The gel phase of the interstitium has also an impeding effect on the flow of fluid (Day, 1952; Watson and Grodins, 1978). Preston et al (1965) demonstrated that the gel is able to decrease the flow of fluid ten million fold. This has the advantage of decreasing the effect of gravity or other forces that may cause fluid movement.

In spite of the restriction of fluid flow, diffusion of small molecular mass substances is almost as great as occurs in the absence of tissue gel (Laurent, 1966). Guyton et al (1975) stated that the rapidity of diffusion within the interstitial space causes the fluids in any given local tissue to be reasonably uniform in character. The tissue fluid proteins however, are hindered in their passage through the tissue gels (Granger, 1974; Laurent and Ogston, 1963). Nevertheless, Guyton et al (1975) concluded that the hindrance is not of major significance as the reduction in diffusion rates is only between twenty and forty per cent.

2.3 Extravascular Circulation

2.3.1 Interstitial fluid and lymph

The formation and circulation of interstitial fluid can be summarized as follows: Fluid is formed from the blood in the capillaries, it then moves through the interstitial space and finally has an exit through the venous capillaries and lymphatics.

The balance of forces, as postulated by Starling (1896), favours a loss of fluid at the arterial end (capillary

filtration), but an absorption of fluid at the venous end of the capillaries (Guyton, 1971; Pappenheimer and Soto-Rivera, 1948).

Capillary filtration and the consequent convective fluid movement as a means of transporting nutrients and wastes to and from cells, is not as important as the process of diffusion (Guyton et al, 1975; section 2.2).

The capillary membrane allows fluid and almost any substance, smaller than the plasma proteins (molecular mass of albumin is approximately 70 000), to pass freely through the slits between the adjacent capillary endothelial cells (Pappenheimer et al, 1951). As the plasma proteins cannot traverse these slits easily, their movement is thus restricted (section 2.3.2).

Guyton et al (1975) showed, by taking the different pressures into account, that more fluid and solutes move out of the arterial capillaries than back into the venous capillaries. Most of this excess fluid and solutes flow into the extremely porous lymphatic capillaries (Mayerson et al, 1962) to be returned to the vascular circulation via the lymphatic system. Electron-microscopical studies by Leak and Burke (1968) and studies by other investigators indicated that the lymphatic circulation is not a passive system, but is responsive to the balance of pressures and movements

in the surrounding tissue, especially muscle (Guyton et al, 1975). Drinker and Field (1931) had postulated that the interstitial fluid from a particular region is similar in composition and protein content to the lymph derived from that region. In actual fact, studies by Garlick and Renkin (1970), where they used endogenous albumin with a graded series of dextrans, showed that the principal barrier to blood-lymph transport is at the blood-capillary wall and not at the lymphatic capillaries, nor within the interstitial space. It was pointed out by Drinker and Field (1931) however, that the original capillary filtrate may differ widely from the lymph because of the exchange of nutrients and wastes between cells and fluid, in addition to a possible modification by the smaller lymphatic capillaries and lymphatic glands. It was indeed later stated by Bogdanikowa and Grabowski (1972) that some of the molecules probably remain in the extravascular space for a long time and may undergo changes due to enzymatic and cellular modifications. This point is also stressed by Libermann et al (1972), namely that interstitial fluid composition is in part determined by the tissue's metabolic needs and the type of metabolic discharge from their cells.

2.3.2 Interstitial Fluid Proteins

Drinker and Field (1931) and Drinker (1946) formulated the concept of an extravascular circulation of proteins from capillaries to lymph. According to these

views, blood proteins move through the capillary endothelium into the interstitial space and can then only return to the venous circulation via the lymphatic system. Other investigators, using labelled proteins, confirmed these views (Abdou et al, 1952; Forker et al, 1952; Wasserman and Mayerson, 1951). The studies by Dewey (1959) and Gitlin and Janeway (1954) have shown by indirect means that the interstitial fluid contains substantial amounts of proteins derived from plasma. Analysis of inter-fibre fluid from guinea pig by Creese et al (1962) showed all the major plasma protein fractions to be present in their samples.

The fact that interstitial fluid proteins can only return to the circulation via the lymphatics and not via the venous capillaries, had been demonstrated by Lewis (1[illegible]). Horse serum was injected intravenously into dogs and it was shown that the foreign proteins appeared in the lymph long before they were noticed in the blood stream. Similar results were obtained by Field and Drinker (1931). Courtice and Simmonds (1949) found that plasma albumin, labelled with T-1824 dye (Evans Blue) injected into the pleural cavities of rabbits and cats, was almost quantitatively absorbed by the lymphatics. Courtice (1971) also supplied evidence against certain views that tissue fluid proteins may enter the blood stream directly through the walls of the capillaries.

Various theories have been postulated in attempts to explain the precise manner in which proteins can move through the capillary membrane in spite of the fact that they are larger in size than the capillary pore (Garlick and Renkin, 1970; Pappenheimer, 1953; Renkin, 1964; Wasserman et al, 1955). Guyton et al (1975) and Schultze and Heremans (1966) discussed some of the theories offered in an effort to explain the movement of proteins through the capillaries.

Brace et al (1977) showed that lymph protein concentration is not always identical to the concentration of interstitial fluid protein. Casley-Smith and Bolton (1973) have suggested that the protein in lymph is concentrated due to the expulsion of fluid back into the tissues as a result of lymphatic compression. The higher protein concentration and consequent higher osmotic pressure of the lymph results in an increased flow of tissue fluid and therefore more protein into the lymphatic capillaries. Guyton (1971) and Guyton et al (1976) agree with the concentrating ability of the lymphatics. However, Rutuli and Arfors (1977) and Taylor and Gibson (1975) could find no proof of the ability of the lymphatics to concentrate proteins.

It would therefore appear that the available data does not prove conclusively that proteins are concentrated in the lymphatics, although Stromberg and Wiederhielm (1976) offered an explanation in an attempt to resolve

the controversy regarding the identity of lymph and interstitial fluid.

Dewey (1959) stated that the rates at which plasma proteins penetrate from the vascular to the extravascular compartments differ from one tissue to another and are roughly proportional to vascularity. The masses of plasma protein contained in the extravascular compartments also differ from one tissue to another. They found, using labelled proteins, that the flux of albumin through skin and muscle is small, but that these tissues contain a significant proportion of the total interstitial albumin of the body.

According to calculations by Schultze and Heremans (1966), eighty to eighty five per cent of the albumin mass leaving the blood will have returned to the blood two days after their escape. However, according to their data, the major flux of albumin through the tissues really involves the minor fraction of the protein whereas the bulk of it is exchanged slowly.

Wasserman and Mayerson (1952) determined the escape rate of albumin in dogs to be 4,9 per cent per hour. Lassen et al (1974) assessed the lymphatic return of albumin at 100 per cent of the intravascular albumin mass per 24 hours. Schultze et al (1972) concluded that most authors agree that total intravascular protein mass completes a passage through the inter-

stitial space at least once a day.

2.4 Interstitial Fluid Studies

2.4.1 Interstitial Fluid Pressure

Since the beginning of 1960 more interest has been shown in the study of the interstitial space. Due the inaccessability of the interstitial spaces, interstitial fluid studies have been difficult to carry out. Guyton (1963) introduced the implanted perforated capsule to measure the subcutaneous interstitial fluid pressure, which was found to be subatmospheric. Most of the methods later developed confirmed the negative capsular registrations obtained by Guyton in 1963 (Eickenberg, 1978; Fadness et al, 1977; Prather et al, 1971). Using subcutaneously inserted needles, Brace et al (1975) also obtained negative interstitial fluid pressures. The wick technique, introduced by Scholander et al (1968), as well as a modification by Ladegaard-Pedersen (1970), all gave negative interstitial fluid pressures in accordance with that originally obtained by Guyton (1963). Other possible methods for measuring interstitial fluid pressure are briefly discussed by Guyton et al (1975).

2.4.2 Interstitial Fluid Sampling

The methods mentioned in the previous section were mainly concerned with measuring interstitial fluid pressure. Recently however, an increasing number of attempts have been made at sampling and analyzing the

fluid obtained from the interstitial spaces. As early as 1938 Maurer inserted tiny capillary tubes into the muscle of the frog in an endeavour to obtain interstitial fluid. This was done after attempts at centrifuging muscle preparations failed because the sample contained (to a large extent) the constituents of the muscle cells. Maurer (1938) collected approximately 1 µℓ of a clear straw-coloured fluid. He also claimed that histological examination showed no damage to the muscle fibres. Manery (1954) doubted whether this was interstitial fluid and suggested that it might have been edema fluid induced by tissue damaged during the introduction of the capillary tubes into the muscles. This would lead to a higher protein content as it was shown that injured capillaries became more permeable to proteins (Cope and Moore, 1944; Menkin, 1956). Creese et al (1962) inserted similar capillary tubes into the muscles of guinea pigs and managed to obtain about 0,1 to 0,2 µℓ of fluid. Results obtained from the intravenous injection of labelled proteins supported their view that it was indeed interfibre fluid that was sampled.

Rutuli and Arfors (1977) used a micropuncture technique (Landis, 1930; Rutuli and Arfors, 1976) for obtaining small volumes of interstitial fluid. These were then analyzed biochemically and electrophoretically (table 2).

The wick method, as used for measuring interstitial fluid pressure, has been applied by various researchers for obtaining interstitial fluid samples (Aukland and Fadness, 1973; Garetto and Hargens, 1976; Lonsman-Poulsen, 1973; Prather et al 1971). This method basically involves the insertion of a cotton thread inside a hollow needle into the tissue. The needle is then withdrawn, leaving the wick, which acts as minute conducting and absorbing tubes, in contact with the tissue elements. Prather et al (1971), after comparing results obtained from the analysis of fluid samples from wicks and capsules, concluded that the injury and consequent edema produced during wick insertion may have an effect on the sample. Rutuli and Arfors (1978), on the contrary, could find no evidence that the wick sample is not representative of interstitial fluid after comparing these results with those obtained from their micropuncture technique.

Haljamäe et al (1974) used a 'liquid-paraffin cavity' technique for obtaining and analyzing interstitial fluid. They compared these results with those obtained by analyzing fluid from implanted titanium capsules. It was concluded that this technique gave a sample more closely resembling interstitial fluid than does the capsule. Although they were satisfied that capsular fluid did not represent an entirely inactive collection of fluid, their results indicated that it did not participate sufficiently rapidly and actively in exchange processes.

A major drawback of their technique is the small volume of sample that is obtained (approximately 50 $\mu\ell$).

2.4.3 Capsular Fluid

In this field of research any method of sampling with the aim of obtaining information concerning the behaviour of the subcutaneous tissue during normal and abnormal physiological conditions, should fulfill the stipulations as laid down by Burke (1964):

1) Sampling must not alter the function of the cells, supporting structures or vessels in the test area.

2) The mechanics of repeated sampling over long periods of time must not alter tissue physiology so that undistorted serial samples may be obtained.

3) The interstitial fluid must flow unchanged from the interstitial space into the collecting unit.

At the present the sampling methods discussed so far had to be rejected in favour of the chronically implanted capsule in an attempt to comply with these requirements. (A chronically implanted capsule is defined by Brace and Guyton, 1979, as one which remains in the animal for longer than 4 weeks.)

There are several adaptations of the perforated capsule technique (Guyton et al, 1975; Guyton et al, 1976).

These vary in size and number of perforations, each apparently suitable for certain specific conditions and locations. It was for example postulated by Guyton et al (1975) that the implanted capsule with the smaller perforations was more effective for a longer period of time as compared to the capsule with the larger perforations. This was due to the fact that granulation tissue did not grow as easily through the smaller perforations and consequently did not fill the interior of the capsule. Guyton et al (1975) have also compared capsules made from different materials and concluded that this factor was not important as long as there is no excessive tissue reaction. They did however, obtain the impression that stainless steel capsules caused more imflammation.

Other types of capsules, besides those mentioned by Guyton et al (1975) have been used by various investigators as discussed below:

Jones et al (1969) used perforated plastic capsules implanted in various tissues to determine the oxygen tension of the interstitial fluid. Katz (1978) used hollow, porous polyethylene capsules which did not appear to induce the growth of granular tissue. This was in an attempt to lessen the amount of protein excluded from the capsule. Multiperforated propylene balls implanted in soft tissue and parenchyma of dogs, were used by Eickenberg (1978) to test the validity of