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COMMENTARY

IS THE "WICK METHOD" APPROPRIATE TO DETERMINE THE PROTEIN OSMOTIC PRESSURE OF INTERSTITIAL FLUID?

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According to the Starling hypothesis, transcapillary fluid exchange is governed by the microvascular hydrostatic and protein osmotic pressure gradients and is usually expressed as

$$F = C\,[(C_p - C_i) - \sigma\,(COP_p - COP_i)]$$

where F = microvascular filtration rate; C = blood capillary coefficient; C_p = capillary hydrostatic pressure; C_i = interstitial hydrostatic pressure; _ = solute reflection coefficient; COP_p = plasma protein osmotic pressure; COP_i = tissue protein osmotic pressure.

Because pericapillary fluid has thus far not been directly sampled, interstitial fluid is usually used to obtain COPi (1). Since 1973 the "Wick method" (2) has become widely employed for this purpose. For example in rats, nylon wicks have been implanted subcutaneously and after their removal the fluid they contain has been considered to represent normal tissue fluid. But even these same authors who introduced the wick method later reevaluated the technique in regards to the inflammatory reaction induced by implantation of the wicks and observed "insertion of the wick greatly increased capillary protein permeability, as demonstrated by rapid transfer of labeled albumin from blood to the wick fluid. The rapid blood-to-wick albumin transport almost ceased within 30 minutes" (3). They also suggested (3) that wick fluid is not necessarily representative of undisturbed interstitial fluid, that the wick technique may conceivably be used for studying inflammatory reactions with mechanical trauma and for testing anti-inflammatory agents and that the rapid blood-to-wick albumin transport might be reduced by indomethacin and cyproheptadine. Finally, they noted that "validation of the wick method in subcutaneous tissue in rats does not guarantee a proper function in other species" and "the true level of colloid osmotic pressure of undisturbed subcutaneous tissue remains uncertain" (3). Despite these cautious conclusions, Noddeland et al used this method to study tissue colloid osmotic pressure in humans (4) as did Bates et al in women with arm lymphedema after treatment for breast cancer (5). Because there are no data concerning histological changes induced by the wick technique or even those caused by simple insertion of a fine needle into normal tissues, we have performed the following studies.

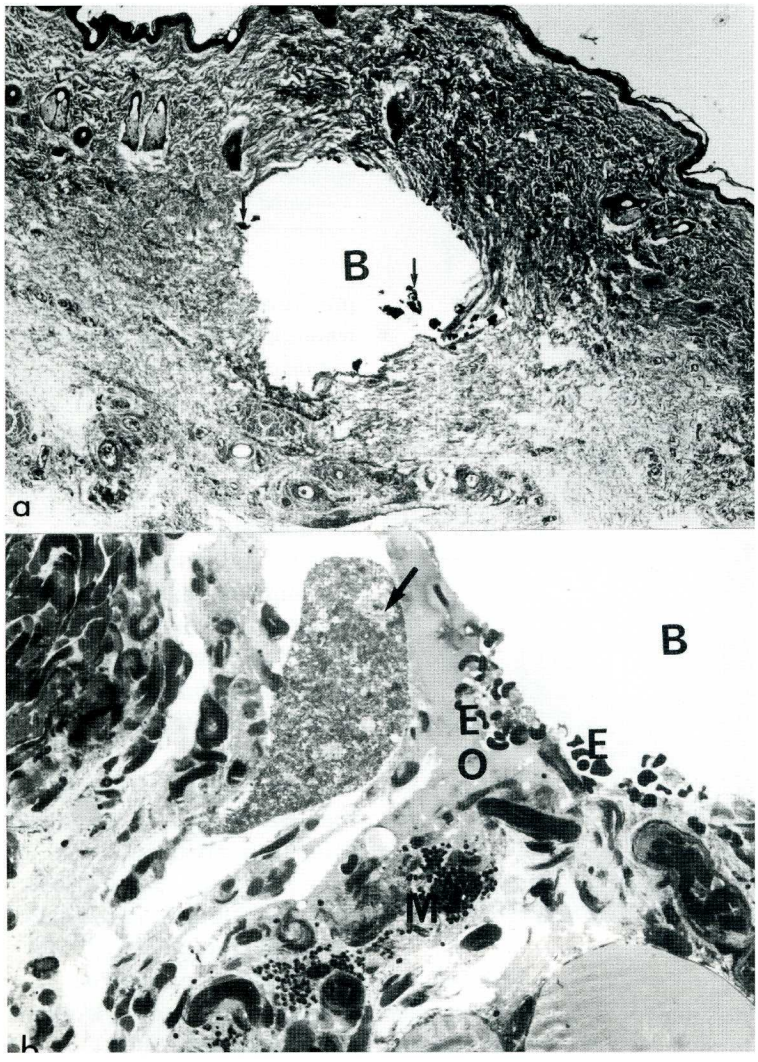

Fig. 1. Histopathology from skin biopsy specimen in a rat after insertion of a 23 gauge needle for 60 minutes. a: Note the artificially created tissue channel (B) with focal marking by previously instilled acryl polymer (arrow). b: In a semi-thin section, the acryl polymer is seen as granules (arrow). Erythrocytes (E) and degranulated mast cells (M) are also seen at the edge of the tissue channel (B) and adjacent free fluid (O). a—H & E x 45; b—Azure -II methylene blue; x180.

Clinical Study

During a radical neck dissection in a 46 year old male patient, adjacent cervical skin was pierced by a 23 gauge needle into the subcutaneous tissue and left in situ for one hour. Subsequently, the skin and surrounding tissue with the needle were excised. The specimen was fixed in 4% formalin and partly in glutaraldehyde for four hours. As the hollow needle was removed, acryl polymer with pigments (Tipp-Ex, Frankfurt, Germany) was instilled into the artificially created tissue channel. Subsequently, tissue fixation was continued for another 12 hours. The specimen was divided into several pieces for impregnation. For semi-thin sections, Azure II Methylene Blue was used for staining. A Siemens EM 10 (Zeiss, Oberkochen) was used for electron microscopy.

Experimental Study

Four male albino rats were anesthetized with Ketancs (Parke-Davis), Rompun (Bayer) and atropine sulphate. After shaving the skin, the skin and subcutaneous tissue were pierced by #23 gauge steel needles and left in situ for one hour. After killing the rat by intracardiac injection of T61 (Hoechst), fixation, imbedding and staining of the tissue specimens were carried out as previously described in the clinical study (vide supra).

Because acryl polymer is black in histologic sections, the tissue channels created by needle puncture were readily identified both by light and electron microscopy. At the edges of these needle channels, erythrocytes and precipitates of fibrin were seen. Large accumulations of erythrocytes were also detected in the adjacent connective tissue.

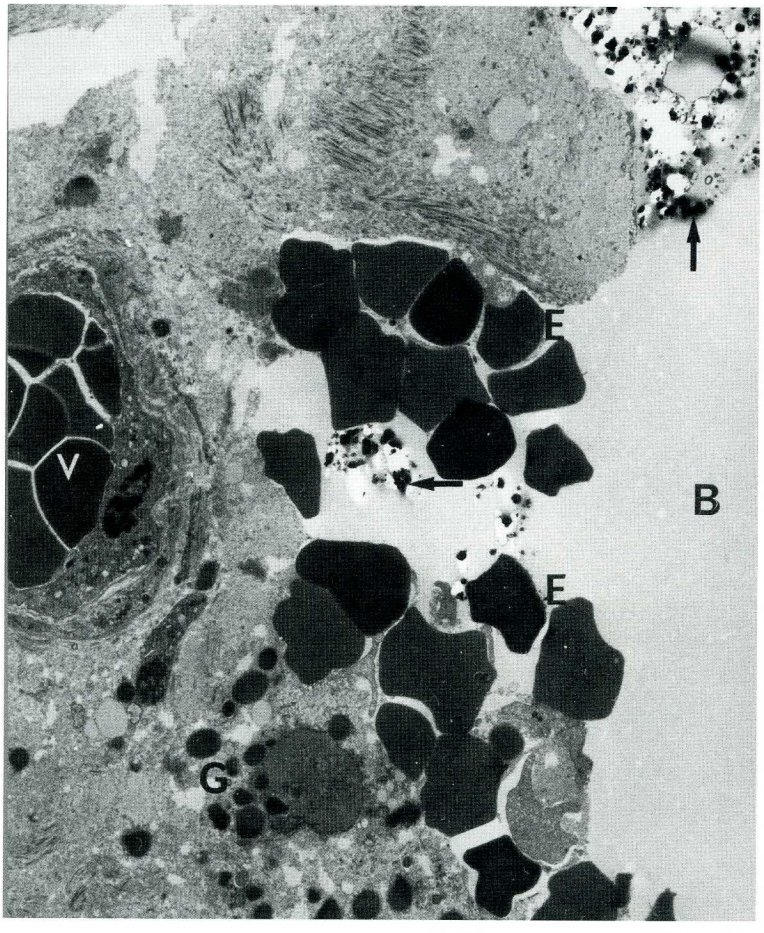

Fig. 2. Electron micrograph of the area shown in Fig. 1b. Note large numbers of erythrocytes (E) near the tissue channel (B). Mast cell granules (G) are also seen in the interstitium. Deposits of acryl polymer are also present (arrows). V—blood vessel; x 10,000.

Connective tissue fibrils were markedly disrupted. Ultrastructurally, disrupted mast cells with their granules lying free in the connective tissue were evident *(Figs. 1-3).*

These results suggest that "tissue fluid" obtained by the wick technique is not representative of *normal* tissue fluid. Needle puncture is associated with gross and microscopic bleeding. If hemoglobin or erythrocytes enter the interstitium and lymphatics, intrinsic lymph pump activity deteriorates (6). Accordingly, protein molecules and other particulates stagnate in the wick area. Introduction of even a fine needle and wick damages superficial and deep cutaneous lymph capillary plexuses, precollectors and lymph collectors, and lymph oozes into the wound site. Fibrin likely seals off the punctured area from the non-traumatized tissues. Damage to plasma cells is further evidence of a significant inflammatory reaction and is seen in the first thirty minutes after initiating the wick method. Mast cells are also directly and indirectly involved in endothelial adhesive function and their "degranulation" suggests interference with other connective tissue cells and β_1-integrin function (7).

Our experiments suggest that wick fluid after its removal contains a mixture of blood serum, inflammatory exudate and lymph and, at best, mixes with adjacent interstitial fluid if indeed any free tissue fluid exists. The protein concentration of wick fluid is likely much higher than normal tissue fluid.

In summary, the protein osmotic pressure of wick fluid should not be regarded as a numerical equivalent to normal interstitial fluid protein osmotic pressure (COP_i). As Aukland and Fadnes note (3), "its [COP_i] true level... remains uncertain."

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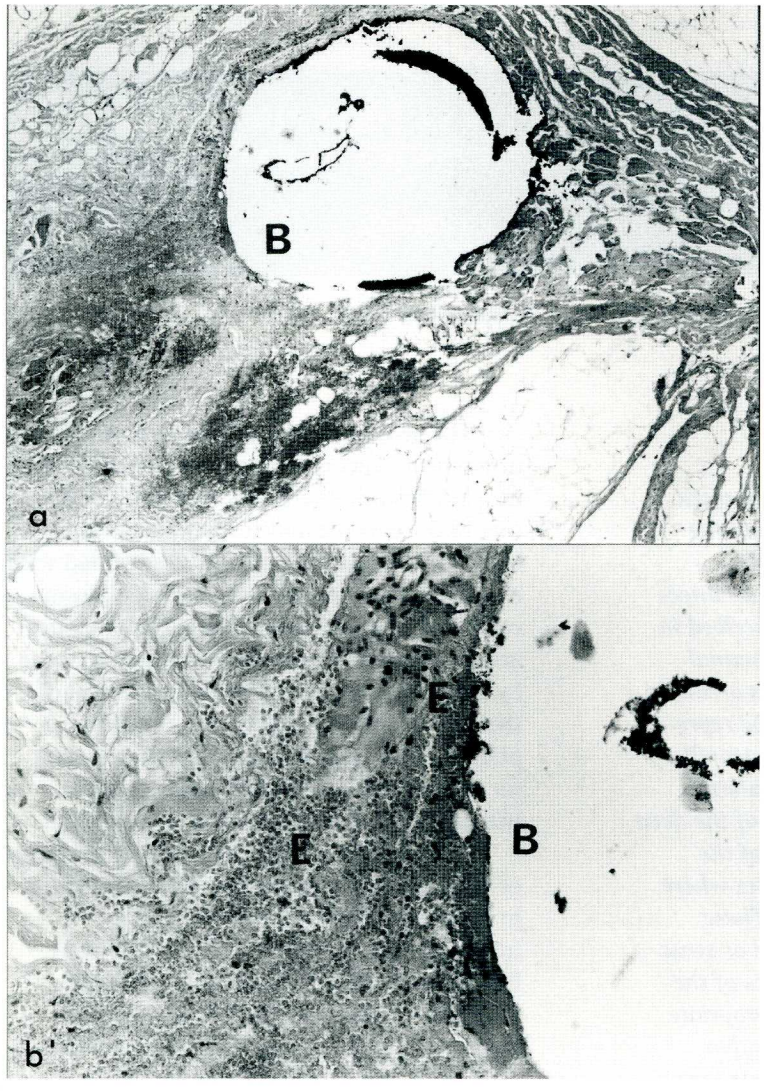

Fig. 3. Histopathology of human skin biopsy specimen after insertion of a 23 gauge needle for 60 minutes. a: Note the tissue channel (B) lined by debris consisting mainly of fibrin (b). A tissue cleft is seen extending into the muscula-ture containing acryl polymer, fibrin, and erythrocytes (E). a—H & E; x45; b—H & E; x180.

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