the capsular method. Libermann et al (1972) used vinyl capsules implanted in the backs of rats to study acid-base values and gas and electrolyte concentrations in the blood and interstitial fluid. Calnan et al (1972) used capsules of various designs and materials implanted subcutaneously and intramuscularly in a series of experiments on greyhounds.

It is however, of physiological importance to establish whether the fluid in the capsule is freely communicating with the interstitial space and therefore representative of interstitial fluid.

Stromberg and Wiederhielm (1970) and Wiederhielm (1968) challenged the idea of free communication of protein between the capsular fluid and the fluid in the surrounding interstitium. Although their objections are directed at the validity of interstitial fluid pressure measurements, they apply to the concentrations of other constituents, for example proteins, of the capsular fluid as well. Wiederhielm (1968) stated that a semipermeable membrane, which can exclude proteins from the intracapsular fluid, is formed around the capsule. This membrane is presumably connective tissue (Granger and Taylor, 1975). Stromberg and Wiederhielm (1970) proposed that hyaluronic acid and protein concentrations are much higher on the outside than on the inside of the capsule. They found that the intracapsular pressure changes when the capsular fluid was re-

placed by fluids with various albumin concentrations. This suggested that the capsular membrane is highly sensitive to oncotic pressure differences as would be expected if the fibrous lining greatly impeded or actually prevented the movement across it's structure.

Guyton (1963) demonstrated the free flow of fluid and protein outward through the capsular lining. Evans Blue or T-1824 dye was injected into the capsule and the rapid outward movement of the dye was demonstrated. As the dye attaches to the plasma protein in the capsular fluid, it was an indication that protein also left the capsule rapidly.

Gibson and Gaar (1970) used I^{125}-labelled albumin to demonstrate that the connective tissue lining of the capsule was permeable to protein. They collected samples of lymph and capsular fluid after injecting the labelled proteins intravenously into dogs. They observed that the lymph and capsular I^{125}-labelled albumin concentrations were identical after 100 hours. Biochemical analysis of the ratio of albumin to globulin indicated that the capsular, blood and lymph samples had approximately the same ratios. These studies are therefore an indication that the capsular membrane is permeable to protein. These results were confirmed by Calnan et al (1972).

Other studies by Brace and Guyton (1979) and Taylor and

Gibson (1975) also failed to show a significant difference between the capsular and interstitial fluids. Eickenberg (1978) concluded from his studies that the fluid inside the capsule communicates extremely well with the fluid in the surrounding tissue. Results by Libermann et al (1972) suggest that capsular fluid represents a local interstitial fluid whose composition is related to the local metabolism of the tissue.

Although the studies therefore demonstrate that the capsule lining is permeable to proteins, they do not give any indication of the degree of restriction of transcapsular movements (compare this with the blood capillaries which, although impermeable to proteins, nevertheless allow them to pass).

Granger and Taylor (1975) carefully removed the connective tissue linings from implanted capsules and determined the restriction offered by the lining to diffusion of I^{125}-labelled human serum albumin. They found that the capsular lining had a reflection coefficient of about 25 per cent for plasma proteins. They concluded that, because of this small reflection coefficient for albumin, it indicated a relatively free movement of albumin across the membranes.

2.5 Choice of Sampling Technique

A capsular method, slightly modified, (section 3.1) was used in this study.

One of the major problems facing the researcher with regard to the interstitial fluid studies, is the inaccessability of the tissue spaces. In the previous section the various attempts at examining conditions prevailing in the interstitial spaces have been briefly discussed. All of these have merit, some possibly more than others. A choice had to be made between these different techniques to find the most suitable one that could satisfy the needs of the present research work (section 2.4.3). Except for the capsules, all the other methods of sampling interstitial fluid lacked the one essential feature necessary for this investigation: they cannot be left in the animals for a considerable length of time and therefore cannot be used for repetitive sampling. This is apart from the possible drawback that most of them only provide minute quantities of sample.

In principle, the chronically implanted capsule provides a large artificial space in the tissues which is assumed to be in complete equilibrium with the surrounding interstitial fluid. It allows for larger quantities of fluid to be obtained for a more fully quantitative biochemical analysis. Repeated sampling of interstitial fluid from a specific area during prolonged periods of normal and abnormal physiology is also possible.

From the preceding section it appears as if the main objection against this sampling technique is not so much levelled at the capsule itself, but more at the lack of free communication between the capsule and interstitial fluid due to the forma-

tion of a semipermeable membrane around the capsule (Stromberg and Wiederhielm, 1970; Wiederhielm, 1968). This membrane would impede the free movement of especially protein into and out of the capsule.

It has however, been proved to my satisfaction that there is sufficient communication between the capsular fluid and the interstitial space and that capsular fluid does represent, at least in part, a local interstitial fluid (Brace and Guyton, 1979; Calnan et al, 1972; Eickenberg, 1978; Libermann et al, 1972; Guyton, 1963; Taylor and Gibson, 1975). It has furthermore been shown that the membrane has a low reflection coefficient (Granger and Taylor, 1975) and that there is an equilibration between capsular fluid and interstitial fluid after approximately 100 hours (Gibson and Gaar, 1970; Taylor et al, 1973).

At the time of this investigation the following views are taken as having been proved:

1) The interstitial space is a two-phase system consisting of a gel phase and a fluid phase.

2) There is free communication between interstitial fluid and capsular fluid in spite of a possible time delay for equilibration of macromolecules.

3) Proteins are not excluded from the capsular fluid.

4) The capsular sampling method is the only known and proven method that complies with the conditions of this research work as outlined in section 2.4.3.

Even though there is a time delay for equilibration between capsular and interstitial fluids, this factor does not affect this investigation, except possibly for the exercise test on the treadmill, as sufficient time was allowed for equilibration (section 3.9).

It must be stressed that the capsule is not unconditionally accepted as the ultimate answer to interstitial fluid sampling. There remain some unanswered questions. Most evidence indicates that there is a lack of submicroscopic organization of ground substance (hyaluronic acid, etc.). How does this effect the actual interstitial fluid sample? There is the relatively large distances from the interior of the capsule to actively metabolizing cells. How does the capillary density per unit volume of fluid affect the actual results? (Haljamäe et al, 1974).

Nevertheless, results obtained with this method compare favourably with those obtained by other sampling means, for example the wick method used by Aukland and Fadness (1973) or the capillary tube method used by Creese et al (1962). (Section 5.1.2).

The capsules used in this study had a certain amount of tissue growth into and around them (plates 1 and 2). It was however, found that even after six months there still remained an open cavity in the centre of the capsule. Capsular fluid protein

concentrations sampled after six months compared very favourably with those of the same rat after six weeks (see section 4.6).

It should be emphasized that the results from this work are for the subcutaneous tissue only and may not be valid for the interstitial fluid of the whole body.

3. MATERIALS AND METHODS

3.1 Implantation of capsules

Male and female white rats, weighing between 250 and 450 grams, were used. The capsules were 2 cm long plastic tubing with an inner diameter of 4 mm and an outer diameter of 6 mm. Both ends of the capsule were left open and no other holes or perforations were made.

The skin on the back of the rat was chosen because it provides an easy access to the loose layer of connective tissue below. It is also very suitable as a test area because the minimum amount of disturbance of the capsule is likely to occur during healing and test periods.

Each rat was anaesthetized with ether, the skin on the back was shaved and cleaned with 70 per cent alcohol. A small incision was made and a small section of the skin was then loosened from the underlying connective tissue layer by means of a blunt instrument. Two capsules were inserted through the incision into the opening underneath the skin and the incision stitched. Aseptic techniques were used throughout. Each animal was immediately given an intramuscular injection of 0,4 mℓ Streptomycin (200 mg per mℓ) and 0,2 mℓ Penicillin (300 mg per mℓ). The animals were housed in individual cages and were given rat cubes (with a minimum protein content of 20 per cent) and water ad libitum.

A period of six weeks was allowed for healing and for tissue

growth in and around the capsule. According to Guyton (1963) four weeks is sufficient for all signs of inflammation to disappear and for complete equilibration between capsular and interstitial fluids. Only animals in which the capsule implantation site had healed without sepsis, were used. Approximately 5 per cent of the animals could not be used due to infection and inflammation. There was some tissue growth into the capsule, but even after six months a large fluid cavity was still present in the capsule (plates 1 and 2).

3.2 Sampling of capsular fluid and blood

Each rat was again anaesthetized with ether. A sample of capsular fluid was obtained by inserting a 23-gauge hypodermic needle through the skin into the centre of the capsule. Slight suction was applied by means of a syringe and approximately 0,1 mℓ of a clear straw-coloured fluid was withdrawn from each capsule. On the few rare occasions when a slightly blood-tinged fluid was obtained, it was discarded.

Blood samples were collected by inserting a 23-gauge hypodermic needle directly into the heart and approximately 0,8 mℓ of blood was slowly drawn into a 1 mℓ syringe (containing a film of heparin - 5 000 units per mℓ). Plasma was obtained by centrifuging the blood in a Christ centrifuge at 2 000 r.p.m. for five minutes.

Blood and interstitial fluid samples, when they were not used immediately, were stored in sealed capillary tubes at -10°C.

3.3 Hematocrit determinations

These were done in a Christ hematocrit centrifuge immediately after the blood sample had been withdrawn.

3.4 Protein concentrations

The total protein content was determined according to Lowry et al (1951) with Folin phenol reagent using bovine albumin as a standard.

3.5 Colloid osmotic pressure

Measurements were done with an electronic colloid osmometer as described by Prather et al (1968). An Amocon PM-10 semi-permeable membrane was used. The instrument was calibrated with a variable mercury column.

3.6 Preparation and isolation of immunoglobulins

3.6.1 Immunoglobulins against rat plasma

The plasma from four rats were pooled and 0,25 mℓ of this was mixed with 0,25 mℓ of Freund's complete adjuvant and subcutaneously injected into the back of a rabbit using a 1 mℓ syringe and a 21-gauge hypodermic needle. This procedure was repeated after fourteen, twenty-eight and forty-two days. Sixty days after the original injection the rabbit was anaesthetized by intravenous injection of 1,5 mℓ thiopentane sodium. By means of a 50 mℓ syringe with an 18-gauge hypodermic needle inserted into the heart, approximately 50 mℓ of blood was carefully withdrawn. Seven days after withdrawing blood, the rabbit was given a booster in-