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LETTER TO THE EDITOR

I wish to draw attention to serious shortcomings in the transmission electron photomicrographs and the statistics in the paper by Cornford and Oldendorf (1). These inadequacies cast considerable doubt on the conclusions drawn by the authors.

Plasma membranes are almost never shown; yet the authors claim depends on seeing them. Plasma membranes may sometimes be sectioned obliquely or difficult to visualize well but general lack of visibility suggests poor staining overall. This deficiency makes junctions almost impossible to detect but their absence in the "terminal endothelial cells" is critical to the authors' proposition. In other words, how can they be sure that the junctions were simply not observed rather than absent. Even when a junction is visible, its identification may be in doubt. For example, in their *Fig. 1b* where "an overlapping endothelial cell seam" (in a blood capillary) is purportedly "indicated by an arrowhead", it looks in fact like rough endoplasmic reticulum. The same may be said of the lymphatic shown in *Fig. 1c*.

In *Fig. 4*, the reverse occurs. Here the lumen is ostensibly completely inside an endothelial cell and "seamless"; yet 1cm below the nucleus (in the photomicrograph) on the left side of the lumen is what appears to be a junction as judged from a highly typical endothelial abluminal extension characteristically found next to a junction. Greater magnification and better staining of the plasma membranes would have made the distinction possible.

Fig. 6 presumably also has no junction, but as displayed, it would be impossible to observe one. Moreover, the "non-striated" fibers attached to the endothelium in fact show evidence of striations and are certainly not "random."

Fig. 7 looks far more like an adipocyte and not "a union of three lymphatics to form a larger one." Again, higher magnification and better staining would make the distinction. Inferior photomicrographs cast doubt on the authors claim and on the schematic diagram *(Fig. 2)*.

Exception is also taken to the statistics shown in *Table 1* and relied upon at length in the text. Allegedly the t-test between the "larger lymphatics" and "smallest lymphatics" had a p value <0.001. Yet, the respective means and standard errors provided are 9.9% (3.6%) and 14.5% (5.7%) on sample numbers of "from 30-35". A 2-tail t-value for these figures is 0.68 or p-0.50! Accordingly, the elaborate theoretical structure which was developed in the Discussion upon this supposed "difference" collapses. The concept is also highly improbable on other grounds (2).

REFERENCES

1. Cornford, ME, WM Oldendorf: Terminal endothelial cells of lymph capillaries as active transport structures involved in the formation of lymph in rat skin. Lymphology 26 (1993), 67-78.
2. Casley-Smith, JR: The structure and functioning of the blood vessels, interstitial tissues, and lymphatics. In: *Lymphangiology*, Foldi, M, JR Casley-Smith (Eds.), Schattauer, Stuttgart (1983), 117-126.

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Reply:

I, too, am somewhat disappointed in the magnification and clarity of the photomicrographs as published since the original photomicrographs do not present these ambiguities. However, in response to Dr. Casley-Smith's analysis, the several "endothelial junctions" as indicated are correctly identified. Moreover, they are not crucial to the observation that small terminal capillaries have a very high mitochondrial content and are likely to be engaged in active transport processes.

Since the terminal endothelial cells are apparently cup-shaped, sections that display the portion of the cell beyond the end of the lumen (bottom of the cup) are of necessity to a degree parallel rather than perpendicular to the lumen of the vessel. Accordingly, the presence of endothelial junctions of the lip of the cup is to be expected. These junctions of small diameter lymphatic capillaries are never "open", which I assume is the thrust of Dr. Casley-Smith's position. Otherwise, I do not appreciate what difference the presence of cell junctions may make.

The photomicrograph of the three convergent small lymphatics also suffers from low magnification, which renders the typical scant basement lamina visible on the exterior of the resultant vessel impossible to see clearly. However, I doubt whether Dr. Casley-Smith has photomicrographs of adipocytes remotely resembling these geometric pattern. Serial sections also showed capillaries before convergence.

The p value of <0.001 is for the non-overlapping values of mitochondrial volume percent found in dermal papillary blood capillaries and the small lymphatics. A $p<0.5$ is not correct even for the small lymphatics compared to the larger lymphatics, which were arbitrarily separated at a diameter which gave two groups of relatively equal size. It is the comparison of the mitochondrial capacity of lymphatic capillaries to blood capillaries that is important here, showing an excess of capacity over the amount that might be expected to maintain endothelial cells confined to the function of defining the walls of a conduit.

Finally, no "elaborate scheme" is intended or described in the paper to explain lymph formation. It is ironic that proponents of the mechanistic theories explaining how extracellular excess fluid is forced into distended, blocked lymphatic vessels in the presence of massive lymphedema, or even into collapsed vessels under homeostatic normal conditions, seem to find the idea that lymph components are actively transported into the lumen too elaborate for belief. I am content that a new avenue of exploration into the basic functioning of the lymphatic circulation has been opened and that future research will benefit thereby.

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