REVIEW ARTICLE

THE ROLE AND FUNCTION OF THE RETICULO-ENDOTHELIAL SYSTEM IN IMMUNOLOGICAL PROCESSES*

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OUR knowledge of the role and functions of the reticulo-endothelial system is very imprecise and uncertain. However, there is considerable interest in immunological processes, that is to say in the resistance of host to infection, and our knowledge is increasing rapidly. That certain types of cells are implicated in the production of immunity was first suggested by Metchnikoff[1]. It is to him that we owe the first comprehensive and plausible theory of the role played by what he named "phagocytosis" in protection of the host against pathogenic invaders. But the existence of an integrated system of cells fulfilling a particular bodily function was postulated by Aschoff and his school. In 1913, Aschoff

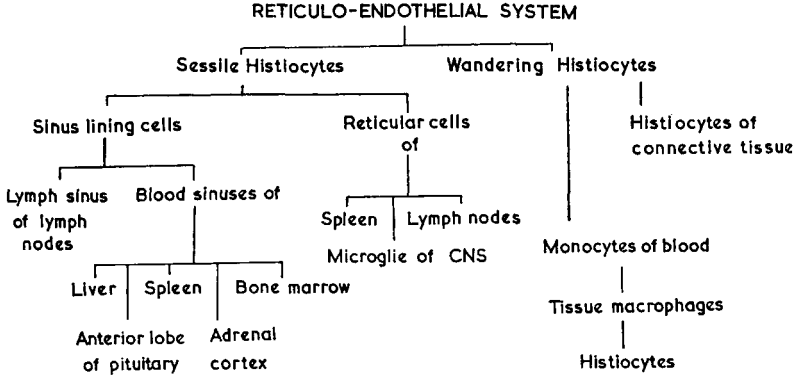

FIG. 1. Aschoff's classification of the R.E. cells.

and Kiyono[2] reported the histological and physiological autonomy of this important system, to which they gave the name reticulo-endothelial system (R.E.S.). Whether Aschoff's hypothesis of the functional unity of this system of cells, based on their ability to ingest and accumulate dyes, will stand the test of time is difficult to predict. His conception has been seriously challenged by some distinguished contemporary histophysiologists with valid arguments[3]. However, for clarity, I shall accept Aschoff's conception of the R.E.S. which is represented in Figure 1.

The histiocytes, which form the R.E.S., are widely but irregularly scattered through almost all tissues and organs. According to the classical conception, they are divided in *sessile* and *wandering* cells.

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Methods for studying the function of all the R.E. cells are not available at present, and in this review, I shall restrict my subject to the study of sessile histiocytes. I propose to ignore the wandering macrophages, irregularly scattered in the connective tissues, which certainly play an important part in the local and general defensive reactions, but which have eluded all attempts at quantitative evaluation.

Sessile histiocytes are concentrated in some important strategic areas of the vascular bed, where they are attached to the endothelia of the sinuses of various organs; liver, spleen, bone marrow and some other glands.

These cells, being in direct contact with the blood stream seem to be particularly active in taking up both inert and living particles from the circulating blood. To stress the importance and the role of this group of phagocytes, Miles[4] proposed to designate it the "systemic defences".

If immunity is defined as the resistance of a body to the effects of pathogenic invaders, the R.E. cells should be considered as one of the most important sources of protection to the host. They act by a double mechanism: (i) phagocytosis, followed by digestion and destruction of bacteria and other foreign debris; (ii) a humoral process, as yet not well understood but which is reflected in an increase of resistance to infections, and which involves, among other factors the production of specific antibodies.

Discussion of phagocytosis and immune reactions mediated by the R.E.S. will form the two main parts of this review.

The Dynamics of the Phagocytic Function

The property of engulfing particles of inert or living material is present even in the simplest animals. Metchnikoff[1] conceived that this process, which in the amoeba, for example, serves primitive nutritional needs, had become, in the course of evolution, an essential process of defence by which pathogenic invaders are combatted.

The importance of phagocytosis is considerable and it is believed by Boyd[5] to be "the greatest single mechanism contributing to natural resistance". Moreover, phagocytosis is not restricted to protection against bacterial infections. Modern physiology points out that the R.E. cells play an important part, by their remarkable capacity of clearance and of storage, in the metabolism of lipids[6], lipoproteins[7], cholesterol[8], haemoglobin[9] and iron[10].

These considerations emphasise the interest of the investigations made on the patterns of the phagocytic function, not only against bacteria, but also against inert particles. Also, the mechanism of phagocytosis of two such different substrates shows some intriguing discrepancies.

The patterns of phagocytosis are studied, in principle, by measuring clearance rates of the particles in the blood and their accumulation in the R.E. cells at specific times after the intravenous injection of a standard colloidal suspension of particles known to be phagocytosed by these cells.

Certain very definite criteria should be strictly observed in the choice of the particulate substances chosen to study the phagocytic activity of the cells[10].

(1) The particles should be phagocytosed by the cells of R.E.S. in contact with the blood and should not be taken up by other cells.

(2) They should normally not cross the capillary barrier.

(3) They should be homogenous in size.

(4) The substance should be non-toxic for the R.E. cells and the host organism.

(5) The substance should be stable *in vitro* and *in vivo* and should not be changed in physical or chemical properties by contact with the blood or after intravenous injection.

(6) The substance should be accurately measurable in the blood and tissues.

The General Patterns of Clearance of Inert Particles

The intravenous injection of a colloid meeting the desired criteria into an animal or a man is followed by a more or less rapid decrease of the particles from the blood stream and their concomitant accumulation in the R.E. cells, mainly in those of the liver and spleen. Figure 2 illustrates the disappearance rates of a suspension of carbon injected at various doses into albino rats. When presented in semilogarithmic co-ordinates by plotting the clearance as the log of the blood concentrates with respect to time, this phenomenon can be expressed by an exponential function (Fig. 3): $C = Co \cdot 10^{-KT}$

or by the equation: $$\frac{\log C_1 - \log C_2}{T_2 - T_1} = K$$

in which C = concentration of the foreign colloid in blood expressed in mg./100 ml., C_1 and C_2 the colloid concentration in the blood at times T_1 and T_2 minutes respectively.

TABLE I

DISTRIBUTION OF COLLOIDS INJECTED INTRAVENOUSLY IN THE RAT

Colloid	Dose mg./100 g.	Per cent recovered				K
		Liver	Spleen	Lung	Total	
Carbon	4	92	4	—	96	0·062
	8	90	4·7	—	94·7	0·026
	16	79	9	—	88	0·013
	32	78	10	2	90	0·006
	48	70	17	1·6	88·6	0·004
C.A.G.^{131}I ..	2	89	trace	—	89	0·118
	4	84	1·5	—	85·5	0·064
	8	83	1·9	—	84·9	0·031
	12	75	3	trace	78	0·022

The constant K of these equations, which defines the slope of the clearance curve in semilogarithmic co-ordinates, is therefore a measure of the clearance of particles and thereby of phagocytosis of the R.E. cells. We have called K the phagocytic index.

The data represented in Figures 2 and 3 show clearly that the main characteristic of the phagocytosis by the R.E.S. of the particles circulating in the blood is that the kinetics of phagocytic activity are affected by the quantity of colloid injected. The mathematical analysis indicates that the phagocytic index K varies inversely with the dose of colloid injected (D). In general, the relationship $K \times D = CT$ is true for different colloidal suspensions except when relatively small numbers of particles are injected[11].

The relationship $K \times D = CT$ has been verified in various animal species and seems to be a general principle. By comparing the phagocytic activity of the R.E.S. in various animal species, it has been found that the R.E.S. of the mouse is the most active; then the rat, guinea pig and the rabbit in order of decreasing activity[12].

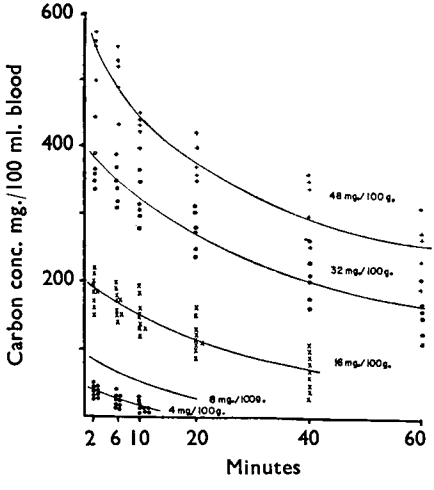

FIG. 2. Blood clearance of various doses of carbon in the rat.

The colloidal particles disappearing from the circulating blood are removed by the cells which line the sinuses of the major organs containing fixed R.E. cells. It may be of some interest to analyse the distribution of the colloidal matter between these organs. The liver is the most important organ, by far, in this respect. As seen from the figures in Table I, the Kupffer cells phagocytise 70 to 90 per cent of injected colloids. The role of spleen is much less important but, in general, the percentage recovered from the spleen increases with the dose. Thus for very small doses of colloids, which are rapidly cleared, the Kupffer cells take up nearly all the injected colloid; however, with higher doses which remain in circulation for hours, the spleen contains more than twice as much per gram as the liver. The reasons for these differences between the liver and spleen may be due to the total quantity of the phagocytes or, even more plausibly, to the circulatory patterns of the two organs.

In addition, species differences are rather important in this respect. While in rats, mice and rabbits, the spleen uptake is rarely as high as 10 per cent, a spleen deposition of over 20 per cent is common in dogs, which have a relatively large spleen compared with the other animals[13].

The role of the bone marrow in the uptake of colloids is similar to that of the spleen. With the usual colloids at average doses, the quantity found in the bone marrow is less than 10 per cent. But, when small particle colloids which disappear slowly from the blood stream are used, the deposition in the bone marrow may be much more considerable, increasing to 50 per cent of the injected material according to Dobson[13]. It should

be added that the high concentrations in the bone marrow have been found only in rabbits.

There is controversy about the part played by the lungs as a clearing organ. In our experiments, using various colloids, no significant deposition in the lungs has been observed, except when the material used was not stable. For example the use of commercial Indian inks for measurements of clearance, produces a gross error. The shellac they contain has potent thromboplastic properties and by the production of microthrombi, causes an accumulation of the carbon in the lungs. In this instance, we are dealing with a simple mechanical filtering effect of the lung capillaries and not with true and active phagocytosis[14].

This same mechanism may explain the observations of Wright[15] concerning the retention of bacteria in the lungs of strongly immunised animals. The bacteria aggregated by the specific antibodies are simply trapped in the pulmonary capillaries. It has been shown that particles, whether inert or living, once trapped in a fibrin net work in lung capillaries, are subsequently phagocytised by the polymorphonuclear cells and carried by them to the R.E. depots in the spleen and liver where they are phagocytised by the R.E. sessile cells.

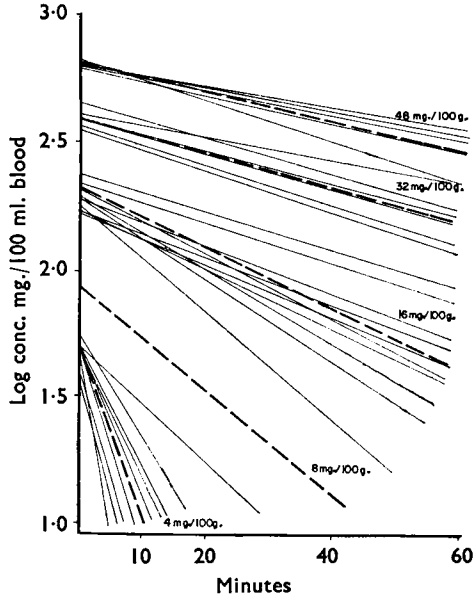

FIG. 3. Blood clearance of various doses of carbon in the rat in semi-logarithmic plot.
Dotted lines indicate the mean values.

$$C = Co.10^{-KT} \quad \frac{\text{Log } C_1 - \text{Log } C_2}{T_2 - T_2} = K$$

Studies with various colloids in different animal species have proved that the kinetics of phagocytosis of particulate substances introduced into the blood stream obey the same general laws, and the differences observed are quantitative only. However, several factors may influence the patterns of the clearance rates, namely the nature of the colloid, the size of the particles, the velocity of the blood flow and the functional conditions of the cells[16,17].

I now propose to discuss the saturating effect of repeated injections of colloids; discriminative phagocytosis and the importance of the stability of the injected colloids in the circulating blood.

The Saturating Effect of Repeated Injections

When the same dose of colloid is repeatedly injected into the same animal, after the particles of the preceding injection have been cleared,

evidence of a saturating effect of the phagocytes is observed (see Fig. 4). These results show clearly that the particles already phagocytised exert a depressive effect on the clearance capacity of the phagocytes towards the same substrate. This saturating effect of the phagocytised colloid is directly related with the so called "blockade" of the R.E.S.

The "Discriminative Phenomenon" in Phagocytosis

When particles of different sizes are injected together, the presence of some colloids in the circulation markedly modifies the rate of clearance of others by the R.E.S. This effect may be, of course, conditioned by the respective concentrations of the two colloids, but the experimental findings suggest that the clearance rate is also influenced by the physico-chemical nature of each colloid[18,19].

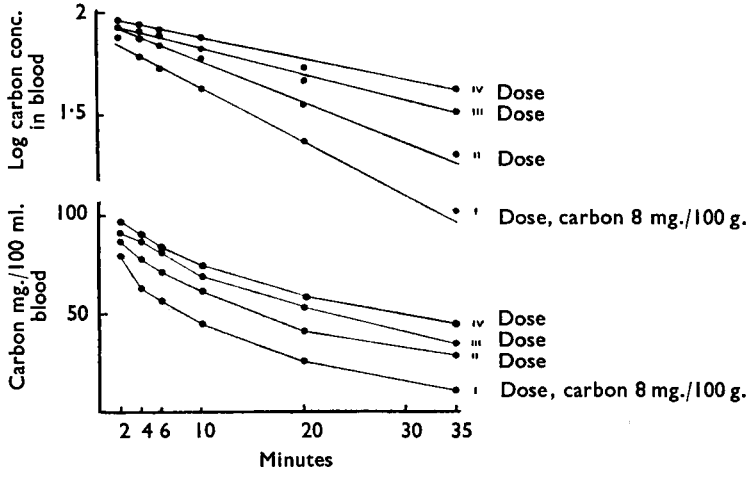

FIG. 4. Blood clearance of 4 subsequent doses of carbon injected intravenously in the rat.

These observations introduce the concept of discriminative phagocytosis, indicating that the avidity of the phagocytes for substances offered simultaneously, may vary widely according to the nature of the colloid. Figure 5 illustrates the phenomenon of discriminative phagocytosis where two different colloids are injected. A carbon suspension (16 mg./100 g.) is injected intravenously and the clearance rate established (K = 0·020). About twenty minutes later, a colloidal heat denatured serum protein suspension (C.A.G.) labelled with ^{131}I is injected (1·5 mg./100 g.). The clearance of carbon almost ceases, as the phagocytes take up the C.A.G. particles rather than carbon. As soon as clearance of the C.A.G. particles is completed, the phagocytosis of carbon particles is resumed at about the same rate as before the injection of the denatured proteins.

The phenomenon of discriminative phagocytosis appears to be a very important and fundamental process. Probably, it governs the turn-over of the lipids, lipoproteins, cholesterol, the clearance of the erythrocytes

and of many other normal and pathological substrates. The experimental example reported here, with a system of two colloids, gives some idea of the complexity of this clearance process when several substrates compete simultaneously. It also provides an explanation of certain pathological processes, resulting from the appearance of a pathological compound, or to the alteration of the clearance capacities of the phagocytes.

Importance of the Stability of the Suspension in the Circulating Blood

In the phagocytosis of erythrocytes, heterologous or denatured homologous erythrocytes are cleared from circulating blood by the R.E. cells,

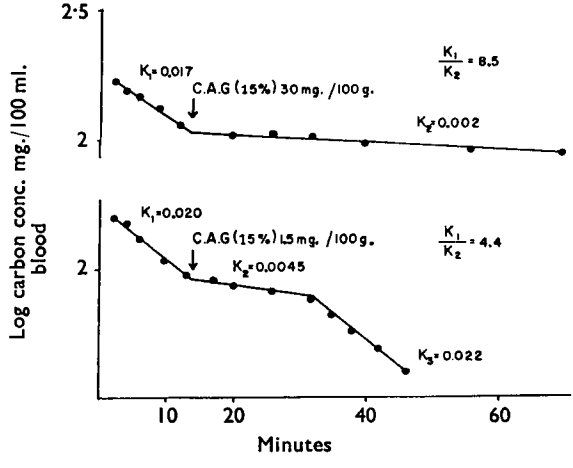

FIG. 5. Effect of the intravenous injection of two doses of rabbit C.A.G. on the clearance of carbon particles in mice for the dose of 16 mg. per 100 g. bodyweight.

mainly those localised in the liver and spleen[20]. In Figure 6 is shown the kinetics of clearance of pigeon erythrocytes labelled with ^{32}P injected into mice and rats, in doses of $\frac{1}{4}$, $\frac{1}{2}$, 1, 2 and 5 billions of cells per 100 g. of body weight. The logarithms of the numbers of erythrocytes per ml. of blood are plotted as ordinates against time as abscissae.

In the mouse, the pigeon erthrocytes are cleared by the R.E. cells according to the general principles already discussed. In rats, however, the kinetics of the clearance are by no means the same. The cause of this strikingly different behaviour of the two animal species, towards the same substrate, is due to immunological factors. Rat serum contains natural agglutinins against pigeon erythrocytes, while such antibody does not exist in detectable amounts in the mouse. The evidence that this interpretation is correct is provided by immunisation of mice against pigeon erythrocytes and thereby creating an identical situation which occurs spontaneously in rats (see Fig. 6).

These findings stress the precautions necessary in interpreting the results of clearance rates for the assessment of the phagocytic dynamics of the R.E.S.

The Clearance Patterns of Bacterial Substrates

The question now arises whether the principles which govern the clearance of inert particles may be applied without restriction to the phagocytosis of bacterial substrates.

In the ingenious studies of Wright[15] on experimental infections, suspensions of various strains of pneumococci were injected intravenously into

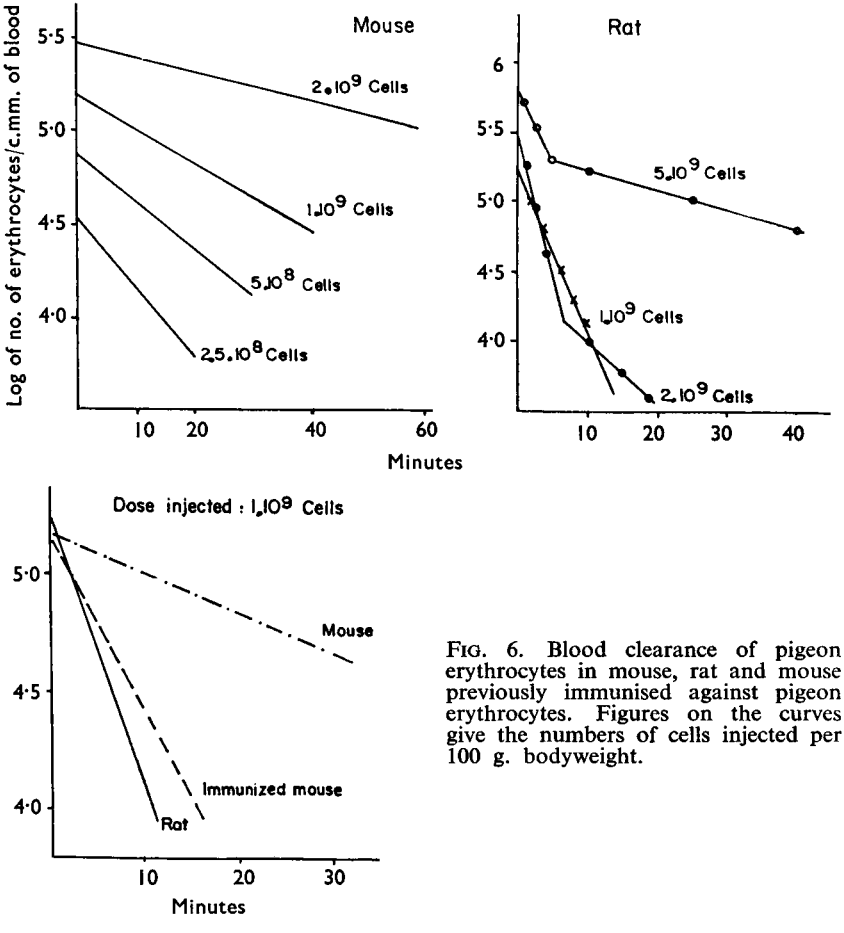

FIG. 6. Blood clearance of pigeon erythrocytes in mouse, rat and mouse previously immunised against pigeon erythrocytes. Figures on the curves give the numbers of cells injected per 100 g. bodyweight.

rabbits and the numbers of the bacteria in the blood counted. In Figure 7, where Wright's results are summarised, logarithms of the number of bacteria per ml. of blood are plotted as ordinate against time as abscissa.

It is evident from these data that the patterns of phagocytosis of living bacteria, while they are grossly similar to the clearance of inert particles, also show significant differences. With living bacteria, clearance is a resultant of phagocytic activity, but also of the virulence of the germs, of their multiplication power, and probably of some other unknown biological factors. To avoid some of these complications, we investigated

the clearance rates of dead bacteria, which being deprived of their virulence and of their capacity of multiplication, can be legitimately likened to inert particles. We used heat killed *Salmonella enteritidis* labelled with ^{51}Cr. The results are in Figure 8, which shows that their clearance follows a rather complex pattern. The curve is likely to be a resultant of at least two different kinetics. Our feeling is that, with both dead or alive bacteria, the lung interferes by liberating some thromboplastic agents. The reason why this is suggested is the peculiar effect of heparin on the clearance rates of bacteria. Pretreatment of animals with heparin, which does not alter the kinetics of clearance of inert colloids, consistently affects the clearance of bacteria. At the present time, these investigations are in hand and the explanation of the particular clearance patterns of dead bacteria can only be an hypothesis.

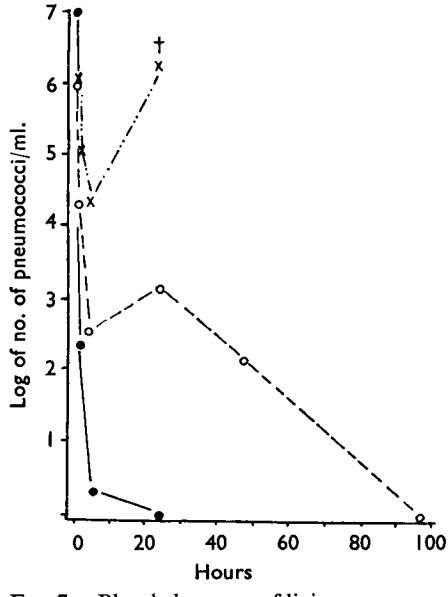

FIG. 7. Blood clearance of living pneumococci according to their virulence in the rabbit. After Wright[15].
●—● Avirulent. o—o Slightly virulent.
×—× Highly virulent. † Died.

The Clearance Patterns of Endotoxins

It has been shown by Bennett and Beeson[21] that endotoxins administered intravenously are rapidly removed from the blood and accumulated in the liver and spleen. More recently, Howard[22] proved that they are phagocytised by the R.E. cells of these organs.

Figure 9 illustrates the clearance rates of intravenously injected endotoxins extracted from Gram-negative bacteria and labelled with radioactive phosphorus. The results indicate that although lipopolysaccharides are rapidly removed from the blood, the clearance does not follow the usual exponential function.

Various investigations suggest that this phenomenon is likely to be conditioned by several factors: (i) the toxicity of the substrate to the phagocytes. After the injection of endotoxin, the R.E. cells are likely to be damaged and impaired. (ii) Thomas[23] has shown that the injection of endotoxins produces a modification of the physical state of the circulating fibrinogen. This is evidenced by the appearance in the plasma, about one hour after the injection of endotoxin, of a large amount of protein which is precipitated in the cold by heparin. This heparin-precipitable protein, is not present in normal rabbit plasma and Benacerraf[24] has recently shown that this denatured fibrinogen is phagocytised by

the R.E. cells. The slowing down of the clearance of endotoxins is perhaps related to the appearance in the blood of the fibrinogen aggregates which are phagocytised preferentially to endotoxins. (iii) The third factor is the heterogenous composition of the lipopolysaccharides. Endotoxin contains several constituents which differ from others in their molecular size. The larger molecular constituents are removed quite rapidly while about 30 per cent of the injected lipopolysaccharides were found to remain in the blood and to be only gradually eliminated in the course of 24 hours.

Factors which Stimulate the Activity of the R.E. Cells

The R.E.S. seems to be autonomous. In general, the activity of the R.E.S. escapes the control of the central and vegetative nervous systems. In fact, it has been shown by perfusion of isolated liver[25] that the phagocytic activity of the R.E. cells obeys grossly the same principles as *in vivo*. Owing to their anatomical situation, the cells are dependent upon the blood supply which is one of the main factors affecting phagocytosis. It has been claimed in the literature that other substances, like histamine or antihistamine drugs[26], exert more or less specific effects on the phagocytosis of the R.E. cells. In our investigations, we have been unable to ascertain such an effect except when, by gross vascular changes, the blood supply to the phagocytic organs has been impaired. On the other hand, the removal of the various endocrine glands has never produced any consistent action. Cortisone at high doses, depresses the recuperation phase of the R.E.S. after injection of a blocking dose of colloid, very likely due to its antiproliferative effects, so does nitrogen mustard[27]. Among the numerous substances and hormones investigated, certain steroids belonging to the oestrogen group were found to have an unequivocal stimulant effect on the R.E.S.[28–30]. Surprisingly enough the enhancing properties of oestradiol and diethylstilboestrol on the R.E. cell activity could be only ascertained in a simple animal species, the mouse. All the attempts to prove a similar effect in other laboratory animal species failed. The reason of this species specificity remains unknown.

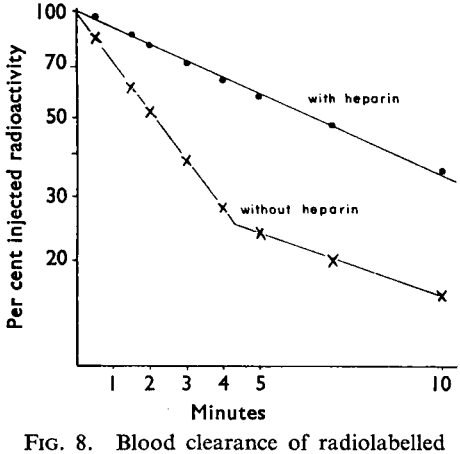

FIG. 8. Blood clearance of radiolabelled *Salmonella enteritidis* in normal and heparinised mice. Dose injected $1 \cdot 10^9$ organisms per 20 g.

At this point, I would like to mention one of the very curious properties of the R.E. cells which is related to the process of stimulation.

I discussed previously the injection of a high dose of a substrate phagocytised by R.E. cells, which is followed, for a short while, by a reduction

of the phagocytic activity. If the colloid does not contain an ingredient toxic to the cells, the animal recovers the normal phagocytic function in 2–3 days. Generally, the phagocytic activity of R.E.S. remains overstimulated during that period. This recovery and overstimulation are always associated with a hypertrophy of the main phagocytic organs namely the liver and spleen. A subsequent injection of the colloidal substrate produces a new and stronger stimulation of the R.E. cells and, by repeating the injections of the colloid, this phenomenon can be remarkably amplified (Fig. 10). This characteristic action on the R.E.S. is even more striking with certain bacteriological constituents especially endotoxins extracted from Gram-negative bacteria.

As shown by Howard and his colleagues[22], a dose as low as 10 μg. of purified lipid produces a typical effect. As with other colloids, a biphasic action is observed with endotoxins, especially when they are administered intravenously. During the first 8 hours, a definite decrease of activity is noticed, followed by a return to normal values. Subsequent injection of endotoxin will enhance the functional activity of the R.E.S. We found recently that more powerful and durable stimulation can be obtained with certain bacilli belonging to the family of mycobacteria[31]. The data in Figure 11 illustrate the action of 1 mg. of B.C.G. in mice. The maximal effect is observed about 2 to 3 weeks after the injection and enhanced rates of clearance are still evident 30 to 40 days later.

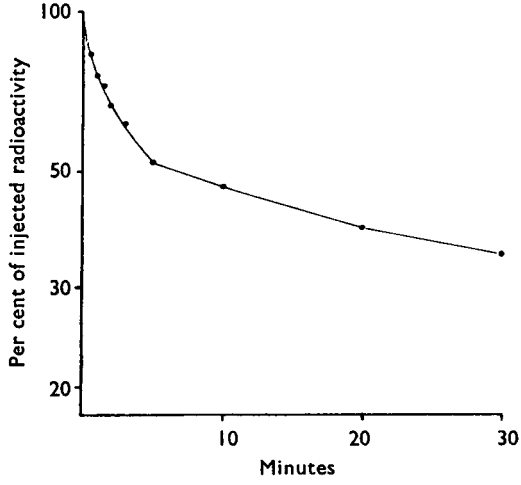

FIG. 9. Blood clearance of radiolabelled (^{32}P) *E. coli* polysaccharide endotoxin in the mouse. Dose 40 μg. per 20 g.

There are two main points which should be emphasised. The modifications of the phagocytic activity are paralleled with the changes of the metabolic activity of the R.E. cells. The metabolic activity of the Kupffer cells has been measured by a new technique which has been recently devised in my laboratory and which consists in the determination of the rate of breakdown of a labelled denatured colloidal protein phagocytised by these cells[33]. We are therefore entitled to infer that changes of the phagocytic function reflects changes in the metabolic (enzymatic) activity of the cell. The second point is the hypertrophy of the liver and spleen always associated with the increased activity of the R.E.S. The gain in weight is not a simple vascular effect but an increase in the protoplasmic mass of the organ. It is very unlikely that the increase in weight which

attains sometimes 50 to 100 per cent or even more is attributable to the multiplication of the R.E. cells only. We are dealing apparently with a special type of regulation of histogenicity which ensures an equilibrium between the number of the R.E. cells and the cellular mass of the organ. The nature and mechanism of this regulation remains unknown.

The discovery of substances capable of stimulating the metabolic and phagocytic activity of the R.E. cells will make it possible to correlate the enhancement of these functions and the other immunological features in

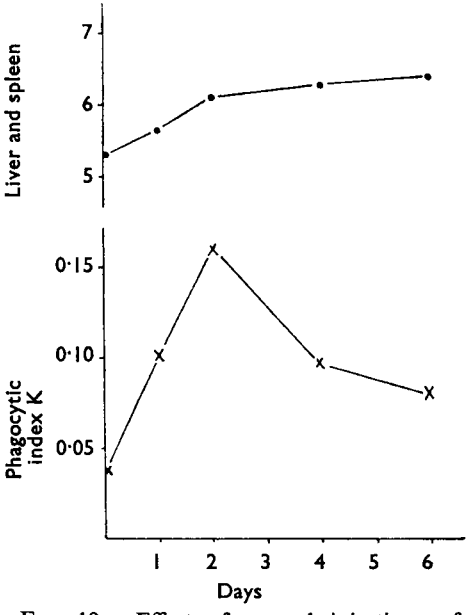

FIG. 10. Effect of several injections of rabbit C.A.G. on the weight of liver and spleen (g./100g.) and on the phagocytic activity of the R.E.S. of the rat measured by the index K for the dose of 8 mg. of carbon per 100 g. in the days following the treatment.

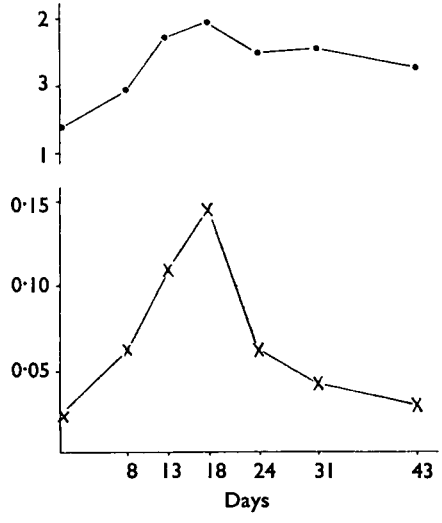

FIG. 11. Effect of infection with living B.C.G. on the weight of liver and spleen (g./20g.) on the phagocytic activity of the R.E.S. measured by the index K for the dose of 16 mg. of carbon per 100 g. in mice.

which the R.E.S. is supposed to be involved. This brings me to the discussion of the second point of my subject, the immunological implications of the R.E.S.

THE RETICULO-ENDOTHELIAL SYSTEM AND IMMUNITY

The sessile R.E. cells may play an important part in the resistance against bacterial infections either by systemic phagocytosis, or through other immunological processes.

Phagocytosis and Immunity

As long as we regard bacteria merely as inert particles, the principles discussed previously concerning the clearance of colloids need only little modification. But, living bacteria are capable of multiplication and it is

the balance between their capacity to multiply and the capacity of tissues to clear them that determines the fate of the host.

It seems that the avirulent bacteria are digested and killed by intracellular enzymes but there is a great deal of evidence that virulent bacteria, when phagocytised continue to multiply vigorously in the cells and it is the cell which dies, liberating living virulent bacteria in the blood stream. In such situation, the sessile phagocytes constitute the ultimate elements available for the defence of the organism.

Furthermore the decisive role of the sessile phagocytes has been established by evidence showing that their efficiency in protection of the organism against infection is greater than that of other tissues, as shown by Dutton[34] recently. He measured the infectivity for mice of graded doses of bacteria introduced by various routes of injection. At critical infecting levels, the dose required to kill the animal by intravenous injection was, in most instances, larger than the subcutaneous killing doses. With *Pneumococci* and *Salmonella typhimurium*, for example, the difference was over 100 fold. The superior defensive power of the sessile phagocytes is probably a matter of number. But whatever the relative defensive value, cell for cell, for a given germ, the defence afforded by the system of the sessile phagocytes is much more effective than that of local tissue lodgement. I fully agree with Miles when he states[4] that systemic phagocytosis is an integral part of the defence from the earliest stage of infection onwards and not only a mechanism which comes into play when local defences have definitely failed.

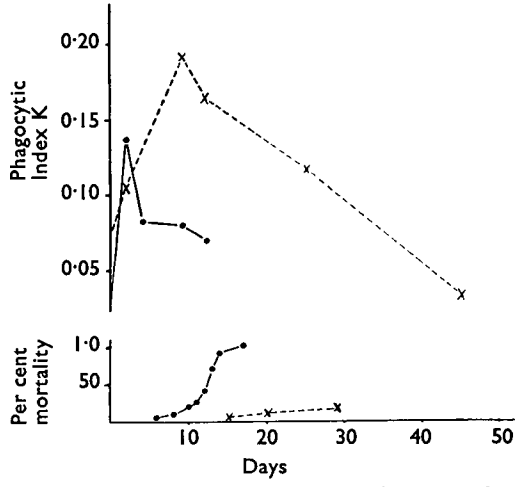

FIG. 12. Effect of an infection with *S. typhimurium* (10^8 organisms intraperitoneally) in control rats and rats treated with horse C.A.G. on the mortality and on the phagocytic index K for the dose of 8 mg. of carbon per 100 g. C.A.G. = 5 injections (50 mg.) in three days intravenously. The animals are infected 24 hours after the last injection.

●—● Control. ×—× Treated.

The Functional State of the R.E.S. and the Resistance to Infections

I shall attempt to provide evidence that experimentally induced modifications of the functional condition of the R.E. cells is related to a change in the resistance of animals to severe infections. Furthermore, the changes of the activity of R.E.S. are reflected in a change in the susceptibility of the animal to certain endotoxins.

As mentioned before, the injection of some colloidal substrates produces an enhancement of the phagocytic and metabolic activity of the R.E. cells. Experiments have been carried out to investigate whether animals whose R.E.S. has been so stimulated, show differences in resistance to severe experimental infections.

Stimulation of the R.E.S. has been accomplished with different substances.

(i) *C.A.G.* In the first group of experiments the R.E.S. was stimulated with a colloidal protein, obtained by heating serum proteins at 60°. This

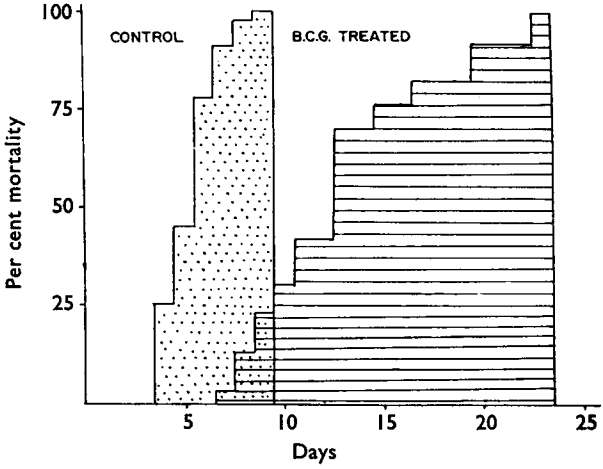

FIG. 13. The effect of B.C.G. infection on the survival time of mice infected with *Salmonella enteritidis*.

colloid is selectively phagocytised by the R.E. cells and repetition of injections causes a significant stimulation of the R.E.S.[35]. The results are shown in Figure 12.

A group of treated and control rats were infected with the same dose of a suspension of *Salmonella typhimurium*, injected intraperitoneally, and the sequences of the infections studied. It is evident from Figure 12 that animals whose R.E.S. has been stimulated previously were able to resist infection much better than the controls, as on the 17th day nearly all control animals died, while 95 per cent of the treated animals were alive. On the other hand, in the treated group, the phagocytic activity of the R.E.S. was greatly stimulated during the course of infection and this hyperactivity lasted until recovery was achieved, while in the control group, a vigorous stimulation of the R.E.S. was observed in the first days, but was shortly followed by a decrease in the phagocytic index until death occurred.

(ii) *Stimulation with B.C.G.* The infection of mice with living B.C.G. leads to a more intense and lasting stimulation of the phagocytic and metabolic activity of the R.E. cells[31]. To assess the development of a mortal infection in treated animals in comparison with the control group,

Salmonella enteritidis (var. *Danysz*) was used. The infecting dose was administered intravenously and was the LD100 for the control group[32].

From Figure 13 it can be seen that although there were no survivors amongst either control or treated animals due to the severity of the infection, the mean survival time was greatly increased in the later group.

By what mechanism does the over-stimulation of the R.E.S. increase the resistance of the animals to infections?

The remarkable studies of Wright put beyond doubt that the increase in phagocytic intensity is at least partly responsible for the increased immunity.

It is also likely that the increase in survival time of animals pretreated with B.C.G. is due to the fact that the multiplication of the phagocytized germ is either prevented or significantly retarded, as B.C.G. enhances not only phagocytosis but also the metabolic rate of R.E. cells[32].

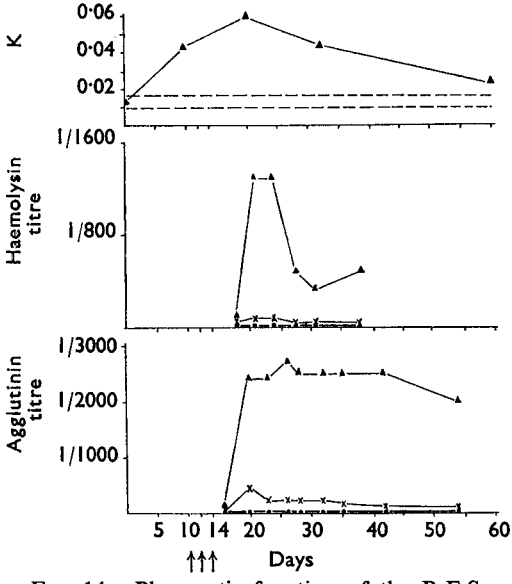

FIG. 14. Phagocytic function of the R.E.S. in mice infected with B.C.G. Antibody production in X—X, control mice, in ▲—▲ mice treated with B.C.G. and in ●—● mice splenectomised and treated with B.C.G. The arrows indicated the injections of antigen.

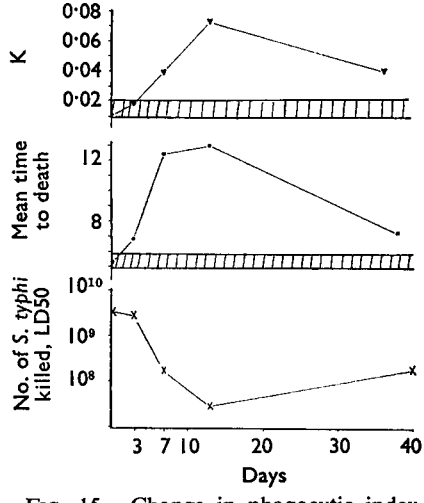

FIG. 15. Change in phagocytic index K of the R.E.S. in resistance to endotoxin LD50 of killed *Salmonella typhi* per 20 g. and resistance to infection with *Salmonella enteridis* (mean survival time after 1000 organisms intra-intravenously) at different times after infection with B.C.G. in mice. The standard deviation of control mice is indicated.

But enhancement of resistance in animals to severe infection may imply the presence of another mechanism: the production of antibody. Experiments in mice, treated with B.C.G., show clearly that the rate of antibody production is also significantly increased[36].

In the experiments summarized in Figure 14, the rates of production of two different types of antibodies—agglutinins and hemolysins—have been studied in normal animals and in animals treated with B.C.G. It is obvious from the data represented that a remarkable increase in antibody titres is observed in the B.C.G.-treated animals, compared with the controls. The antibody response to the two different antigens is enhanced and this observation suggests that this reaction is a general feature.

The role of the R.E.S. in the production of the antibody is evidenced by removal of a part of the R.E.S. by splenectomy. It is obvious from data presented in Figure 15 that the titre of agglutinins and hemolysins is considerably reduced in splenectomized animals, even in those which have been treated with B.C.G.

I am aware that the spleen contains besides R.E. elements many other cells, to which an important role has been conferred in the production of antibody. But a glance at the modern literature shows that the morphologists are far from being in agreement on the genealogical relationships between the R.E. cells and the other types of cells, such as immature plasmocytes, to which a main role in production of antibody has been attributed, and I do not propose to involve myself in such a discussion.

Correlation Between Stimulation of the R.E.S. and Resistance to Endotoxins

Endotoxins, as we have seen, are removed from the circulation by the R.E. cells. They have, *per se*, a potent stimulating action on the R.E.S. and resistance to endotoxins[23] has been considered by several authors to develop as a result of this action. Endotoxins, on the other hand, increase the resistance of animals to infections, as proved recently by Rowley and his colleagues[37].

Whether and to what extent resistance to infections, that has been enhanced by administration of B.C.G. to animals, has some parallel increase in resistance to endotoxins has not yet been clarified. The results obtained show that such a correlation does not always exist[38].

As shown in Figure 15, B.C.G.-treated animals, which have an enhanced phagocytic activity, and also an increased resistance to infections, become highly sensitive to endotoxins. The lethal dose of endotoxin in B.C.G.-treated animals is about 100 times lower than in normal controls. These findings are apparently in conflict with current opinion about the relation between the functional state of the R.E.S. and sensitivity to endotoxins. But the opposition is more apparent than real.

Our recent investigations suggest that the increased susceptibility of B.C.G.-treated animals to endotoxin, results from a particular state of immunity caused by the tubercle bacillus. The phenomenon of enhanced susceptibility to endotoxins is related to the increased vulnerability of the blood vessels observed in animals infected with tubercle bacilli and is similar to a generalised Shwartzman phenomenon.

As a matter of fact this susceptibility to endotoxins is only observed when stimulation of the R.E.S. is induced with the tubercle bacillus, but not when oestrogens or other colloids are used.

Thus from the work discussed in this article it is apparent that the R.E. cells form the keystone of immunity. By their powerful and practically unlimited phagocytic capacity, the sessile phagocytes form the fundamental system for clearing bacterial endotoxins from the circulating blood. In turn, the phagocytised substrates stimulate the activity of the cells, inducing a kind of self-stimulating process, the possibilities of which are limited only by the toxic effects of the ingested material on the phagocytes and their proliferative capacity.

Stimulation of the phagocytes may be associated with an increase of the metabolic enzymatic activity of the R.E. cells. It may be deduced that intracellular antibacterial mechanisms, still largely unknown, may be affected in similar manner. This may provide the explanation of the increased resistance to infections found in animals whose R.E.S. has been over-stimulated.

If phagocytosis is beyond doubt one of the means by which the R.E. cells are involved in the enhancement of immunity, our recent investigations show pertinently that animals whose R.E.S. has been stimulated are able to respond by an increased antibody production. Our results indicate that, in the laboratory animals, when antigens are administered intravenously, the spleen is the main organ involved in antibody production, while the Kupffer cells form essentially the clearance system.

For many of the experimental observations reported here I am as yet unable to offer a firm or definite explanation. These are completely new problems and no doubt some of our present hypotheses may be questioned by to-morrow's findings.

However, it is my conviction that the R.E.S. is of considerable importance and I believe that the now widely conducted and intensive investigations will underline this.

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