen to the panel, it's apparent that more and more we're getting into the error that so many have made with cardiac output—that is, thinking that the heart regulates cardiac output when indeed it's the mean vascular pressure and the peripheral circulation. The heart only regulates cardiac output in heart failure. What we should recognize, and I only raise this again because Dr. Johnston at times would slip in lymph flow and lymph contractility on the ordinate as if they were interchangeable, one should remember that in hemorrhagic shock there's almost no cardiac output but the heart is beating "to beat the band." It's

vigorous as hell and yet there's no flow because there's no inflow. Similarly in the lymphatic system, and I know Dr. Roddie approached this issue, if there's no presentation of fluid no matter how vigorously the lymphatics are contracting, there obviously cannot be any lymph propulsion. I would only interject again some caution as these physiologic studies are elucidated that one cannot equate contractility with lymph flow. Finally, when one observes the lymph heart in frogs, which I suppose is somewhat analogous or perhaps a forerunner of contractile lymphatics, it's apparent that the rhythmicity is very irregular. Indeed, when I've looked at Olszewski's diagrams in a few places, the contractility was very irregular and unpredictable in the lymphatics. I was surprised, Dr. Roddie, to see how regularly your lymphatics contracted.

DR. RODDIE: I have a number of points. First, everyone would agree it would be foolish to generalize to say that all lymphatics in all animals behave in this way. Besides, Dr. Courtice from Australia feels that the sheep is not even an animal and he thinks their lymphatics are atypical. [Dr. Morris injected: an Australian ground louse!] But, I think that people who have worked with these a long time find that recording no contractions is easy because they are tender little vessels and they can be made to not contract with some ease. Usually the more you work with them and the more gentle you are and the more you learn, the more likely you are to see contractions. The question on the propagation of contraction is an interesting one because one thing is fairly clear. Quite a large section of the wall gets its contraction together. These are well coordinated things. It doesn't seem to be due to nerves because neurotoxins don't abolish it. And where the endothelium seems to contract alone, how is the impulse read there? On the point you raised about inflow and output. That may be true, but there's an interesting concept Guyton would put forward, maybe the more the lymphatic contracts the more it sucks out the interstitium and therefore the more goes out the far end. There's not a lot of evidence for that, but I don't think it can be ruled out yet. The last point was on Dr. Casley-Smith's why, when there's no movement, you get lymphedema. Could it be that really the most potent thing in getting lymph to flow has something to do with tissue movement and that movement provides the force to go from the interstitium to the lymphatics and once you get paralysis whatever this mechanism is won't work.

DR. JOHNSTON: I just have one quick point concerning the dissociation between lymph formation and lymph flow. On all too many occasions, we've been faced with necrophysiologic experiments with dead animals in which lymph continues to flow for up to an hour after the animal has died. So very clearly and from what Professor Roddie has already told us with the nerve stimulation experiment, you can have no perfusion to an organ and death and yet lymphatics continue to pump fluid away presumably for as long as there is fluid to pump. The other thing I'd just like to say in passing, I have no problem with other mechanisms of lymph transport at all. I'm certain that all these comments are correct, that there are tremendous variations in the mechanisms by which lymph moves through a lymphatic, but I have been rather impressed with the work that Professor Roddie has done that shows very nicely that external compression is not a very good way to move lymph. I'm therefore a bit leery that anything other than intrinsic contractile mechanisms work in most tissues. There are probably some exceptions, but this is a particularly good mechanism to move it. If you expose an intestinal lymphatic and beat the hell out of it, fluid doesn't move very well but when the vessel is contracting in its coordinated way, fluid moves. So, extrinsic forces may play a role, but I tend to think in general, it's probably minor. Whether that's not true in skeletal muscle, I just don't know.

DR. M. WITTE: But the formation of a lot of lymph, whether or not you have a lot of contractility, is also a major factor making lymph flow. In the cirrhotics (I'm a thoracic duct and major lymphatic duct watcher in man), although we do see rhythmic changes in pressure at around 10-12 beats/min, at a rate that correlates with no other vital function. Many of these patients basically have just distended lakes of lymph where I doubt there's any contractility. The valves are incompetent. The lymphatic flows when you cannulate it and lymph pours out.

DR. OLSZEWSKI: Briefly, referring to the paper of Dr. Johnston and the influence of endotoxin on the contractility of lymph vessels. I think these interesting data corroborate what we see in physiological experiments after injection of certain antigens into the skin. After an initial phase of increased contractility, there is a decrease for 24 even 48 hours. Would you just briefly speculate what would be the chemical mediator because I'm quite sure it's not endotoxin itself?

DR. JOHNSTON: We've put a considerable effort into defining the pharmacological effects of a wide variety of potential mediators in this system, where you have a reservoir and outflow catheter and you apply a transmural pressure to an isolated vessel and then add the mediator to the lumen. Strangely enough, we have at our disposal pharmacologic agents that enhance mechanical activity and that depress mechanical activity. With only one exception, all these compounds depress lymph pumping. In other words, they shift the transmural pressure-flow relationship to the right. These include the important mediator, interleukin-1, which seems to be a powerful depressor of lymphatic function. Interestingly enough, the only mediator we've found in preliminary studies of 10 or 15 vessels that stimulates lymphatic pumping is cachectin or tumor necrosis factor, which is reputed to be produced from endotoxin. In fact, one can initiate almost all of the features of septic shock

with cachectin. This is the only compound we've tested that can actually shift the curve to the left. The answer to Dr. Olszewski's question is really open. It could be prostanoid compounds, interleukin-1, tumor necrosis factor perhaps initially. We haven't done experiments to deplete leukocytes or pharmacologically modify the donor animal. At this time, we can only guess at the answer.

DR. C. WITTE: If I could just say one thing, Dr. Johnston, about the flow of lymph after death. I'm somewhat skeptical of your point in that the partial pressure of oxygen in lymph will drop to 0 within 30 seconds, far more quickly than in the bloodstream which is rich with hemoglobin. In terms of energy availability or aerobic metabolism, it's non-existent. Moreover, really the only duct that continues to flow spontaneously is the thoracic duct and what happens with ischemia is vigorous contraction of the bowel that I'm sure propels lymph flow.

DR. JOHNSTON: We've done popliteals, prefemorals, and intestinals. Popliteals and prefemorals in particular tend to flow for quite some time.

DR. C. WITTE: In edematous states or in a normal animal?

DR. JOHNSTON: No, in just a normal animal in which you want to do an experiment who expires before you can complete it.

DR. C. WITTE: Your cannulae flow a lot more vigorously than mine.

DR. M. WITTE: In the next discussion segment, we will return to some of these questions for which knowledge is incomplete and speculation is limitless.