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Research article

## Plasma proteins in a standardised skin mini-erosion (I): permeability changes as a function of time

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### Abstract

**Background:** A standardised technique using a suction-induced mini-erosion that allows serial sampling of dermal interstitial fluid (IF) for 5 to 6 days has been described. In the present study, we studied permeability changes as a function of time.

**Methods:** We examined IF concentrations of total protein concentration and the concentration of insulin (6.6 kDa), prealbumin (55 kDa), albumin (66 kDa), transferrin (80 kDa), IgG (150 kDa) and alpha-2-macroglobulin (720 kDa) as a function of time, using an extraction pressure of 200 mmHg below atmospheric.

**Results:** At 0 h after forming the erosion, mean total IF protein content (relative to plasma) was  $26 \pm 13\%$  (SD). For the individual proteins, the relative mean concentrations were  $65 \pm 36\%$  for insulin,  $48 \pm 12\%$  for albumin,  $30 \pm 19\%$  for transferrin,  $31 \pm 15\%$  for IgG and  $19.5 \pm 10\%$  for alpha-2-macroglobulin. At 24 h, the total IF protein content was higher than at 0 h ( $56 \pm 26\%$  vs  $26 \pm 13\%$ ;  $p < 0.05$ , diff: 115%), as were some of the individual protein concentrations: prealbumin ( $50 \pm 24$  vs  $25 \pm 13\%$ ;  $p < 0.05$ ), albumin ( $68 \pm 21$  vs  $48 \pm 12\%$ ;  $p < 0.05$ ) and IgG ( $55 \pm 30$  vs  $31 \pm 15\%$ ;  $p = 0.05$ ). In the interval 24 h to 96 h the concentrations were relatively unchanged.

**Conclusions:** The results indicate that fluid sampled at 0 h after forming the erosion represents dermal IF before the full onset of inflammation. From 24 h onward, the sampled fluid reflects a steady state of increased permeability induced by inflammation. This technique is promising as a tool for clinically sampling substances that are freely distributed in the body and as a model for studying inflammation and vascular permeability.

### Background

A technique that allows serial sampling of dermal IF from a suction-induced mini-erosion for 5 to 6 days has been previously described [1–3]. Studies of interstitial glucose and other metabolites in healthy and diabetic subjects

during oral glucose tolerance tests performed at an interval of 2 days have indicated firstly, that such fluid samples are representative of dermal IF, and secondly, that the composition of the fluid was altered over time, perhaps because of an increased permeability [3].

The erosion is characterised by an intact dermis with local hyperemia that begins when the epidermis is being detached and is sustained over several days [5–7]. Complete reepithelialisation occurs within a week [7]. It is not yet known to what degree inflammation and concomitant changes in permeability affect the sampling possibilities from the erosion. Therefore, the aim of this study was to assess the permeability changes in the erosion as a function of time by serial measurement of the interstitial concentrations of plasma proteins with molecular weights ranging from 6 to 720 kDa. The results of the present study indicate that fluid sampled at 0 h after forming the erosion represents dermal IF before the full onset of inflammation. From 24 h onward, the sampled fluid reflects a steady state of increased permeability induced by inflammation.

## Materials and methods

### Subjects

The study was undertaken in consenting volunteers after approval by the Central Oxford Research Committee and in accordance with the principles of the Declaration of Helsinki. Participants were seven healthy subjects (four men and three women) aged 27–41 years (mean 35.7 years). All but one were nonsmokers. The experiments took place at an ambient temperature of 20–22°C.

### Formation of the erosion

One erosion was made on the volar side of the forearm of each subject using a technique described in detail elsewhere [1]. A suction cup set to operate at -200 mmHg (Epiport AB, Sweden) was adhesively applied to the skin. Chemical warming pads (43-C) (Ingkro, Jönköping, Sweden) with protective foam rubber were used to heat the site during suctioning. An epidermal vesicle of 6-mm diameter was formed in approximately 1 h and its roof was removed with a pair of tweezers.

### Sampling of IF

The same type of suction cup as described above was used for serial extractions of IF from the erosion. Excess IF was absorbed with a piece of gauze before applying and disengaging the sampling suction cup. Extraction was performed at 0, 24, 48, 72, 96 and 120 h after formation of the erosion; fluid samples were taken at each time point by aspirating the contents of the suction cup into a syringe when approximately 60 µl had accumulated, or after a maximum 120 min of suction. All samples were collected in plastic microtubes containing 7 µl of EDTA and weighed (Sartorius Analytic, Göttingen, Germany) before storage at -70°C. Each subject's erosion was covered with adhesive film (Tegaderm, 3M, St Paul, Minnesota, USA) between samplings and for 4 days after the end of the experiment.

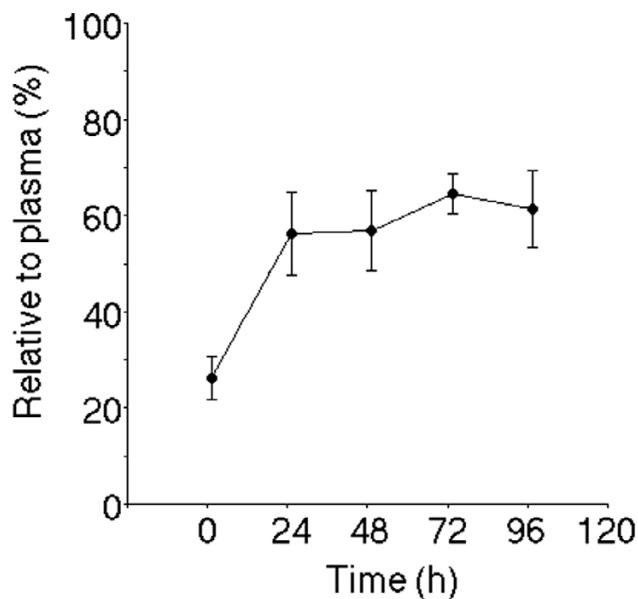
### Venous blood samples

A venous blood sample (5 ml) was collected from an antecubital vein into a heparinised vacutainer tube approximately 60 min after the start of each IF sampling. The blood was centrifuged at 2500 rpm for 10 min. The supernatant was then centrifuged at 5000 rpm for 10 min at 4°C (Heraeus, Hanau, Germany) and frozen at -70°C.

### Methods of analysis

The total protein concentration was determined using a Micro-BCA protein assay reagent kit (Pierce, Rockford, Illinois, USA). Insulin (6.0 kDa) was measured by radioimmunoassay (ultrasensitive insulin RIA kit, Linco, St Charles, Missouri, USA).

Prealbumin (55 kDa), albumin (66 kDa), transferrin (80 kDa), IgG (150 kDa) and alpha-2-macroglobulin (720 kDa) were analysed by quantifying bands in SDS-PAGE gels. All samples were run in SDS-PAGE, 7.5% unreduced gels. The plasma and suction samples were diluted 1/15 in PBS-0.5 mM EDTA and 20 µl of this dilution was added to the gels. For each subject, plasma and suction samples from the same day were run in the same gel. A densitometer with an image analysis program (Computing Densitometer and Image Quant, Molecular Dynamics, Seven Oaks, Kent, England) was used to quantify the individual proteins in the gels.



**Figure 1**  
Total protein concentration (mean ± SEM) in IF relative to plasma as a function of time.

### Diffusion sample

In one volunteer on a separate occasion, an additional forearm erosion was made, over which a diffusion chamber containing 1.5 ml of saline solution was adhesively applied. Samples were taken at 40, 80, 120 and 160 min and run in an SDS-PAGE (Figure 5).

### Calculations and statistical analysis

Concentrations in IF were calculated and presented as relative values to those in plasma on the same day, and expressed as either mean  $\pm$  SD or mean  $\pm$  SEM. Wilcoxon's signed rank test for paired values was used to determine the significance of IF/plasma differences in concentration within and between days after Friedman's test had been performed. The area under the curve (AUC), calculated with a trapezoidal method, was used in all comparisons between the concentrations of different proteins over time (0–96 h) and expressed in area units (AU). Wilcoxon's signed rank test for paired values was used to determine the significance of differences between the AUCs of the different proteins. Many statistical comparisons were performed on the data, thereby increasing the risk that some of the significant differences could be attributed to chance. A *p*-value of  $< 0.05$  was considered significant.

## Results

### General observations

Most subjects felt a tingling sensation when the erosion was formed and during the initial seconds of each sampling procedure. There were no local complications. The IF samples were always clear and yellow in colour and were never blood contaminated. From day 2 onward, petechiae were noted in the erosions. Epidermal regeneration was complete within a week in all cases. Sample volumes were generally in excess of that needed for analysis; at 120 h, however, reepithelialisation prevented IF sampling in four of the seven volunteers. Data at 120 h were therefore excluded from analysis.

### Total protein concentration

Total protein concentration in interstitial fluid relative to plasma is shown in Figure 1 as a function of time. Compared to the value at 0 h ( $26 \pm 13\%$ (SD)), the protein concentration was significantly higher at 24 h ( $56 \pm 26\%$  (SD);  $p < 0.05$ , diff: 115%), 48 h ( $57 \pm 25\%$ ;  $p < 0.01$ , diff: 119%), 72 h ( $64 \pm 12\%$ ;  $p < 0.01$ , diff: 146%) and 96 h ( $61 \pm 22\%$ ;  $p < 0.05$ , diff: 135%). There were no other significant differences in total protein concentration between days.

### Insulin, albumin, transferrin, prealbumin, IgG and alpha-2-macroglobulin concentrations

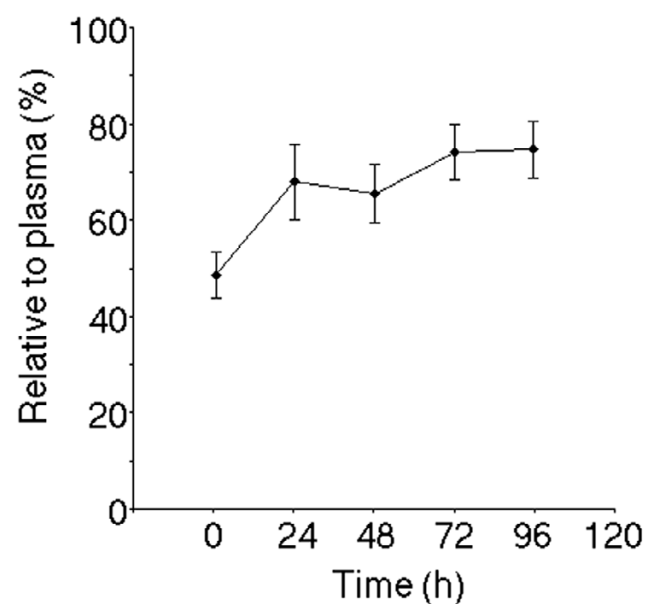
The protein concentrations for all six proteins over time (0–96 h) are shown in Table 1. Baseline interstitial values at 0 h for five of the proteins together with comparative

data from other studies are given in Table 2. All values are given relative to those in plasma at the same day. The relative concentrations (mean  $\pm$  SEM) of two of the variables from 0 to 96 h are shown in Figures 2,3.

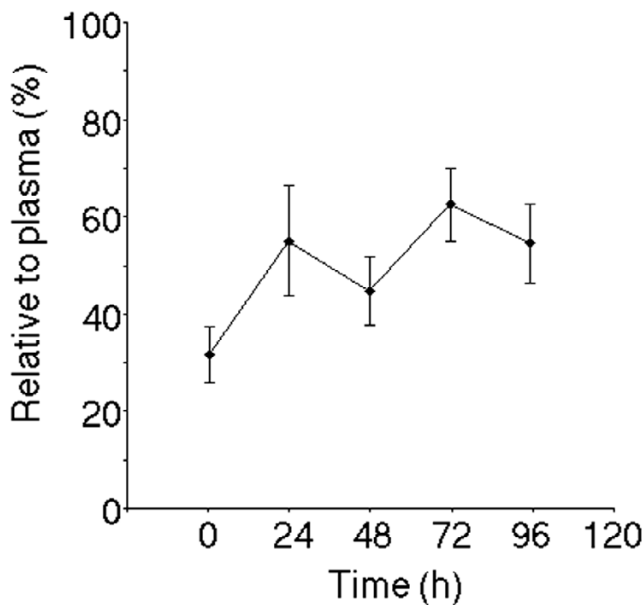
Due to a failure in the handling of samples, insulin was not measured in one volunteer at 0 h; in two volunteers at 24, 72 and 96 h; and in three volunteers at 48 h. No significant differences were found for insulin concentration between days.

For albumin, compared to the value at 0 h, the concentration was significantly higher at 24 h ( $p < 0.05$ , diff: 42%), 48 h ( $p < 0.05$ , diff: 38%), 72 h ( $p < 0.01$ , diff: 54%) and 96 h ( $p < 0.05$ , diff: 56%). There were no other significant differences in albumin concentration between days. For transferrin, compared to the value at 0 h, the concentration was significantly at 72 h ( $p < 0.05$ , diff: 103%) and also higher at 48 h ( $p < 0.05$ , diff: 50%). There were no other significant differences in transferrin concentration between days.

For prealbumin, compared to the value at 0 h, the concentration was significantly higher at 24 h ( $p < 0.05$ , diff: 100%), at 48 h ( $p < 0.05$ , diff:100%) and at 72 h ( $p < 0.05$ , diff: 217 %). Finally, prealbumin concentration was significantly higher at 72 h than at 48 h ( $p < 0.05$ , diff: 56%).



**Figure 2**  
Albumin (66 kDa) concentration (mean  $\pm$  SEM) in IF relative to plasma as a function of time.



**Figure 3**  
IgG (150 kDa) concentration (mean  $\pm$  SEM) in IF relative to plasma as a function of time.

For IgG, compared to the concentration at 0 h, significantly higher values were obtained at 24 h ( $p < 0.05$ , diff: 77%) and 72 h ( $p < 0.01$ , diff: 103%). The concentration was also significantly higher at 72 h than at 48 h ( $p < 0.05$ , diff: 40%). IgG concentration at 96 h was not significantly higher than on day 1 ( $p = 0.063$ ). There were no other significant differences in IgG concentration between days.

Alpha-2-macroglobulin could only be quantified in three volunteers at 0 h, because the bands in the SDS-PAGE were at the very border of the gel. There were no significant differences in alpha-2-macroglobulin concentration between days.

#### Comparison between different substances

At 0 h, the relative concentration of albumin was significantly higher than that of transferrin ( $p < 0.05$ ), prealbumin ( $p < 0.05$ ) and IgG ( $p < 0.01$ ). alpha-2-macroglobulin was quantifiable only in three subjects at 0 h. There were no other significant differences between relative concentrations of the individual proteins on day 1.

When comparing the concentration of different substances over time by comparing area under curve (AUC), the concentration of albumin ( $2.83 \pm 0.64$  AU) was significantly higher than that of transferrin ( $2.13 \pm 0.62$  AU;  $p < 0.05$ ) and IgG ( $2.16 \pm 0.59$  AU;  $p < 0.05$ ). The concentration of prealbumin ( $2.07 \pm 0.73$  AU) was also significantly higher than that of transferrin ( $2.13 \pm 0.62$  AU;  $p < 0.05$ ). For alpha-2-macroglobulin, AUC could only be calculated

in three subjects. There were no other significant differences.

#### Sample extraction rates

The sample extraction rate was (mean  $\pm$  SEM)  $1.4 \pm 0.2$   $\mu$ l/min at 0 h,  $1.9 \pm 0.2$   $\mu$ l/min at 24 h,  $1.9 \pm 0.4$   $\mu$ l/min at 48 h,  $2.6 \pm 0.4$   $\mu$ l/min at 72 h and  $1.8 \pm 1.3$   $\mu$ l/min at 96 h.

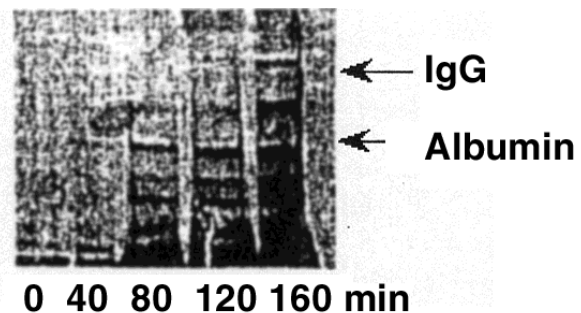
#### Diffusion sample

The SDS-PAGE gel for the erosion diffusion sample is shown in Figure 4.

#### Discussion

The present study showed that at 0 h, the samples obtained from the erosion reflected protein concentrations in interstitial fluid before the full onset of an inflammation-induced increase in permeability. Samples taken in the subsequent time interval from 24 to 96 h indicated a stable state of increased permeability.

The sampling technique used in this study is an extension of the suction blister technique described by Kiistala in [8]. Morphological studies of the erosion have shown that the suction-induced split always occurs through the lamina lucida of the epidermal basement membrane and that the underlying dermis with its microcirculatory network remains intact [5,6,8]. Studies using the laser Doppler technique to assess local superficial blood flow during the formation of an erosion have shown low values indicating a decreased blood flow, then a transient early increased blood flow 10–15 min after applying suction; this is followed by a hyperemic reaction initiated when the epidermal vesicle is formed and continuing unaltered after the end of the suction stimulus [5]. The hyperemic reaction is believed to be induced by a neurogenic inflammation triggered by the detachment of the epidermis with its thin, non-sensory nerve branches [4,5]. In neurogenic inflam-



**Figure 4**  
SDS-PAGE gel of a sample from a diffusion sample (0–160 min).

**Table 1: IF concentrations of the six plasma proteins (means  $\pm$  SD).**

Time (h)	0	24	48	72	96
Insulin(6,6 kDa)	65 $\pm$ 36	71 $\pm$ 24	66 $\pm$ 39	83 $\pm$ 38	68 $\pm$ 26
Prealbumin(50 kDa)	25 $\pm$ 13	50 $\pm$ 24 *	50 $\pm$ 15 *	78 $\pm$ 36	66 $\pm$ 19
Albumin (66 kDa)	48 $\pm$ 12	68 $\pm$ 21 *	66 $\pm$ 16 *	74 $\pm$ 15 *	75 $\pm$ 16 *
Transferrin(80 kDa)	31 $\pm$ 19	47 $\pm$ 25	42 $\pm$ 14 *	63 $\pm$ 22 *	49 $\pm$ 16
IgG (150 kDa)	31 $\pm$ 15	55 $\pm$ 30 *	45 $\pm$ 19	63 $\pm$ 20 *	55 $\pm$ 21
alpha-2-macroglobulin (720 kDa)	20 $\pm$ 10	17 $\pm$ 9	15 $\pm$ 4	21 $\pm$ 9	21 $\pm$ 10
Total Protein	26 $\pm$ 13	56 $\pm$ 36 *	57 $\pm$ 25 *	64 $\pm$ 12 *	64 $\pm$ 12 *

\*Indicates a significant difference ( $p < 0.05$ ) as compared to 0 h.

mation, peptides such as histamine, serotonin and bradykinin induce endothelial cell contraction producing endothelial cell gaps leading to increased permeability [21,22]. Substance P and neurokinin A are also active in inducing protein extravasation and that calcitonin gene related peptide induces a neurogenic vasodilation [9]. There is evidence of a biphasic sequence of events for this change in permeability [10]. Initially, the increase in permeability is caused by an increased amount of endothelial gaps in venules; later, from about 8 hours to several days, capillary leakage is predominant. The first phase may be induced by peptides involved in neurogenic inflammation, whereas the second phase may be caused by the inflammatory reaction itself [10,11].

Transport of plasma proteins over the endothelium from the vascular space to the interstitium is considered to be a passive process governed by the laws of convection and diffusion. There is evidence showing that the permeability of most microvascular walls can be described in terms of a two-pore system: a high frequency of small pores that restrict protein passage, and a number of large pores-endothelial gaps, permitting the passage of macromolecules [12].

The interstitial space itself is characterised by collagenous, reticular and elastin fibers and an amorphous ground substance consisting of glycosaminoglycans that, at physiological pH, are negatively charged and exert a high osmotic force. The pH in the erosion may be low, as indicated by lactate levels approximately 50 to 100% higher than those in plasma [3]. These factors may also affect the passage of plasma proteins, depending on their chemical and electrical characteristics. The degree of lymph flow is also an important factor in the concentrations of proteins in interstitial fluid; high flow increases the clearance, giving lower protein concentrations [12]. Nothing is known, however, about the state of microlymphatics in a suction-induced skin erosion.

### Findings at 0 h

The SDS-PAGE gel in Figure 5 illustrates the efficacy of passive transport. Even large proteins like IgG diffuse quickly into the cell, giving clear bands in a volume of 1.5 ml within 120 min. At 0 h, the total protein concentration in the extracted samples was 26  $\pm$  13% of that in plasma. This value corresponds quite well with findings of other studies on total protein concentration in IF [8,14].

Table 2 shows the relative concentrations of individual proteins in IF relative to plasma on day 1, along with comparable data from other studies. For insulin, values at 0 h correspond well to values obtained with a microdialysis technique [15] and with lymph measurements in dogs [16]. For the remaining proteins, the IF/plasma ratios at 0 h are similar for albumin and alpha-2-macroglobulin, whereas the ratios for prealbumin, transferrin and IgG are slightly lower than those found in other studies on interstitial fluid [13,17,18]. This may indicate that the samples obtained have an increased water fraction [11]. It also indicates that, at 0 h, the restricting function of the vascular endothelium against larger proteins is basically intact. The comparatively small differences in concentration ratios for macromolecules with molecular weights in the range of 60–720 kDa compare well with other studies of interstitial fluid, and with Garlick and Renkin's results concerning lymph/serum concentration rates for dextran molecules with molecular weights between 60 and 500 kDa [19]. In general, our results are in agreement with the two-pore theory of vascular permeability.

### Findings over time

From 0 to 24 h, the total protein content increased approximately twofold before stabilising (Figure 1). Curves for the individual proteins are in general very similar to that of the total protein concentration, with an increase in IF concentration from 0 to 24 h and relatively unchanged levels thereafter. This may be explained by an increased number of endothelial gaps. A tendency to increase at ap-

**Table 2: The IF concentrations relative to plasma (%) of five of the individual protein variables at 0 h, tabulated with corresponding values obtained through other sampling techniques.**

	Insulin	Albumin	Transferrin	IgG	alpha-2-Macro-globulin
Svedman et al *	65	48	30	31	20
Svedman et al (11)**	45	59	43	46	18
Rossing et al (17)***		54	48	43	20
Vermeer et al (18)***	90	29			18
Ader et al (16) †	53				
Jansson et al (15) ††	56				

\* IF extracted from an erosion at -200 mmHg. \*\* IF extracted from an erosion at -50 mmHg. \*\*\* IF obtained from suction blisters. † IF obtained by lymph cannulation. †† IF obtained by microdialysis.

proximately 72 h was also seen. This may be ascribed to an increased number of papillary loops [5]. It is not known whether the new capillaries are immature in their endothelium and perhaps more permeable to large-sized proteins, but this could explain the obtained results. Insulin was the only exception for which no significant differences were found between days, but the statistical material was small. An earlier study with larger statistical material, using the same technique, showed a significant increase in relative insulin concentration between days 1 and 3 from 49 to 74%.

The fact that the IF/plasma ratio for individual proteins stabilised at levels not exceeding 70% for any of the proteins, along with the reproducibility of the findings, indicate a functioning lymphatic drainage. This also indicates that the suction-induced epidermal split did not significantly disturb the initial lymphatics.

If the erosion technique had produced excessive cellular damage, bands representing intracellular molecules would have been seen in the SDS-PAGE gels. No bands were found in the interstitial fluid samples that did not exist in the plasma, indicating relatively little cellular damage in the erosion. The variability of concentrations in IF as compared to the variability in plasma was not presented, because the quantification from SDS-PAGE gels only allows comparison within a gel when a dilution series is run on it. The variability that could be calculated from our material would represent the sum of the variability in plasma, IF and the SDS-PAGE method in itself, and is therefore of little interest. The erosion and IF extraction techniques could be of clinical interest, primarily for serial measurement of freely distributed substances such as glucose and electrolytes, and as a model for studying inflammation and vascular permeability. The model could also be useful for studying the mechanism of neurogenic inflammation involved in diseases in other organs than skin. In addition, the erosion has been used for transder-

mal administration of dextrans in a wide size range during 24 h [20]. The present study suggests that the erosion could be used for systemic delivery of even larger molecules and for as long as 5 days.

## Conclusions

A simple method using a standardised erosion for serial measurement of plasma proteins in dermal IF is reported. At 0 h, the concentration of plasma proteins reflects interstitial fluid composition before the onset of an inflammatory-induced increase in permeability. In the time interval from 24 to 96 h, the values characterise a state of increased permeability. The findings are in accordance with a neurogenically induced inflammatory reaction.

The lower concentrations of proteins in IF than in plasma indicate a functioning lymphatic drainage.

Clinically, the technique shows potential for the serial measurement of freely distributed substances and as a model for studying inflammation and vascular permeability.

## Abbreviations

IF Interstitial fluid

AUC Area under curve

AU Area units

## Competing interests

None declared

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