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Anatomy of the Interstitial Tissue

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Summary

Aspects on composition and function of the interstitial tissue have been given. The glycosaminoglycans of the interstitium have at physiological pH a net negative charge and are osmotically active. They restrict free diffussion through the interstitium. The ground substance phase can be further subdivided into a colloid-rich subphase and a colloid-poor subphase. The latter seems to constitute the true tissue fluid phase of the interstitium. The functional importance of the interstitium on exchange processes between the vascular and the cellular compartments is discussed. Changes in aggregation and hydration of the ground substance change the physico-chemical properties and the functional characteristics of the interstitium.

The interstitial compartment surrounds most cells of the organism. It consists of fibrillar structures embedded in a tissue fluid containing amorphous ground substance. The fluid volume of this compartment is approximately three times that of plasma and the exchange rate of fluid between these two extracellular compartments is rapid. All substances that are exchanged between blood and tissue cells have to pass through the interstitium. The interstitial content of acid macromolecular components may be assumed to affect the composition of the capillary filtrate reaching the cells. An evaluation of the true environmental milieu of tissue cells therefore makes it necessary to take the physico-chemical properties of these various interstitial components into consideration. Structural and functional aspects of the interstitium will be dealt with in the following presentation.

The fundamental structure of the interstitial phase is schematically shown in Fig. 1. The main components are fibers and the interstitial ground substance, which may be subdivided into a colloid-rich and a water-rich phase.

Fibers

Three types of fibers – collagenous, reticular and elastic ones, are present in the interstitium. These fibers are synthetized by mesenchymal cells, fibroblasts, osteogenic and chondrogenic cells, and possibly also smooth muscle cells (elastic fibers; 1). Molecular collagen is secreted from the cells into the interstitial ground substance, where it is polymerized to collagenous and reticular fibers (2). The reticular fibers form thin networks around cells and they are also closely related to basement membranes and the stroma of e.g. lymphoid organs. Collagenous fibers of various dimensions are demonstrable in the interstitial phase of most tissues while the networks of elastic fibers are not so common. The functional importance of the various fibers is mainly mechanical support. The isoelectric points of the fibrillar constituents lie between neutrality and pH 5.0 (3). Therefore, at physiological pH the number of charged sites is small and the influence of these fibrillar structures on the distribution of other ions relatively limited.

Ground substance

The amorphous ground substance or gellike matrix of the interstitium is produced by the same cell types as the fibrillar components. It contains several different glycosaminoglycans (mucopolysaccharides) the more common ones of which are hyaluronic acid, chondroitin-4-sulphate, chondroitin-6-sulphate, dermatan sulphate and keratan sulphate. The proportions of the different glycosaminoglycans vary in the interstitium of various tissues. Hyaluronate is present in most places, while the chondroitin-sulphates are mainly present in cartilage and bone. The glycosaminoglycans are composed of repeat units of two different saccharides,

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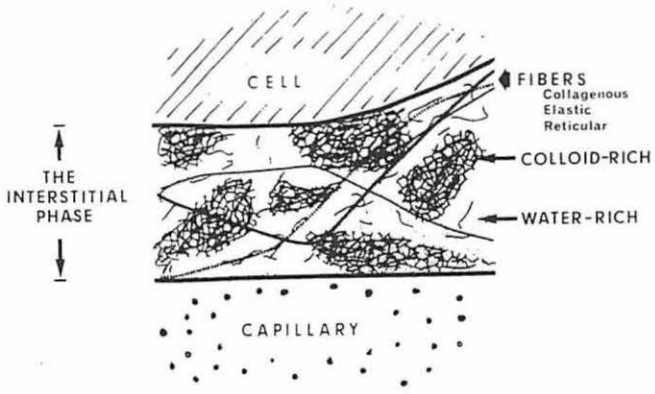

Fig. 1 Schematic presentation of the anatomy of the interstitium.

usually hexosamine and hexuronic acid. The disaccharide units contain charged anionic groups such as carboxylate and sulphate groups. The density varies from one (hyaluronate) to four (heparin) of these anionic groups per disaccharide unit. The mucopolysaccharides have low isoelectric points (between pH 2.0 and 3.0) and at physiological pH there is consequently a high density of negative colloidal charge within the interstitial ground substance.

It has been suggested, however, that the extent of aggregation of the polymers varies within the interstitium as schematically shown in Fig. 1. On the basis of this concept a colloid-rich water-poor phase coexists with a water-rich colloid-poor phase (4). This hypothesis predicts the existence of areas with highly aggregated ground substance and a high negative charge density alternating with areas which have a low content of disaggregated ground substance and a low negative charge density (Figs. 1 and 2). In the highly aggregated areas the charge density and the large domains of the macromolecules will result in steric exclusion of other large molecules (5). Therefore, it is considered that e.g. plasma proteins passing the capillary walls will be mainly restricted to a random network of interstitial channels corresponding to the colloid-poor water-rich areas (6, 7, 8). The relative mobilities and the distribution of water as well as of small diffusible cations and anions in the interstitium will also be affected by the high negative charge density of the glycosaminoglycan aggregates (3, 4).

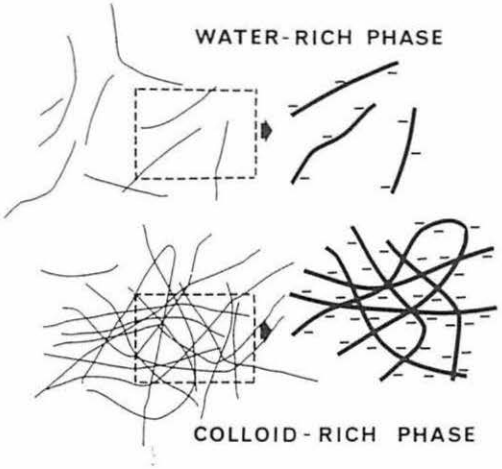

Fig. 2 Density of charged colloidal macromolecules in the water-rich and the colloid-rich interstitial phases.

Important functional effects of the colloidal phase are restriction of free diffusion, binding and immobilization of cations and anions, and involvement in ion-exchange reactions. It is obvious that the extent of these interstitial effects is dependent upon the degree of colloidal aggregation and tissue hydration (Fig. 3). Oedema causes an increased hydration and depolymerization of the ground substance. The result will be a decrease in the colloidal-dependent restriction of free diffusion as well as in the binding capacity of cations (9). Tissue dehydration, on the other hand, increases the colloidal density and thereby restricts free diffusion and increases the binding capacity of cations (10).

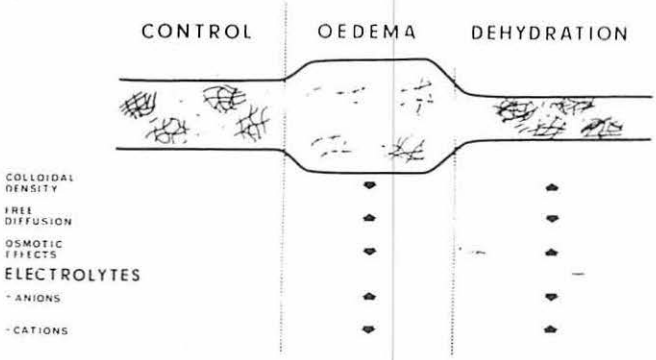

Fig. 3 Changes in interstitial functional characteristics in commection with tissue oedema or dehydration.

Interstitial tissue fluid

The existance of an extracellular fluid representing a mere dialysate of blood was questioned already in 1960 by *Gersh* and *Catchpole* (4). It seems reasonable to believe that the free fluid phase i.e. the colloid-poor water-rich phase is the true interstitial fluid. This fluid phase will receive products from the vascular as well as from the intracellular compartments and its composition will be modified by the equilibrium with the colloid-rich phase. It may be assumed that cellular membranes are mainly in contact with this movable fluid phase and this phase, therefore, represents the true environmental milieu of tissue cells.

Techniques for direct sampling of nanoliter quantities of this interstitial tissue fluid phase have been developed (11) and the protein (12) as well as the electrolyte (11, 13) composition of the fluid has been studied using micromethods (14, 15).

The glycosaminoglycan content of this movable tissue fluid phase is shown to be low in comparison to that observed in fluid obtained from implanted capsules. The content in such implanted capsules may be assumed to represent total interstitial content (13). The low content of glycosaminoglycans in the fluid obtained with the micropuncture techniques, therefore, favours the concept that it is a true representative for the colloid-poor water-rich interstitial fluid phase.

The electrolyte content of the interstitial fluid is also markedly different from a hypothetical ultrafiltrate of plasma. The tissue fluid: plasma ratios of K^+ and Na^+ are considerably larger than 1.0 and that of Cl^- is smaller than 1.0 (Table I). This higher content of K and Na in tissue fluid is probably due to binding effects on cations caused by the colloidal anionic polyelectrolytes of the interstitium. The low Cl content is consequently due to the presence of these colloidal anionic polyelectrolytes.

Table 1 The distribution of electrolytes (K, Na and Cl) and proteins between plasma (P) and interstitial tissue fluid (TF). (From ref. s. 11, 12, 13 and unpublished data)

	TF	P	TF−P	TF:P
K mmol/l	4.67	3.78	+0.89	1.25
Na mmol/l	153	142	+11	1.08
Cl mmol/l	91.2	99.5	−8.2	0.92
Total protein	–	–	–	0.32
Albumin	–	–	–	0.36
Globulin	–	–	–	0.24

The total protein content as well as the albumin: globulin ratio of the sampled fluid are also in agreement with hypothetical interstitial fluid concentrations (12).

Experimental data obtained from direct analyses of sampled interstitial tissue fluid, therefore, are in agreement with the hypothesis that the colloid-poor water-rich phase is the true environmental milieu of the cells. The nutrients to and metabolites from cells pass mainly through this phase, but the interplay with the colloid-rich phase prevents major fluctuations in the cellular milieu. Extensive loads of e.g. metabolites during tissue hypoxia are buffered in the interstitium due to ionic binding to and ion-exchange reactions with the colloid-rich phase. This interplay between the two interstitial phases may be considered as an important de-

fence mechanism which prevents extensive changes in the milieu outside the cell membrane and thereby also major cellular functional disturbances in emergency situtations.

Disturbances of interstitial homeostasis

The extent of aggregation and disaggregation of the interstitial ground substance will affect the interstitial homeostasis. Hormones, e.g. estrogen, androgen and cortisone, have been shown to affect the amount as well as the state of aggregation of ground substance. Disaggregation of the colloides lowers the density of the colloidal charge and increases the osmotic effect. Thereby tissue water content increases and an edema situation is initiated (4, 16). Inflammatory reactions increase the vascular permeability and also cause a disaggregation of the interstitial ground substance. Hyaluronidase and collagenase induced decomposition of interstitial components will dramatically increase the spread of substances through the interstitium. Such changes favour activiation and movement of fixed tissue macrophages as well as the migration of inflammatory cells such as monocytes, leukocytes and lymphocytes into the interstitial tissue.

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Discussion

Lasson: How would you explain your findings of higher K^+ and Na^+ in tissue fluid than in plasma?

Haljamäe: It is usually considered that there is a distribution of electrolytes between the vascular compartment and the interstitial compartment according to a Donnan equilibrium. The effects of the interstitial ground substance are, however, not taken into consideration. Therefore a lower content of potassium and sodium is assumed to exist in the interstitial fluid than in blood plasma. In the interstitium, however, you have a higher content of colloidal mucopolysaccharides than in the plasma. The charged macromolecules of the interstitium will affect the distribution of all small cations and anions. Especially in situations with changing tissue hydration mucopolysaccharides seem to participate in ion-exchange reactions of major importance for the control of the milieu at the cell membrane level.

Aukland: If you could compare local capillary plasma ionic contration with that of interstitial fluid your values might be closer than when you compare plasma from large veins.

Haljamäe: What we are using is the regional venous blood. This is the closest what we can get with-out disturbing the homeostasis across the capillary wall. We are also trying to study the local interstitial change by the use of other techniques. As I mentioned, we are using local tissue pH registrations and comparing to simultaneous regional blood changes. We are also trying to compare interstitial electrolyte changes with changes of cellular transmembrane potentials.

I would also like to come back to the question about capsular fluid. We have tried to analyze capsular fluid and we used titanium capules which we implanted subcutanously in rabbits. We compared the electrolyte and the glycosaminoglycan composition of the capsular fluid with that what we call "true interstitial fluid". We found profound differences in composition and I think that the results point to the fact, that in the capsule there is a large pool of fluid, and the number of capillaries per volume of fluid is very small. Therfore, in the capsule we do not probably have a suborganization into a colloid-rich and a colloid-poor phase. The other thing we found was that if we injected labelled substances into the blood, the exchange rate to the capsular fluid was small compared to "true tissue fluid". Therefore we do not think that the capsular fluid is representative for tissue fluid. It is a big lake, but what happens on the shores of that lake does only to a minor extent affect its composition. The sampling procedure of "true interstitial fluid" I described in 1970 in a paper in Acta Physiologica Scandinavica. The sampling is performed under liquid parafin. Subcutanous tissue fluid is obtained from connective tissue by the use of thin capillaries. With this technique it is possible to get nanoliter quantities of the fluid. A fresh surface area, i.e. untouched, is always used to prevent capillary leakeage. The electrolyte and protein content of repeated samples from the same animal is very constant indicating a good reproducibility.

Question: Is there a difference in the composition of lymph and of the tissue fluid collected with your method?

Haljamäe: I think that fluid within the lymphatic vessels does not reflect the changes in the interstitial fluid. In the lymphatics we do not have the equilibrium between the two colloidal phases, which we have in the interstitium i.e. between the colloid-poor and colloid-rich phase. Therefore, lymph does not reflect changes in the immediate cellular milieu to such an extent as does local interstitial fluid.

Olszewski: During micropipetting you damage cells. Is not the high potassium centent the result of release of this ion from damaged cells?

Haljamäe: If the difference in potassium distribution was only due to cellular damage during sampling then at the same time, the sodium content would have been affected. Due to entry into the cells sodium would decrease. What we have found was a high content of potassium as well as of sodium and a low content of chloride in interstitial fluid.