CHAPTER I

INTRODUCTION

The Reticuloendothelial System (R.E.S.) is a major host defense system participating in the vascular clearance and intracellular degradation of blood-borne foreign and effete autologous particulate matter (Saba, 1970).

The Reticuloendothelial System (R.E.S.) as described by Aschoff is composed of various groups of mesenchymal cells which are widely scattered throughout the body which are characterized by their common ability to engulf particulate substances and colloidal suspensions. Their unity as a cellular system has therefore a physiologic property rather than a morphologic basis. Indeed the component cells of the system show considerable cytologic heterogeneity.

Aschoff's concept will suffice to say, however, that not all endothelial and reticular cells are phagocytic and that the term "macrophage system" may be more appropriate. The slightly modified representation of Aschoff's concept of the R.E.S. (Aschoff, 1924) is shown in Fig. 1. As indicated, the system includes fixed and wandering "macrophages", and excludes the polymorphonuclear leukocyte or so called "microphages". Of specific interest in this study, will be the fixed macrophages localized in the blood sinuses of the liver, lung, spleen, and bone marrow, since it is these cells that participate to a major degree in the clearance of particulate

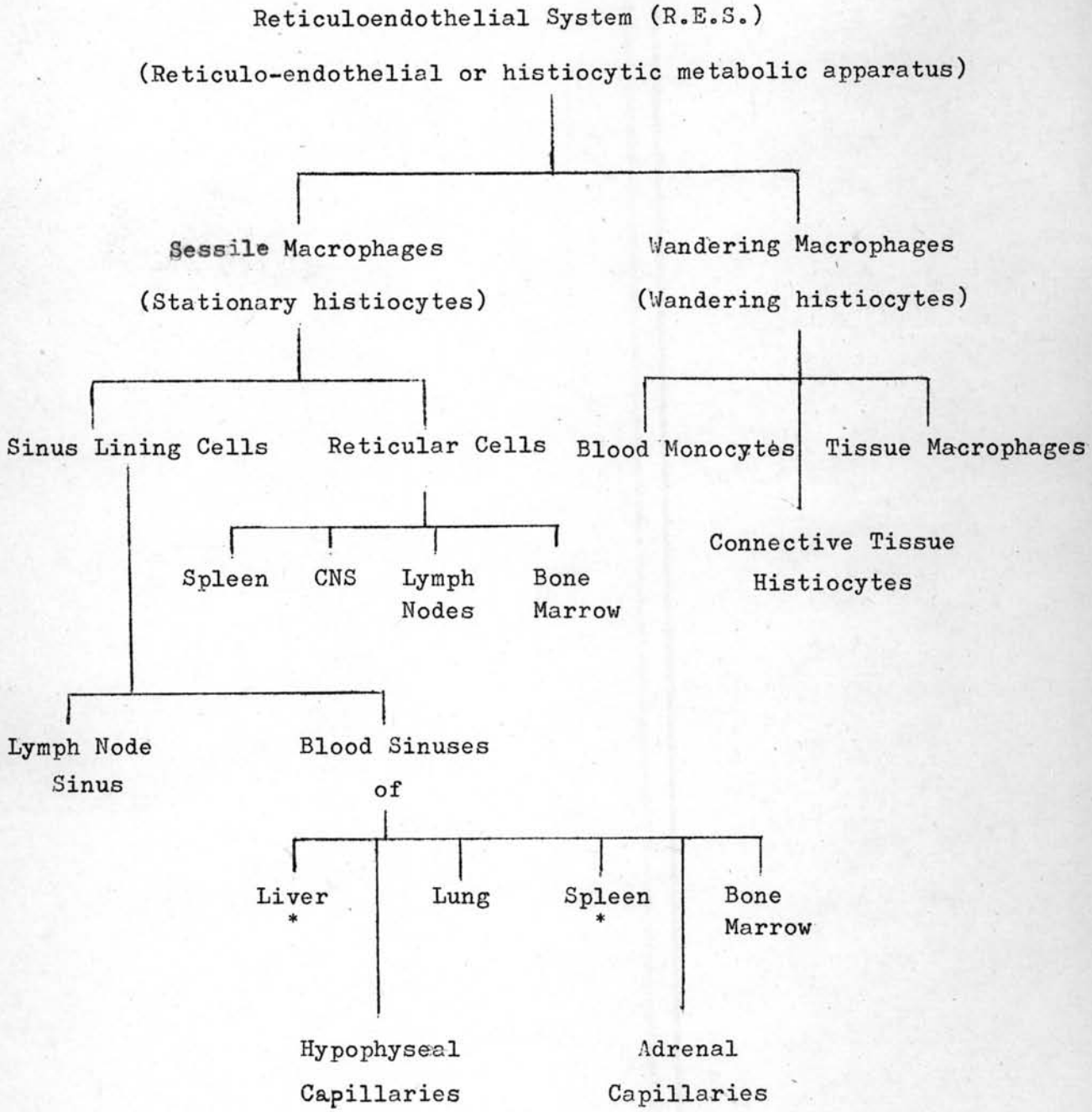

Fig. 1 Slightly modified version of Aschoff's concept of R.E.S. Liver and spleen (*) are major organs participating in clearance of particulate matter from blood.

matter from the blood (Benacerraf, Biozzi, Halpern, et al, 1957; Dobson and Jones, 1952). The majority of cells in this group are located in the sinusoids of the liver (Kupffer cells 80-90 per cent) and in the red pulp of the spleen (5-15 per cent). The remainder (about 5 per cent) is found in the bone marrow, adrenals, pituitary gland and pancreas. Following the formulation of the concept of the R.E.S. as a discrete functional system, interest developed concerning the potential role of the R.E.S. in a variety of physiological and pathological processes (Jaffé, 1931).

These R.E. cells are concerned with both defense against micro-organisms invading the blood and with several normal physiologic activities of the liver and the spleen. The role of the R.E.S. is well established in the destruction of the effete or damaged erythrocytes (Miescher, 1957; Mollison, 1959) and in the metabolism of iron (Vannotti, 1957), cholesterol (Friedman, 1954; Roseman, 1960; Neveu, 1956), lipid (Biozzi, Stiffel and Mouton, 1963; Di Luzio and Riggi, 1964), proteins (Benacerraf, Halpern, et al, 1955; Thorbecke, Maurer and Benacerraf, 1960), bile pigments (Durmont, Stertzer and Mulholand , 1962) and hormones (Berliner, Nabors and Dougherty, 1964). The Kupffer cells play an important role in the complex metabolic activity of the liver in close relationship with parenchyma cells. The macrophages of the spleen probably also participate in the immunologic activity characteristic of this organ by phagocytizing antigens and then transmitting the "antigenic information" to adjacent lymphoid cells which are thereby capable of initiating antibody synthesis (Biozzi, Stiffel, Halpern

and Mouton, 1960; Fishman, Hamilton and Bond, 1963). These R.E. cells actively phagocytize particulate substances which come into contact with them via the circulating blood. A first distinction must be made between the phagocytosis of simple colloidal suspensions such as carbon and complex particle such as bacteria and red cells. As a general rule, serum opsonins play a decisive role in the phagocytosis of most bacteria and red cells (Benacerraf, Sebestyen and Schlossman, 1959; Biozzi et al, 1961; Halpern et al, 1957; Wardlaw and Howard, 1959). On the contrary, simple colloids are phagocytized by the R.E.S. without the intervention of serum opsonins acting as a rate-limiting factor (Biozzi et al, 1963; Biozzi and Stiffel, 1963).

The principle of measuring phagocytic function of the R.E.S. is based on the kinetics of clearance from the blood stream of a colloidal suspension injected intravenously. The suspensions used for this purpose must have the following properties.

(1) The colloidal particles should be exclusively phagocytized by R.E. macrophages lining the blood vessels.

(2) The size of the particles should be large enough to prevent them from crossing the capillary wall or leaving the circulation by ways other than phagocytosis.

(3) The particle should be homogeneous in size, perfectly stable in the circulation and not toxic either for macrophages or for the whole organism.

(4) The colloidal particles should be accurately measurable

in the blood and in the tissue by chemical or physical methods.

(5) The test particles should not be taken up by cells other than those investigated. That is they should be specifically cleared from the blood by the R.E.S.

Consequently the rate of phagocytosis of appropriate dose of simple colloids affords a direct measurement of the phagocytic activity of the R.E. cells. The study of phagocytosis of simple colloids could give useful information about the phagocytic and metabolic function of R.E. cells of the liver and spleen. The kinetics of clearance from the blood of simple colloids can also be applied to the measurement of liver blood flow (Dobson and Jones, 1952; Benacerraf et al, 1957).

It has been well established that an estimate of the phagocytic activity of the reticuloendothelial system can yield useful information in the investigation and management of a variety of pathologic states (Halpern, 1959; Biozzi et al, 1958).

Many different colloids including carbon (Halpern et al, 1953; Biozzi et al, 1953), saccharated iron oxide (Benacerraf et al, 1954), gold (Halpern et al, 1958) and heated-aggregated serum protein labelled with ^{131}I (Benacerraf et al, 1955; Benacerraf et al, 1957; Halpern et al, 1958) have been employed. Furthermore, the dynamics of phagocytosis is the same for all these colloids in the animal species so far investigated, i.e., mouse (Miescher, 1957), rat (Biozzi et al, 1953), guinea-pig (Stiffel et al, 1954), rabbit (Halpern et al, 1954), chicken (Stiffel et al, 1954), dog and man (Halpern et al, 1956; Biozzi et al, 1958). It is interesting

to note that for human, only investigations using the aggregated serum albumin has been used.

The characteristics of an ideal test agent for this determination are quite specific, for example, the material is not only necessary to be safe from an immunologic and microbiologic standpoint, but also have to be uniform and predictable in the plasma. Heated denatured human serum albumin, labelled with ^{131}I (^{131}I-AA) has been shown to be a suitable agent for this purpose. It is phagocytized very efficiently by the R.E. cell when injected intravenously, and because of its lack of toxicity, its stability in the blood and absence of antigenicity in the homologous animal species, it can be injected in large and small doses to explore both the phagocytic activity of the R.E.S. and to measure liver blood flow in laboratory animals and in man (Breiner, 1968; Benacerraf et al, 1957; Halpern et al, 1956).

The carbon particles can not be metabolized by the R.E. phagocytes and persist in the organs while the serum albumin labelled with ^{131}I is rapidly catabolized by enzymes within the phagocytes. As a result of this intracellular digestion, the radioactivity is liberated from the R.E. cells, returns to the circulation and is eliminated in the urine (Biozzi et al, 1958). The percentage of particles fixed in the spleen increases with the dose of colloid injected, while the reverse is true for the liver. This phenomenon is due to the difference in blood flow of these two organs which is related to their different size and vascularization.

The excellent studies by Benacerraf et al, Biozzi et al, Dobson and Jones, and Biozzi and Stiffel, showed that approximately 85 to 95 per cent of the injected colloidal particles will become localized in the hepatic and splenic macrophages, while the remainder becomes localized primarily in the lungs and bone marrow.

Studies on the chemical control of the phagocytic function of the R.E.S. have been developed very fast in the past few years. It is possible to experimentally stimulate or depress the phagocytic activity of the R.E.S. For example, glucan (Riggi and Di Luzio, 1961), zymogen (Heller, 1960), estrogen (Nicol and Bilbey, 1960), endotoxin (Benacerraf and Sebestyen, 1957), BCG (Bacellus Calmette Guerin) (Halpern, Biozzi, Stiffel, et al, 1959) and others are potent R.E. stimulants; while methyl palmitate (Blickens and Di Luzio, 1965; Di Luzio and Wooles, 1964), ethyl palmitate (Stuart and Davidson, 1964; Stuart, Biozzi, Stiffel, et al, 1960), cortisone (Weiner, Magaretten and Spiro, 1963)and antilymphocyte serum (A.L.S.) (Pisano, Patterson and Di Luzio, 1969; Grogan, 1969) are potent R.E. depressants. While the mode of stimulation and/or depression of the R.E.S. with the most of this agents remains to be determined, it is now apparent that stimulation or depression of the R.E.S. may have a humoral or cellular basis (Saba and Di Luzio, 1968). The ability to identify specific compounds and utilize them in inducing a state of R.E. hyperphagocytosis or hypophagocytosis is most definitively demonstrated by the findings of Di Luzio and co-workers on glucan-induced R.E. stimulation (Riggi and Di Luzio, 1961) and methyl palmitate-induced R.E. depression (Blickens and Di Luzio,1965).

The potential value of specific R.E. depressant and R.E. stimulation compounds is suggested by the observations that chemically induced hyperphagocytosis will significantly increase resistance to tumor growth and experimental infection (Dobson and Jones, 1952). While hypophagocytosis can delay renal homograft rejection (Kauffman, Humphrey, Hanback, et al, 1967) and depress the immune response (Di Luzio and Wooles, 1964).

A variety of simple lipids has been studied for their depressing on enhancing effect on R.E.S. phagocytosis. In 1960, Stuart has shown that an intravenous injection of a colloidal solution of ethyl palmitate [$CH_3(CH_2)_4COOC_2H_5$, Mol.Wt. 284.84] caused necrosis of spleen and suppression of the phagocytic function in mice (Stuart, 1960). These results were confirmed and further experiments have shown that an injection of ethyl palmitate caused prolonged survival of human rod cell in mice (Buchanan and Mc Gregor, 1964).

These findings are of considerable importance because they open up the possibility of treating certain haemolytic syndromes where the increased red cells destruction appears to be "hyperactivity" of the R.E.S. The objective of the present studies is to ascertain the liver blood flow and the phagocytic activity of the R.E.S. in rhesus monkeys treated with ethyl palmitate.