Lymphology 30 (1997) 111-115

COMMENTARY

THE PROBLEM OF GAINING ACCESS TO INTERSTITIAL FLUID. AN ATTEMPT TO RATIONALIZE A WICKED DISCUSSION ON WICKS

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In a Letter to the Editor of *Lymphology* (1) and a Commentary in the same journal (2), Földi and coworkers expressed disbelief in the wick method for collecting interstitial fluid for measurement of protein concentrations and colloid osmotic pressure.

In our view, their explanation brings little information on the principle of the method and how it has been tested, and we find it especially deceptive that no studies made after 1977 were mentioned. In the interest of readers who might consider measuring interstitial colloid osmotic pressure (COP) or protein concentration, we provide a brief but somewhat more complete account.

The "wick method," which was introduced in 1973 (3), is briefly as follows: a multifilamentous nylon thread ("wick"), about 1 mm thick, is soaked in 0.9% saline and stitched under the skin for a length of 5-7 cm. After 1 hour to allow for equilibration with interstitial fluid, the wick is pulled out, the protruding ends cut off, and the middle part immediately transferred either to a test tube containing 2-3 ml saline for elution and measurement of protein concentrations (3), or centrifuged under oil to obtain undiluted fluid for measurement of COP (4).

Földi's evidence against the accuracy of the wick method seems to be the following:

1. It fails to confirm the axiomatic increase in interstitial fluid COP in lymphedema (1).

2. Electron micrographs demonstrate red blood cells in the needletrack after one hour implantation of a 23 gauge cannula (2).

3. Citation of several reservations noted in our early studies.

As to the last point: Whereas the first two quotations are accurate, the third one, "the true level of colloid osmotic pressure of undisturbed subcutaneous tissue remains uncertain" is not to be found in the claimed reference (5). Nevertheless, the citations may illustrate our skepticism and critical attitude in 1977. It also reflects the fact that no other method to sample interstitial fluid in normals was available at the time.

Several problems were obvious. First, admixture of blood due to the traumatic insertion of the wick. Like Földi et al (2), we made histological sections and saw some red cells (6), but more importantly, we measured quantitatively the hemoglobin content of a large number of wicks and found that in most the addition of hemoglobin contributed to less than 5% of the total protein concentration (3). Much more disturbing was the finding of a marked transitory increase of capillary protein permeability and leakage of proteins from plasma into the wick fluid. In extensive studies, Fadnes et al (5) showed, however, that reduction of protein leakage by anti-inflammatory agents often led to lower wick fluid COP, but at the same time, the wick fluid hydrostatic pressure fell to more

negative values. The interpretation of this and other experimental findings was that the wick fluid attained an osmotic equilibrium with surrounding undisturbed tissue, and that some protein leakage was necessary to compensate for the comparatively large volume of protein-free fluid introduced by the saline-soaked wick.

From these studies we concluded that the original wick fluid technique gave a reliable measure of interstitial fluid COP in rat subcutaneous tissue but reserved judgment regarding the protein composition of wick fluid. Because much of the protein found in the wick fluid was derived from plasma during the transient increase of capillary permeability, we could not exclude the possibility that the protein composition of this exudate deviated from that in undisturbed interstitial fluid (5). However, the findings of albumin/globulin ratios similar to that of peripheral lymph suggested that the deviation, if any, must be small. We also emphasized that the conclusion we reached in the rat subcutis should not be transferred to other tissues or species without further studies.

In the following years, the wick method was thoroughly tested in rat skeletal muscle (6), as well as in human subcutaneous tissue (7). [The reference to the latter study given by Földi et al (2) is incorrect.] Wick fluid from rat skeletal muscle contained an admixture of a plasma-foreign protein (8), probably derived from damaged muscle fibers. More recently, Wiig et al found that this admixture can be avoided by an improved implantation technique (9). It is worth noting, however, that the admixture did not result in a different COP.

In spite of the many reassuring findings referred to above we maintained our critical attitude to the method. Can one really rely on oncotic equilibration to compensate for the initial inflammatory increase of protein extravasation, and the introduction of protein-free fluid using the wick? After all, many measurements of protein content in peripheral lymph have given lower values, possibly not always because of overhydrated tissues.

We therefore decided to get rid of, or minimize the transcapillary protein and fluid movements caused by the inflammatory response to wick insertion. This can be achieved by sampling wick fluid from uncirculated rat skin. The blood volume in this tissue is only 2% of the extracellular fluid volume (10), which means that even a completely free escape of protein from plasma would have a negligible effect on interstitial fluid protein concentration. Accordingly, we implanted saline loaded subcutaneous wicks shortly after killing the anesthetized rat with i.v. KCl (11,12) and found that less than 3% of the wick albumin was derived from plasma. However, the COP of the wick fluid was lower than that obtained in awake rats (12), clearly because of dilution by the introduced saline. We therefore tried to match the interstitial fluid COP by introducing several wicks with plasma diluted with saline to total protein concentrations ranging from 20 to 50 mg/ml (11,12). As shown schematically in *Fig. 1*, wicks primed with low protein fluid increased during implantation, whereas those starting with high concentrations were diluted. By interpolation of the observed final values we could then determine the COP of the serum dilution which would remain unaltered during implantation, and accordingly represent interstitial COP (COP_i). Similar results were obtained by 15, 30 and 60 min implantation. Most importantly, the absolute values obtained with this "crossover technique" tended to be slightly higher, not lower, than those obtained with 1 hour saline loaded wicks implanted for 1 hour *in vivo* (12). Similar values were obtained with dry wicks implanted after circulatory arrest, but their low fluid volume required more technical skill and preferably handling in a humidified incubator. We regard these studies during circulatory arrest to be the best evidence for the validity of the wick method until now.

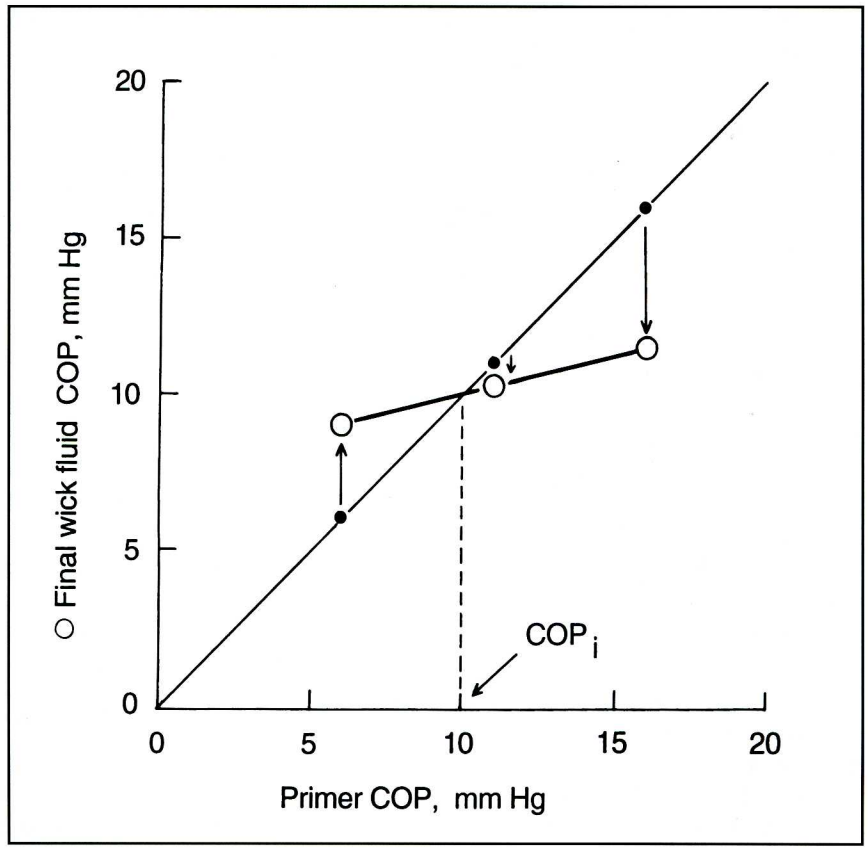

Fig. 1. Estimation of interstitial colloid osmotic pressure (COP_i) by the "crossover technique." Three wicks soaked in serum dilutions with COP = 6, 11 and 15 mmHg (closed circles). Wick fluid COP after 30 min subcutaneous implantation in a dead rat (open circles). Arrows show alteration in COP during implantation.

Can we judge the wick method by the results? If we ask for confirmation of absolute values the answer is no, simply because we have no reference method to serve as a gold standard. However, some qualitative validation may be obtained by comparison to other methods with other possible sources of error.

Thus, in rabbit leg skin, Fadnes (13) found fair agreement between wick fluid and prenodal lymph over a wide range of COP in control conditions and during volume expansion. Moreover, the average COP in the rat subcutis measured with an implantable osmometer did not deviate from that obtained in wick fluid (14). In human skin the wick method has been directly compared to the blister suction technique (15,16), the latter probably more handy for use in clinical studies. Overall the blister fluid had a 15-30% lower COP, presumably because suction increases the filtration of low protein fluid (17). However, the correlation between the methods seems satisfactory. Finally, the COP obtained using the wick technique in post-mastectomy lymphedema has been confirmed by fluid obtained by direct puncture (18,19).

Some qualitative evaluation may also be obtained by comparing responses to experimental perturbations or disease states with that predicted from known physiological mechanisms. Up to now, the wick method has been used by at least 10 groups in Europe and the USA with remarkably consistent results. Some clinical studies may be of special interest — subcutaneous measurements have been made in nephrotic syndrome (20,21), liver cirrhosis (22), heart failure (23,24), diabetes (25,26), premenstrual syndrome (26), normal pregnancy and pre-eclampsia (28,29), in leg edema after arterial reconstruction

(16,30), and during extracorporeal circulation (15). In all these studies, the results were qualitatively predictable or at least physiologically explainable. For instance, the marked fall in wick fluid COP in heart failure agrees well with that observed in lymph. If the "high" normal COP obtained under normal conditions was a result of excessive plasma leakage, as Földi et al (2) seems to believe, how is that prevented by venous stasis or heart failure?

Still, the quantitative validity of the results must rest on the experimental evidence, which undoubtedly is much more extensive than that obtained for any other method for sampling interstitial fluid. More references, especially to animal experiments may be found in several review articles (31-33)

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