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An electrophoretic investigation of rat interstitial fluid proteins

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1. INTRODUCTION

What is interstitial fluid? Schultze and Heremans (1966) summed up by stating: 'Interstitial fluid is a deceptively simple name for an astonishingly complex physical entity'.

Although, as will be seen in the review of the literature, there is some agreement amongst various researchers regarding certain aspects of interstitial fluid and the interstitium, the concept 'interstitial fluid' is still not fully understood from morphological, compositional and functional points of view. However, with the advent of new scientific equipment (e.g. electron microscope) and better investigational methods, older theories regarding certain facets of interstitial fluid and the interstitial space were proved, disproved or had to be altered to suit the latest hypothesis or experimental evidence. The inaccessability of the interstitial area is probably one of the major factors contributing to the differences in opinions amongst many authors regarding this space.

The lack of sufficient data does not make interstitial fluid less important. On the contrary, interstitial fluid plays an extremely important role in the body. Schultze and Heremans (1966) even considered the extravascular circulation as biologically more important than the vascular one. The cells of the body, being in close contact with interstitial fluid, depend on this as the medium to supply them with their nutrients and to remove their metabolic end-products. The cells in the body therefore depend on the interstitial fluid for their very survival and any abnormality in the composition of this fluid will have a direct bearing on the proper

functioning of these cells.

Proteins play a major role in this life-sustaining function of the interstitial fluid. These interstitial fluid proteins have been shown to be similar to the main protein species that occur in the plasma by means of specific immunochemical precipitation reactions (Schultze and Heremans, 1966). The functions of the proteins (plasma and interstitial fluid) are as manifold as the diversity of the proteins themselves; for many components the role remains to be discovered. The primary functions of these proteins include the maintenance of colloid osmotic pressure, pH, electrolyte balance; the transport of metal ions, fatty acids, steroid hormones, drugs etc.; their ready availability as a nutritional source of amino acids for the tissues; the regulation of cellular activity and functions of hormones; and defence against invasion through the action of antibodies (Putnam, 1960).

Albumin, the major secretory protein synthesized by the liver, plays a major role with regard to the above functions. One of the important functions of albumin is the maintenance of proper osmotic balance throughout the body; others are related to its ability to bind various and diverse substances. Alterations in albumin mass due to changes in synthesis or catabolism in disease or alterations in albumin distribution between intravascular and extravascular compartments may have profound effects on health.

Although albumin is probably one of the best studied of the plasma proteins today, numerous questions still remain unanswered. Some of the questions, which include those from Rothschild et al

(1979), are the following: How do albumin and the other proteins pass from the capillary into the interstitial fluid? What influences the movement of albumin and the other proteins to and from the extravascular sites? How frequently does a single molecule make this trip? How are the protein molecule altered during this passage? Much more research will have to be carried out before these and other questions can be answered.

It can thus be said that knowledge obtained by ascertaining the composition of interstitial fluid can supply useful information regarding cellular metabolism and can allow for the interpretation of observed exchanges between extracellular and intracellular body compartments. Under normal physiological conditions the interstitial fluid reflects the activity of the tissue elements bounding the interstitial space and under disturbed or abnormal conditions the attempts of these elements to maintain a biochemical equilibrium.

The present research work was an attempt to obtain more information regarding this very important aspect of physiology, namely the interstitial fluid and its proteins.

This research work was done with the following as guide-lines:

1) The electrophoretic separation of plasma and interstitial fluid proteins into different fractions.

2) The determination of the concentration of each of these fractions.

3) The determination of the alterations in the concentrations of each of these fractions under the following conditions:

   (a) Cold exposure.
   (b) Heat exposure.
   (c) Exercise.

4) The immunoelectrophoretic identification of a possible modification of the albumin molecule during it's journey through the extravascular space.

5) The separation of albumin into two fractions by means of disc electrophoresis and crossed immunoelectrophoresis.

6) The possible location of a protein that may be exclusive to interstitial fluid.

As mentioned earlier, the major problem with studying interstitial fluid is the inaccessability of the interstitial space, although from the review of the literature it can be seen that there are a few techniques for obtaining samples supposedly representing interstitial fluid. After due consideration it was decided to use the chronical implanted capsule for obtaining subcutaneous interstitial fluid. For the purpose of this study the capsule served as the best means of obtaining interstitial fluid. Although no claim is made as to the authenticity of the capsule for obtaining interstitial fluid, it is the only technique for obtaining a sufficient volume of sample supposedly representing interstitial fluid. The capsule offers at least a means of probing into the composition

and functional mechanisms of interstitial fluid whereby some of the deductions and speculations made by other workers regarding the interstitial space, may be confirmed or disregarded.

2. REVIEW OF LITERATURE

(Concluded April, 1980).

An excellent review concerning interstitial fluid has been published by Guyton et al (1975). Reviews on the plasma proteins have been published by Putnam (1960) and Schultze and Heremans (1966).

2.1 Classification

According to Edelman and Leibman (1959) approximately 55 per cent of the estimated body water in the young adult male is intracellular fluid, whilst the remaining 45 per cent is classed as extracellular fluid.

The term extracellular fluid designates a variety of fluids, e.g. interstitial fluid, the plasma portion of blood, fluid in the lymph, fluid in the gastrointestinal tract, cerebrospinal fluid, ocular fluid and fluid in the potential spaces of the body (Guyton et al, 1975). The interstitial fluid fraction represents the greatest portion of these fluids. According to the calculations of Edelman and Leibman (1959), 20 per cent of the total body water is present as interstitial-lymph fluids whereas plasma represents about 7,5 per cent.

2.2 Interstitial fluid characteristics

Certain studies considered interstitial fluid to be a free fluid independant of the intercellular substances (Manery, 1954). Other investigators, like Snashall (1977), thought it

to be a one-phase system.

However, the interstitium is now generally accepted as being a two-phase system composed of free fluid and gelled fluid (Casley-Smith and Vincent, 1978; Gersh and Catchpole, 1960; Guyton et al, 1971, 1975; Schultze and Heremans, 1966, Stromberg and Wiederhielm, 1976; Watson and Grodins, 1978). Various researchers had pointed out that the relationships between the two phases are very labile and that certain conditions and substances are known to affect this (Catchpole, 1966; Gersh and Catchpole, 1960).

The tissue spaces contain collagen fibres interwoven in the form of bundles among the cells and other tissue structures. They form a lattice-work in which the tissue elements are dispersed. Hyaluronic acid and other mucopolysaccharides (ground substance) are found in the openings of the collagen fibres (Gersh and Catchpole, 1960; Guyton et al, 1975). The ground substance entraps the tissue fluid and causes it to take on a gel-like consistency (Schubert, 1964). Therefore, when fluid flow does occur, it takes place in the form of small rivulets that flow along surfaces of cells or large tissue fibres (Clark and Clark, 1933). Very small quantities of free fluid are possibly present in small vacuoles located throughout the gel (Gersh and Catchpole, 1960). It was shown by Casley-Smith and Vincent (1978) that tissue channels (the water-rich phase) do exist in the interstitial tissue of the rat ileum, knee joint capsules and kidneys and that these are not just vacuoles.

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