CHAPTER V

DISCUSSION

In using the term R.E.S. in the present studies, it did not imply any specific type of mesenchymal cells but was defined here as all factors participating in the phagocytosis of the ^{131}I-AA.

In the present studies, ^{51}Cr-labelled human red blood cells were given intravascularly to the normal and the ethpalm monkeys for studying the erythrophagocytosis. These labelled red cells were probably removed from the circulation by two mechanisms i.e. intravascular haemolysis and erythrophagocytosis by the reticuloendothelial system especially by the liver and spleen. Since only about 11 per cent of the radioactivity of the dose given was found in the plasma at 180 minutes after injection, therefore only a small part of the ^{51}Cr-labelled human red cell destruction seems to have occurred intravascularly in these monkeys. These findings were similar to the results obtained from mice injected with pigeon red cells (Benacerraf et al., 1957) and from dogs injected with human red cells (Areekul et al., 1973). The pigeon red cells were found to be phagocytized by the R.E.S., usually 90% being removed in the liver and spleen according to the same law that governs the phagocytosis of foreign particles e.g. carbon and aggregated human serum albumin.

The mean value of the half-disappearance time (T½) of ^{51}Cr

labelled human red cells in ethpalm monkeys were found to be longer than those of the normal monkeys. These findings were in accordance with results reported earier in mice treated with ethyl palmitate as measured by the clearance of ^{51}Cr-labelled human red cells (Stuart, 1960; Buchanan and Mc Gregor, 1964), and those in dogs treated with ethyl palmitate as measured by the clearance of ^{51}Cr-labelled human red cells (Areekul et al., 1973). These indicated that there was a suppression of the functional activity in all R.E. cells which were in contact with the blood stream, i.e. the phagocytic activity in both the liver and the spleen must be depressed by the ethyl palmitate. Since the liver has a higher phagocytic activity than the other organs of the R.E.S., it seemed likely that the depressing effect was exerted mostly on the liver. This impression was confirmed by the finding of decreased liver blood flow and by histological sections (See discussion below).

Since the labelled aggregated human serum albumin has been shown to be phagocytized by the R.E. cells lining the blood vessels particuarly by the Kupffer cells of the liver or the reticular cells of the spleen, and was metabolized immediately as shown by the prompt reappearance of the radioactive free iodine in the blood (Benacerraf et al., 1957; Halpern et al., 1954; Benacerraf et al., 1955). Therefore it is possible to administer large quantities of this material to study the phagocytic capacity of the R.E.S. which could not be obtained by measurement of the clearance of minute quantities of nonmetabolized meterials.

It has been shown in the previous reports that the blood clearance of ^{131}I-AA followed an exponential function of the time and its rate approached a limiting rate as the dose increased. This limiting rate is the maximal rate at which the R.E.S. could phagocytize the ^{131}I-AA, regardless of the dose given. In other words, as the number of administered ^{131}I-AA was increased, a point was reached at which the product of the dose and the fractional clearance rate became constant. This characteristic of ^{131}I-AA was observed in normal rhesus monkeys, rats and in other experimental animals studied with colloidal carbon and ^{131}I-AA (Areekul et al., 1973; Biozzi and stiffel, 1965). The dose of 5.0 mg/Kg which was much higher than the critical dose of 1.61 mg/Kg (Areekul et al., 1973) was therefore administered to study the phagocytic activity of the R.E.S. in normal rhesus monkeys in the present studies. The mean value of the phagocytic index, K, of 10 normal rhesus monkeys was found to be 0.056 min^{-1} (range 0.036-0.082).

It has been shown that when subcritical doses of colloid are injected, the concentration reaching the liver through the portal vein was so small that all the colloid was cleared in a single passage (Dobson and Jones, 1952; Halpern et al., 1956). Therefore the colloidal concentration in the blood of the hepatic vein should be zero. In fact, the injection of colloid directly into the portal system followed by catheterization of the hepatic vein has shown the presence of very little concentration of colloid in different animal species (Benacerraf, et al., 1957; Halpern et al., 1958).

The measured constant K_e (K_{max} expressed as natural logarithms) will represent the fraction of the total blood volume which is cleared of colloid during this time i.e. corresponds to the sinusoidal liver blood flow, this value estimated from the ^{131}I-AA 0.03 mg/Kg in 9 normal rhesus monkeys was found to be 107.2 ml/min or 1.00 ml/min/gm of liver weight.

Sinusoidal liver blood flow decreased considerally in monkeys received 10%, 15% and 20% ethyl palmitate. This was probably due to the congestion and hydropic degeneration of the liver cells and the several degenerative changes at portal area and periphery central vein as shown in the histological examination.

The phagocytic index which was an overall measurement of the phagocytic activity of the R.E.S. showed no significant difference from the values of the normal monkeys.

Microscopic examination of the liver in monkeys receiving 10-20% ethyl palmitate emulsion shows the degenerative change i.e. cloudy swelling, hydropic degeneration and fatty metamorphosis of the liver. The histological section of the spleen showed no significantly pathological lesion. It has been shown that ethyl palmitate induced specific destruction of the spleen in mice, rats, guinea-pigs and rabbits and caused no pathological changes in the liver (Stuart, 1960; Buchanan and Mc Gregor, 1964; Prosnitz et al., 1969). This discrepancy could be due to, (1) the particle size, amount and the interval between the dose of ethyl palmitate administered and (2) the different strains and species of animals,

the previous experimental animal studies were on mice, rats, guinea-pig and rabbits (Prosnitz et al., 1969).

The effect of ethyl palmitate on the functional activity of the reticuloendothelial system (R.E.S.) have been studied in various animal species. Ethyl palmitatedepressed reticuloendothelial phagocytic function as measured by clearance of colliod carbon particles (Stuart, 1960). The removal of homologous red blood cells from the circulation, also largely dependent on the phagocytic function of the R.E.S., is inhibited by ethyl palmitate. In one study the survival time of human red cells in the circulation of mice was prolonged from 50 minutes ($T_{½}$) to over 400 minutes by pretreatment of the animals with ethyl palmitate (Buchanan and Mc Gregor, 1964).

The distribution and the fate of ethyl palmitate in the body of various animal species are not well understood. The physicochemical properties of this compound prevent its passage through the capillary barrier and therefore inhibit entry to lymph gland and thymus. There was also evidence that the liver phagocytes were affected because of the low values of the phagocytic index in mice, but no significantly pathological lesion was observed, presumably due to efficient detoxification by the parenchyma (Stuart, 1960). It has been suggested also that the mechanism of action is probably related to a critical concentration of ethyl palmitate within the phagocytes which is followed by cell damage and release of cytotoxic material into the susceptible lymphoid

tissue of the spleen in mice (Stuart, 1960). The selective organ of destruction in dogs was found to be the liver which showed dose dependence while there were no pathological changes in the spleen even when there were no pathological changes in the spleen even when the dose of ethyl palmitate was increased to 30% (Areekul et al., 1973).

Numerous investigations of the effects of methyl palmitate in mice have been carried out (Blickens and Di Luzio, 1965; Morrow and Di Luzio, 1965; Di Luzio and Blickens, 1966; Blickens and Di Luzio, 1965; Di Luzio and Wooles, 1964). This compound not only depresses R.E.S. phagocytic activity but also inhibits both primary and secondary antibody responses (Morrow and Di Luzio, 1965; Di Luzio and Blickens, 1966; Di Luzio and Wooles, 1964). Phagocytosis depression,however, is not believed due to either release of methanol or tissue saturation with lipid (Blickens and Di Luzio, 1965). Although direct comparisions have not been published; inhibition of total body R.E.S. function by methyl palmitate probably is more pronounced than that produced ethyl palmitate.

A variety of simple lipids has been studies for their depression or enhanceing effects on R.E.S. phagocytosis. Stuart and Davidson (1964) have shown that small doses of cholesterol oleate may stimulate the R.E.S., where as large doses may depress R.E.S. function (Stuart and Davidson, 1963; Stuart and Davidson, 1964).

The reasons for variation in intensity of response to ethyl palmitate among different animal strains and species are not known

but probably relate mostly to differences in splenic pulp architecture and splenic blood flow. Alterations in particle size, dosé, and variations in injection schedules profoundly influence the splenic response to ethyl palmitate. The ultimate induction with ethyl palmitate of either permanent or transient splenic oblation along with immunosuppression and depression of R.E.S. function in many animals species, however, should permit the performance of a wide range of important basic studies.