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# A comparative study of the proteins in the body fluids of animals

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Other proteins higher in concentration in females than in males, are $\alpha_1$-lipoprotein, ceruloplasmin and immunoglobulins -G and -M, while prealbumin, albumin, orosomucoid, haptoglobin, $\beta_2$-lipoprotein and $\beta_1$-globulin were found to be higher in males than in females. (Ganrot, 1972).

During an investigation of serum protein changes during human pregnancy, it was observed that there is an additional precipitin line in the fast $\alpha_2$-globulin region when using only anti-pregnancy serum (Hirschfeld & Söderberg, 1960; Afronso & Farnham, 1962; Pedreira et al, 1962; Cooper, 1963; Afronso & De Alvarez, 1964; Studd et al, 1970; Stimson & Eubank-Scott, 1972).

The human has only one $\alpha$- macroglobulin trypsin-binding fraction, isolated and described by Schönenberger et al, (1958), while two distinct serum $\alpha$-macroglobulins ($\alpha_1$ and $\alpha_2$) have been described in normal rabbits (Picard & Heremans, 1963; Got et al, 1965; Knight & Dray, 1968; Berthillier et al, 1968). Dog serum was also shown to contain two trypsin-binding fractions, namely $\alpha$-macroglobulin and $\alpha_1$-antitrypsin (Ohlsson, 1971). Electrophoretically these two trypsin-binding proteins resemble the two $\alpha$-macroglobulins of rabbit serum. The human trypsin-binding protein has been identified as $\alpha_2$-macroglobulin and corresponds to $\alpha_1$-macroglobulin of the rabbit and to a normal protein in the rat which has not yet been identified (Berne et al, 1971).

One of the first workers to describe a "blue globulin" i.e. ceruloplasmin, was Pedersen (1945). The possible roles for ceruloplasmin have been suggested and summarized by Putnam (1975). Firstly ceruloplasmin may act as a ferroxidase, secondly it is responsible for copper transport and it may also aid in the maintenance of hepatic copper homeostasis. In the rat (Williams et al, 1974) and the pig (Roeser et al, 1970) ceruloplasmin appears to be essential for the flow of iron from reticuloendothelial cells to transferrin.

Another main metal-binding protein is transferrin which is capable of combining with iron, copper and zinc. Transferrin has the mobility of a $\beta$-globulin and comprises as much as 4,5% of the plasma proteins. Twenty or so inherited variants of transferrin have been noted, but none have been implicated in a pathological condition (Schultze & Heremans, 1966; Putnam, 1975).

The final plasma proteins to be mentioned are the $\gamma$-globulins (antibodies). The association of antibody activity with the $\gamma$-globulin fraction of serum was described many years ago by Tiselius and Kabat. In each species the immunoglobulin molecules can be subdivided into different classes on the basis of the structure of their "backbone". This is a heterogeneous group which mainly differ in physiochemical, antigenic and biological properties. For example, in the human there are five structural types called the immunoglobulins-G, -M, -A, -D and -E. They are abbreviated to IgG, IgM etc. Immunoglobulins have molecular masses between approximately 150 000 and 900 000. The latter two globulin types (D and E) are minor classes in comparison to the other classes. IgG could be grouped into four subclasses now termed $IgG_1$ to $IgG_4$. Their differences lie in the heavy chains.

Each major class can be distinguished from the others on the basis of subunit polypeptide structures. All the immunoglobulins appear to have a structure comprised of two types of polypeptide chains which have been termed light (L) and heavy (H). A light chain may be linked by a disulphide bond to a heavy chain to give a basic monomere (LH). Most classes of immunoglobulins are in the form of the dimer $(LH)_2$ (Roitt, 1971)Disulphide bridges appear to contribute appreciably to the shaping of the immunoglobulin molecule and they are considered to be of crucial importance for the stabilization of the antigen-combining site of antibodies. The class of the immunoglobulins is determined by the nature of the heavy chain ( $\gamma$ for IgG, $\alpha$ for IgA, $\mu$ for IgM, $\delta$ for IgD and

$\varepsilon$ for IgE) but a molecule of any class may have a pair of identical light chains of either the $\kappa$ (kappa) or the $\lambda$ (lambda) type, though not a hybrid mixture. Purified IgG antibodies, when visualized in the electron microscope by negative staining, can be seen to be Y-shaped molecules which swing open on combination with antigen. The unique structural characteristic of immunoglobulins is the division of their polypeptide chains into variable (v) and constant (c) regions, which determine respectively the specificity of antibodies and the characteristic class difference and various biological effector properties. The v region of both chains are involved in the antibody combining site.

The immunoglobulins may also be differentiated by their centrifugal and immunoelectrophoretic behaviour although they have a very wide range of electrophoretic mobilities within each class, ranging from $\gamma$ - to $\alpha_2$-globulin in the case of IgG due to different net charges on the different immunoglobulin molecules (Roitt, 1971). Immuno-globulin -G comprises approximately 80% of the total plasma immunoglobulins (White et al, 1968) and is particularly a-bundant in body fluids where it combats micro-organisms and their toxins. Immunoglobulin -A is the major immunoglobulin in sero-mucous secretions such as saliva, tears, nasal fluids, colostrum and secretions of the lung and gastrointestinal tract, where it defends external body surfaces. Although IgM is a minor constituent of human plasma (White et al, 1968), it is an extremely efficient agglutinator which is produced early in immune responses. It is often referred to as the macroglobulin-antibody because of its high mole-cular mass (900 000 Roitt, 1971). The low concentration of IgE in serum is increased in parasitic infections and is responsible for symptoms of atopic allergy. The biological properties of IgD are not yet well defined, but there are preliminary reports of IgD antinuclear-antibodies and of antibodies to BSA (Bovine Serum Albumin) in gut fluids (Roitt, 1971).

The synthesis of antibodies can briefly be summarized as follows (Guyton, 1976): The lymphocytes in normal lymphoid

tissues are distinctly divided into two separate populations, although they look alike when studied under the microscope. The one is responsible for forming the sensitized lymphocytes that provide cellular immunity and the other is for antibody formation providing humoral immunity. These two immunological reactions may occur when an antigen enters the body. With humoral immunity the antibody acts by direct combination with and neutralization of bacterial toxins by coating bacteria to enhance their phagocytosis etc. The production of sensitized lymphocytes, which have the antibody on their surface, are effectors of cell mediated immunity, expressed in such reactions as the rejection of tissue transplants.

Both of these types of lymphocytes are derived originally in the embryo from lymphocytic stem cells in the bone marrow. A number of these descending stem cells migrate to the thymus gland where the sensitized lymphocytes are preprocessed. For that reason they are called T-lymphocytes and are responsible for cellular immunity. Another population of stem cells migrate to an unknown area of the body, possibly the liver or spleen. Gut-associated lymphoid tissue such as the tonsils, appendix and Peyer's patches, have also been included as possible candidates (Roitt, 1971). Bursa-equivalent-lymphocytes were discovered in birds in which this preprocessing occurred in the bursa of Fabricius, a structure not found in mammals. The B-lymphocytes are destined to form antibodies and are responsible for humoral immunity.

The T- and B-lymphocytes tend to be localized in separate parts of the lymphoid tissue. Upon entry of a foreign antigen, the B-lymphocytes enlarge and undergo morphological changes to form lymphoblasts which further differentiate to form plasmablasts, which proliferate and undergo morphological changes to form plasma cells. The mature plasma cell produces $\gamma$-globulin antibodies at an extremely rapid rate. The antibodies are secreted into the lymph and are carried into the circulating blood.

The antibody can act in three different ways to protect the body against invading agents. These are by direct attack on the invader in one of several ways, agglutination, precipitation, neutralization, lysis etc. Secondly the complement system can be activated to destroy the invader and thirdly by activation of the inflammatory reaction that changes the local environment around the invading antigen and in this way probably prevents its action (Guyton,1976;p82).

1.1.2 Lymph

The lymphatic system can be regarded as an accessory route by which fluids can be transported from the interstitial space back into the blood stream, generally via the thoracic duct. Lymphatics were discovered by Asellius in 1627, but have recently been studied more comprehensively with the aid of the electron microscope (Palay & Karlin, 1959b; Fraley & Weiss, 1961; Casley-Smith, 1962, 1964). The lymphatics mainly carry large particles and proteins which are not or cannot be directly absorbed by the blood capillary. (Drinker, 1946; Courtice & Morris, 1955). On the basis of studies using vital dyes, lymphatics are thought to be more permeable to large molecules than blood capillaries as studied by Yoffey and Courtice (1956), Palay and Karlin (1959a) and French et al (1960). It must be realized, however, that this extravascular circulation does not apply to all plasma proteins to the same extent. In general the smaller protein molecules e.g. albumin are present in larger amounts in lymph compared to larger protein molecules (Drinker & Yoffey, 1941; Wasserman & Mayerson, 1952; Korner et al, 1954; Courtice & Morris, 1955; Mayerson, 1963; Jacobsson & Kjellmer, 1964; Schultze & Heremans, 1966; Rusznyak et al, 1967; Reichel, 1970, 1971, 1974; Renkin, 1970; Courtice, 1972). The similarity between the plasma proteins and the lymph proteins of the same animal has also been demonstrated by Perlman et al (1943) and Yoffey and Courtice (1970). Concentration differences mainly depend on the molecular sizes (Mayerson, 1963; Rusznyak et al,

1967; Renkin, 1970; Reichel, 1970, 1971, 1974; Courtice, 1972). Besides the molecular sizes this is also determined by the types of capillaries in the various organs (Reichel et al, 1976). Most of the proteins leaving the vascular system cannot re-enter the system by the same route since the hydrostatic pressure against which it will have to move, is too great.

Lymph collected from various areas differs in albumin content, suggesting that capillaries are only partially permeable to albumin, and that the permeability varies in different areas (Shirley et al, 1957). This does not necessarily signify differential permeability, but may rather indicate the difference in surface area of capillaries actually functioning, but with normal leakage of protein (Wasserman & Mayerson, 1952). The rates at which the plasma proteins penetrate from the vascular compartment to the extravascular compartments, also differ greatly from one tissue to another (Dewey, 1959).

The precise mechanism by which proteins are transferred across the bloodvessel wall to the tissue spaces and ultimately to the lymphatic vessels, is still uncertain (Quin & Shannon, 1977). From recent findings it seems reasonable to conclude that the transcapillary passage of plasma macromoles is essentially based on diffusion and filtration, either occurring through pores and/or leaks by a process involving capillary endothelial cells or by random confluences of micropinocytotic vesicles, fenestrae of cytopempsis (Karnovsky, 1962; Landis & Pappenheimer, 1963; Mayerson, 1963; Winne, 1965; Jennings & Florey, 1967; Garlick & Renkin, 1970; Simionescu et al, 1972). Grotte (1956) indicated that there are two kinds of functional pores in the walls of capillaries and this pore theory is also supported by Shirley et al (1957), Landis and Pappenheimer (1963) and Crone (1974). These two pore systems could be explained by a large number of small pores which more or less completely restrict albumin, and a small number of

larger pores with no selective restriction.

Lymph is generally accepted as interstitial fluid that flows into the lymphatic with no gradient between the two (Drinker & Yoffey, 1941; Courtice & Morris, 1955; Keyl et al, 1965; Bell et al, 1969; Gärtner et al, 1973; Hargens & Zweifach, 1976; Rutili & Arfors, 1977). In recent reports (Casley-Smith & Bolton, 1973; Casley-Smith, 1976) it has been suggested that as a result of lymphatic vessel compression, lymph is ultra-filtered through the endothelium, thus increasing the protein concentration of the lymph, as compared to the interstitial fluid concentration.

All tissues of the body, with the exception of a few, have lymphatic channels. The exceptions are the superficial portions of the skin, the central nervous system, deeper portions of peripheral nerves, the endomysium of muscles and bones (Guyton, 1976).

Although many electron microscopic investigations of blood capillary endothelium have been made (Maynard et al, 1957; Moore & Ruska, 1957), there are only a few recorded studies on lymphatic endothelium (Palay & Karlin, 1959a; French et al, 1960; Papp et al, 1962; Cliff & Nicoll, 1970). The most important features of absorbing lymphatics are a poorly developed or absent basement membrane, and the presence of openings, or gaps, in the endothelium lining of the walls that may be up to 10$\mu m$ across (Palay & Karlin, 1959a & b; French et al, 1960; Casley-Smith & Florey, 1961; Casley-Smith, 1964; Leak & Burke, 1966; Russnyak et al 1969; Cliff & Nicoll, 1970; Leak, 1971; Taylor et al, 1973). Papp et al (1962), however, did not find any example of intercepted endothelial in the collecting lymphatics during their study of lacteals. No gaps have been found or reported in the endothelial layers of the transport channels (Cliff & Nicoll, 1970). Valves have been reported in the transport channels. The structure of the lymphatic valves is extremely delicate being only two thin layers of endothelium separated by a

narrow zone containing fine fibrillar material.

It is also thought that the gaps identified are sites where the lymphatic endothelical cell junctions have opened in a manner of "flap valves" in response to the tissue hydrostatic pressure which is higher than the lymphatic pressure, to permit the passage of materials into the lymphatic lumen (French et al, 1960; Casley-Smith, 1962, 1964; Papp et al, 1962; Leak & Burke, 1966). Any rise in the intra-lymphatic pressure would close the flap valves to prevent the passage of fluid out of the lumen. The functioning of a lymphatic system operating as described, would be basically independent of absolute interstitial fluid pressure as long as a centrally directed pressure gradient exists.

Moreover, Leak and Burke (1968) and Cliff and Nicoll (1970), found anchoring filaments connecting the small lymphatics to the surrounding connective tissue, representing the mechanism responsible for opening the lymphatic capillaries under conditions of increasing tissue pressure as originally proposed by Pullinger and Florey (1935).

Up to a molecular mass of 600 to 2 300 the lymphatic endothelial offers no hindrance to the rapid and free permeation of most substances (Mayerson et al, 1962). In addition, vesicular or phagocytic transport systems have been reported for greater particles (French et al, 1960; Casley-Smith & Florey, 1961; Papp et al, 1962; Rusznyak et al, 1969; Cliff & Nicoll, 1970).

The transport channels contain smooth muscle cells in their wall which are responsible for the propulsion of lymph. These cells are not innervated and this probably indicates a myogenic control (Burnstock & Merrillees, 1964; Cliff & Nicoll, 1970). These intrinsic contractions have been well documented in the guinea pig (Florey, 1927; Pullinger & Florey, 1935; Smith, 1949), in the rat (Webb, 1933; Pullinger & Florey, 1935; Smith, 1949), the mouse, rabbit and dog (Smith, 1949).

It would appear from various reports that a definite normal structure for the lymph nodes is almost an impossible condition (Frey, 1875; Gulland, 1894; Schumacher, 1896; Bunting, 1905; Jolly, 1909, 1910; Lewis, 1909; Clark, 1912; Godbille, 1915; Job, 1915, 1922; Bach & Lewis, 1973). Age, sex, physical condition and the topographical anatomy are a few factors which determine the structure of the nodes. It has been shown by Beh et al (1974), Quin et al (1974) and Quin and Shannon (1977) that the lymph node may contribute 30 to 50% of the protein output observed in the efferent lymph. These workers did their study by using the popliteal node in the thigh. After antigen injection an increase in lymph flow and protein concentration was observed in the efferent lymph from the popliteal node. This increase could not entirely be accounted for by changes in afferent lymph to the node, or by immunoglobulin synthesis at the node (Smith & Morris, 1970; Quin et al,1974; Quin & Lascelles, 1975). These changes are undoubtedly associated with an increase in the vascular supply to the node (Herman et al, 1972; Hobbs & Hay, 1975). It is also suggested that these proteins accompany the continual movement of lymphocytes across the wall of the vessels of the node, as has been shown in the dog (Chapman & Bopp, 1970), the mouse (Schoefl, 1970; Mikata & Niki, 1971; van Deurs et al, 1975) and rat (Nopajaroonsri et al, 1974). It should be considered that all lymph passes through at least one lymph node and that this may be as much as eight to ten (Yoffey & Courtice, 1970). Not only do molecules leave the capillaries in the nodes, but small molecules are also able to diffuse into the blood stream (Mayerson et al, 1962), while macromolecules are retained in the lymph (Mayerson et al, 1962; Casley-Smith, 1973). There are numerous reports on the cannulation of lymphatic vessels in many animals such as the dog (White et al, 1933; Mayerson et al, 1962; Schad & Brechtelsbauer, 1977a, b), cat (Jacobsson & Kjellmer, 1964), rabbit (Lewis & Westcott, 1968; Lewis, 1969; Boyels et al, 1970;Jasani & Lewis, 1971; Lewis & Yates, 1972; Bach & Lewis, 1973; Tibes et al, 1974, 1977), mouse (Friedman, 1957) and even in

the human (Courtice et al, 1951; Engeset et al, 1973, 1977; Olszewski et al, 1977).

Considerable variation in both the rate of lymph flow and the concentration of protein in lymph from various regions of the animal body have been reported in the literature (Drinker & Field, 1931; White et al,1933; Field et al, 1934; Warren & Drinker, 1942; Sugarman et al, 1943; Courtice et al, 1951; Nix et al, 1951; Jacobsson & Kjellmer, 1964; Bach & Lewis, 1973; Engeset et al, 1977; Olszewski et al, 1977). Passive movements (White et al, 1933; Ladd et al, 1952; Jacobsson & Kjellmer, 1964; Tibes et al, 1977), exercise (Ranke, 1865; Barcroft & Kato, 1915; Kjellmer, 1964; Jacobsson & Kjellmer, 1964; Engeset et al, 1977; Olszewski et al, 1977; Schad & Brechtelsbauer, 1977a), high and low temperatures (Lewis & Westcott, 1968; Lewis, 1969; Lewis & Yates, 1972; Bach & Lewis, 1973; Olszewski et al, 1977), nerve stimulation (Lewis & Yates,1972), immunological injury (Jasani & Lewis, 1971), pressure (Vreim et al, 1976b; Olszewski et al, 1977), pain (Jones & Shannon, 1972) etc. have been shown to change the lymphatic flow rate and protein concentration. An overall tendency, although of different degrees, is an inverse relationship between lymphatic flow rate and lymph protein concentration which agrees with Drinker's theory (1933) when capillary permeability is constant (White et al, 1933; Benson et al, 1955; Landis & Pappenheimer, 1963; Courtice & Sabine, 1966; Arthurson et al, 1969; Garlick & Renkin, 1970). No correlation between the lymphatic flow rate and protein concentration was found by Engeset et al (1977), but this could be due to the fact that experimentation was done during the night or first thing in the morning and that time was required to wash the protein-rich lymph formed during rest out of the interstitial space and lymphatic vessels before the period of activity. The accumulation of proteins in lymph at night might have played a role here.

Most studies on lymphatics have been performed in animals which were anaesthetised. This may result in significant alteration in thoracic duct lymph composition (Cain et al, 1947; Browse et al, 1974). Other studies were conducted in conscious animals with thoracic duct fistulae in which there is the disadvantage of continuous loss of fluid and protein (Brown & Hardenbergh, 1951; Doemling & Steggerda, 1960; Nelson & Swan, 1969; Giradet & Benninghoff, 1973; Schad & Brechtelsbauer, 1977a). There still remains a paucity of information regarding the influence of anaesthesia on both concentration and flow rate in the lymphatics (Polderman et al, 1943; Quin & Shannon, 1975).

The lymphatic flow would be expected to be higher in conscious than anaesthetised animals because of the action of the "tissue pump" (Allen & Vogt, 1937; Polderman et al, 1943; Casley-Smith, 1967; Leak & Burke, 1968; Quin & Shannon, 1975; Schad & Brechtelsbauer, 1977b) and because anaesthesia is likely to block the intrinsic pumping mechanism of the lymphatic vessels (Hortsmann, 1959; Zweifach & Prather, 1975; Guyton et al, 1975). A third possibility could be because of circulatory changes, such as decreased net capillary filtration (Guyton, 1965; Gibson & Gaar, 1970; Guyton et al, 1976). Lymphatic flow could also be reduced by the re-uptake of fluid from the interstitial spaces into the vascular compartment (Quin & Shannon, 1975). Authors who also found lymphatic flow exceeding the reported values for anaesthetised animals by 50% are Watkins and Fulton (1938), Shafiroff et al (1943), Furneaux and Ham (1970), Pilon and Bittar (1973) and Schad and Brechtelsbauer (1977a).

It could be expected that the lymphatic protein concentration may be lower and the albumin/globulin ratio higher in conscious as compared to anaesthetised animals because of the reduced blood flow during anaesthesia(Yoffey & Courtice, 1956; Rusznyak et al, 1969; Schad & Brechtelsbauer, 1977a).

Thus it seems that the suppression of lymphatic return of fluid and protein by anaesthetics is mainly due to immobilization of the animal and that passive movement will enhance lymphatic flow significantly as reported by White et al (1933), McCarrell (1939), Yoffey and Courtice (1956), Rusznyak et al (1969) and Schad and Brechtelsbauer (1977b). This could also mean a reduction of the extravascular pool of interstitial fluid.

### 1.1.3 Interstitial Fluid

It is at the capillaries that the main function of the circulation occurs, namely, interchange of nutritients, respiratory gases and cellular excreta between the tissues and the circulating blood. The space between the capillaries and tissues is called the interstitial fluid space and is filled with both a liquid and a gel medium. By far the most important means by which substances are transferred between the plasma and interstitial fluid (IF) is by diffusion (Guyton, 1976). There is no evidence that active transport is involved in the exchanges across the membrane, enabling us to consider IF as a transcellular fluid (Fulton, 1955; Bland, 1963).

Interstitial fluid composition is partly determined by local metabolism. Different regions may have different IF compositions depending on their own tissue metabolic needs and the type of metabolic discharge from their cells (Luciani, 1901; Cannon, 1927; Winton & Bayliss, 1955; Bland, 1963).

Interstitial fluid is composed of both a free fluid and fluid in a gel state (Clark & Clark, 1933; Gersh & Catchpole, 1960; Guyton et al, 1975). Most work on IF was done to measure pressure of the fluid. Two entirely separate ranges of values have been obtained. One range being a slightly positive pressure (McMaster, 1946; Kjellmer, 1964; Wiederhielm & Weston, 1973) and the other being moderately negative (Guyton, 1963; Anas et al, 1968; Hopkinson et al, 1968; Scholander et al, 1968; Ladegaard-Pedersen, 1970; Stromberg & Wiederhielm, 1970). The positive pressures have

recently been identified as total tissue pressure, while the negative values have been identified as interstitial fluid pressure (Guyton et al, 1975).

Different methods have been used for determining interstitial fluid pressure. Direct measurements have been performed by the implantation of perforated capsules which accumulate fluid, by a wick method, a skin capsule method and a few other related methods. These methods have also been used for interstitial fluid analysis e.g. pH, $Po_2$, $Pco_2$, ionic values, proteins etc.

The wick method has been developed for studying interstitial protein concentration and has been described by Scholander et al(1968), Stromme et al (1969), Poulsen (1973), Aucland and Fadnes (1973), and Aukland and Johansen (1974). The method described by Aukland and Fadnes (1973) is a 3cm long and 0,5mm thick nylon thread which is sewn into subcutaneous tissue and left for 35 to 240 minutes. The middle part is quickly transferred to a test-tube containing 2ml saline and then weighed. Total protein, albumin and haemoglobin concentrations were measured in the eluate.

Local tissue fluid (extracellular fluid) within the subcutaneous tissues has been sampled with a liquid paraffin cavity method described by Haljamäe (1970). This is done by making a 2 to 3cm incision through the epidermal and dermal layers under a layer of liquid paraffin. The edges of the incision are immediately widely separated. The incision is kept covered with paraffin to avoid evaporation. Micropipettes are used for the sampling of fluid. Both the wick and liquid paraffin methods involve incisions through the skin and the problems of intracellular fluid escaping at the incision, haemorrhage and inflammation have not been taken into account when doing protein concentrations. According to Aukland and Fadnes (1973) the possibility of too high values which might be the result of the insertion of the wicks which may produce bleeding or local inflammation with increased capillary permeability to pro-

teins is not applicable in their study which they have proved by doing a haemoglobin test. Haljamäe et al (1974) concluded that the compositional and functional characteristics of the directly sampled fluid fit the requirements of a hypothesized true IF far better than does capsular fluid.

A similar type of method is the skin capsule (Guyton, 1965). In using this procedure the skin is sucked up into a cup by a temporary vacuum. An adhesive material, previously applied to the cup, then holds the skin in this position for 24 or more hours. After this period free fluid is found in the section of tissue that has been held up in the cup. A curved syringe needle inserted through the skin, curves upward into the cupped area. Results obtained according to this method are not as constant as those measured with the perforated capsule. A possible explanation for these erratic results could be the compression of the surrounding tissue by the edges of the cup. Equilibrium time for the cup method is also much longer than that required for the capsular technique (Guyton et al, 1975).

The perforated capsule technique has been used most widely and employs a perforated capsule implanted in a tissue (Guyton, 1963; Floyer, 1969; Libermann et al, 1972; Lucas & Floyer, 1973; Haljamäe et al, 1974). Following implantation, fibrous tissue and new vessels grow into the capsule through the perforations. After 12 days, the entire wall of the cavity is lined and the lining is approximately 1 to 2mm in thickness. Thus the fluid inside the cavity is not in contact with the capsule itself, but instead it is a true tissue space (Guyton, 1963; Jones et al, 1969). It was shown that the intracapsular fluid is in dynamic equilibrium with the extracapsular IF (Guyton, 1963; Meyer et al, 1968; Guyton et al, 1971).

Guyton (1963) showed that four weeks is the optimal period of time for signs of inflammation to disappear and air from the capsule to be absorbed and replaced by IF.

Besides inflammation, which is the most difficult problem to prevent, it has been suggested that the capsule might develop a membrane which is impermeable to protein, and that this might cause an osmotic effect at the membrane thus creating a pressure in the capsule which is not a true measure of IF pressure. Guyton (1963) contradicted this possibility by using a dye (T-1824) which attaches to the proteins.

In general the concentration of the protein in the IF is about 20 g/l in most tissues of the body, as high as 60 g/l in the liver (Rusznyak et al, 1967) and as low as 0,25 g/l in cerebrospinal fluid (Davson, 1960). Interstitial fluid protein concentration has been measured at 25g/l for rabbits (Haljamäe et al, 1974), 37 g/l for rabbits (Stromberg & Wiederhielm, 1976), 19,6 g/l in dogs (Guyton, 1963), 24 g/l and 34,4 g/l in rats (Libermann et al, 1972; Aukland & Fadnes, 1973) respectively.

Up to now the perforated capsule technique has been the most widely accepted method for the sampling of interstitial fluid.

### 1.1.4 Cerebrospinal Fluid

Cerebrospinal fluid (CSF) is the fluid found in the ventricles of the brain, in the cisterns around the brain, and in the subarachnoid space around both the brain and the spinal cord. All these chambers are connected with one another (Guyton, 1976). In certain regions, notably the cisterna cerebello medullares (cisterna magna), the space is exceptionally large, and this constitutes a useful sampling locus for studies on experimental animals and man. The main function of CSF is to cushion the brain within its solid vault. The determination of total protein content is one of the most useful diagnostic laboratory procedures for the neurologist (Merritt & Fremont-Smith, 1937; Kirk et al, 1974).

The CSF within the ventricle is formed continuously by choroid plexuses and originates from blood plasma (Merritt & Fremont-Smith, 1937). The choroid plexuses are protrutions of the vascular system into the ventricle, covered with ependyma which has become morphologically and functionally differentiated from its neighbouring regions and is called the choroidal epithelium (Davson, 1976). Although the choroidal capillaries have been shown to be highly permeable to large molecules, such as iodinated serum albumins, the epithelial layers are sealed laterally to form tight junctions (Reese & Karnowsky, 1967; Brightman & Reese, 1969; Burgess & Segal, 1970; Carley & Maxwell, 1970; Cserr et al, 1972) and it is this morphological feature that constitutes the barrier to the passage of dissolved material from blood to CSF. Ever since Ehrlich's early observation that cerulin-s, which freely stains most body tissue, does not seem to penetrate the brain, there has been a growing body of evidence that there is a great selectivity regarding the entry of substances from blood into brain tissue.

Obviously CSF is then not a simple filtrate from the capillaries, but a choroid secretion. A functional leak from fenestrated capillaries at the root of the choroid plexus has been described as a pathway to bypass the barrier (Brightman & Reese, 1969), as well as a widespread transfer across the arterioles (Westergaard & Brightman, 1973). Active and carrier-mediated transport (Eidelberg et al, 1967; Davson, 1970b; Bito & Myers, 1970; Katzman & Pappius, 1973; Davson & Hollingsworth, 1973), molecular polarity (Crone, 1960) and neurochemical alteration of entering molecules at the endothelial level (Bertler et al, 1964; Bjorklund et al, 1969) are among many factors which are components of the barrier system as well as certain arteriolar endotheliums capable of vesicular transport (Westergaard & Brightman, 1973; Amtorp & Sorensen, 1974).

Cerebrospinal fluid is not exactly like other extracellular

fluids, since the sodium concentration is 7% greater than the concentration in other extracellular fluid, while both the glucose and potassium levels are much decreased in CSF (Guyton, 1976). The total protein content of CSF has been found to vary between 0,15 and 0,65 g/l (Denis & Ayer, 1920; Marron, 1941; Abelin, 1943; Pappenheimer et al, 1962; Klatzo et al, 1964; Kirk et al, 1974). Much higher protein concentrations in CSF have also been reported by Kabat et al (1942) and Lamoureux et al (1975). They reported values between 2,7 and 3,5 g/l in normal adults. High CSF protein values are commonly observed in newborns (Levinson, 1950; Davson, 1970a) and in subjects of advanced age, as reported by Schultze and Heremans (1966).

It has been noticed that the electrophoretic protein pattern of CSF is somewhat different from that of lumber CSF e.g., the high proportion of prealbumin in ventricular CSF (Schultze & Heremans, 1966). The plasma proteins in CSF are in proportion to their concentration in serum and in inverse proportion to their molecular mass (Schultze & Heremans, 1966; Laurell, 1972). Plasma proteins of high molecular mass are almost completely absent in CSF (Kabat et al, 1942; Rosenthal & Soothill, 1962). According to Kabat et al (1942) the mobilities of the CSF components correspond closely to those of blood. The prealbumin band is barely discernible on plasma electrophoresis, but in concentrated CSF this component normally produces a strong band with a slightly higher migration rate than in plasma (Laurell, 1972). It has been suggested that tryptophan-rich prealbumin and transferrin may be produced in normal CSF to some extent (Parker & Bearn, 1962; Schultze & Heremans, 1966).

Antisera raised against normal human plasma proteins, demonstrate that many of the CSF proteins are identical with those in plasma (Schultze & Heremans, 1966). Fifteen plasma proteins were determined in CSF by means of rocket immunoelectrophoresis (Bock, 1973a).

Several authors have found that there are at least 9 or 10 antigenic constituents in CSF that are not demonstrable in normal unconcentrated plasma (Clausen, 1961; MacPherson & Cosgrove, 1961; Denker & Swahn, 1962; Laterre & Heremans, 1963; Laterre et al, 1964; Link, 1967; Bergman et al, 1968). These antigens were studied by various quantitative immunological techniques and originate predominantly from brain tissue (Bock, 1973b).

A fluid formed continuously must be drained away and therefore almost all the CSF formed is reabsorbed into the blood through special structures called arachnoidal villi (Shabo & Maxwell, 1968a, b; Alksne & Lovings, 1972; Tripathi & Tripathi, 1974; Guyton, 1976). This system has unidirectional valves resulting in passive outflow (Welch & Friedman, 1960). Thus these projections may be compared to a lymphatic drainage, large enough to permit a relative unrestricted flow of fluid and solutes of large molecular weights (Davson et al, 1970, 1973).

### 1.1.5 Aqueous Humor

Aqueous humor is the part of the eye fluid which lies in front and to the sides of the lens. It is continually being formed and reabsorbed. For many years aqueous humor was considered to be a secretion of the ciliary process (Duke-Elder, 1927), based on a simple filtration process dependent on hydrostatic pressure differences (Leber, 1903; Henderson & Starling, 1904, 1905). Today the capillary pressure is considered to play a minor role in the formation and circulation of aqueous humor, as already reported by Langham (1958).

The formation of aqueous humor by the ciliary processes is much the same as that of CSF by the choroid plexus (Guyton, 1976). The anatomical and histological features of the system through which aqueous humor circulates, is fairly well understood (Dvorak-Theobald, 1934, 1955; Friedenwald, 1936; Ashton, 1951, 1952; Ashton & Smith, 1953; Carpenter, 1954;

Flocks, 1956; Ashton et al, 1956). Numerous reviews on the permeability of the blood-aqueous humor barrier have been published (Duke-Elder and Davson, 1948; Friedenwald, 1949; Adler, 1950; Kinsey, 1950; Davson, 1953; Stjernschantz et al, 1973; Neupert and Lawrence, 1970; Schmut and Zirm, 1974). The possibility of a parasympathetic nervous control for the blood aqueous barrier has been reported by Uusitalo et al (1974).

The most striking difference between the chemical composition of aqueous humor and plasma is in the concentration of the proteins. Both in man and laboratory animals such as rabbits, the protein concentration is less than 1% of that in plasma (Kinsey, 1951; Langham, 1958; Adler, 1959; Kuhlman & Kaufman, 1960; Anjou & Krakay, 1961; Krause & Raunio, 1969; Takase, 1970; Dernouchamps et al, 1974; Schmut & Zirm, 1975 etc.). Higher protein concentrations have been reported in rats by Davson (1956) and Stjernschantz et al (1973), and in humans by Hemmingsen and Other (1967). It should be borne in mind that the latter two authors collected samples from post mortem specimens. Francois and Rabaey (1960) found that the composition of aqueous humor changes very rapidly after death. As in the case of CSF, a high albumin/globulin ratio for aqueous humor has been reported as a result of the blood-aqueous barrier which restricts the movement of certain higher molecular mass proteins (Francois et al, 1958; Francois & Rabaey, 1960; Krause & Raunio, 1969; Neupert & Lawrence, 1970; Stjernschantz et al, 1973). Several authors have reported albumin/globulin ratios for normal aqueous humor to be similar to those of the respective sera (Moore, 1945; Svensson, 1946; Sallman & Moore, 1948).

Apart from a few results (Hagen, 1920; Löwenstein, 1920), most authors working on paracentesis of the eye, found an increase in total protein concentration and a decrease in albumin/globulin ratio levels of the aqueous humor until they equalled that of serum levels (Dieter, 1925; Kronfeld et al, 1941; Adler, 1965; Yangaizawa, 1965; Neupert & Lawrence, 1970).

In the eye there is no separate system of lymphatic vessels (Eisler, 1930; Duke-Elder, 1961) which has raised much speculation as to the drainage routes for proteins of the intraocular fluid. Schlemm's canal at the circumferential annular space of the human eye which lies within the sclera, has been described by Langham (1958). A similar but not identical drainage system in the form of an irregular series of collecting vessels, which borders the trabecular meshwork, has been noticed in animals (Troncoso & Castroviejo, 1936; Troncoso, 1942; Greaves & Perkins, 1951).

## 1.2 ELECTROPHORETIC METHODS FOR THE STUDY OF PROTEINS

In this study electrophoresis was the method of choice for the study of proteins in body fluids. Electrophoresis is the movement of ions through a medium under the influence of an electric field (Michaelis, 1909). A wide variety of supporting media have been used. The passage of fluids through the pores of a gel may be due to hydrostatic pressure, electroendosmosis, or a combination of both of these effects. Fortunately, however, with the generally used acrylamide gels, electroendosmosis does not occur, or is so slow compared to the mobility of the proteins, that it may be ignored (Gordon, 1975).

### 1.2.1 Polyacrylamide Gel Electrophoresis

Polyacrylamide gel electrophoresis (PAGE), also used in this study, provides a versatile high resolution method for fractionation and physico-chemical characterization of molecules on the basis of size, conformation and net charge (Raymond & Weintraub, 1959; Davis, 1964; Ornstein, 1964; Narayan *et al*, 1964). Since 1968, electrophoresis using acrylamide gels has become one of the most commonly used techniques in all laboratories concerned with proteins, enzymes and nucleic acids (Gordon, 1975). Although the apparatus required to carry out electrophoresis is relatively simple, the time required for gel preparation according to

the method of Davis (1964) is considerable (Gordon, 1975). This is due to the use of three separate gel layers each containing a different buffer. This method was simplified by Hjertén et al (1965) by the introduction of a single homogeneous gel containing buffer of the same molarity and pH.

The name of "disc electrophoresis" was given to this method because of the extreme sharpness of the protein zones separated on the gels (Gordon, 1975). Originally, PAGE was carried out at either very high pH (Raymond & Weinstraub, 1959; Ornstein, 1964), or very low pH (Potts et al, 1967). This ensured that all proteins moved in the same direction, but minimized differences in net charge and reduced efficiency of charge fractionation (Hendrick & Smith, 1968). The pH in the region of the gel in which separation occurs should be such that the maximum difference of mobility exists between the different molecular species undergoing separation (Phelps & Putnam, 1960). For this reason a pH of 8,6 is generally used. Above pH 8,6 denaturation may be found to occur. The ionic strength is usually set at 0,1M (sometimes 0,05M) because at higher electrolytic concentrations the conductivity of the buffer would cause the development of an undesirable amount of heat through the Joule effect and also an inconveniently slow migration rate of the proteins. At lower concentrations the buffering capacity would become too slow (Schultze & Heremans, 1966).

The gels are formed by polymerizing acrylamide monomers in the presence of methylenebisacrylamide, which acts as a cross-linking agent. By adjusting the total acrylamide concentration, the average pore radius of the polymers can be varied between 0,5 and 3nm (Bishop et al, 1967). The work of Fawcett and Morris (1966) on the effect of varying concentration of bisacrylamide in gels from 6,5 to 20% total acrylamide concentration, provides considerable data in this regard. It is this pore size variability which is mainly responsible for the versatility of PAGE.

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