Studies on the Relationship between the Infection Therapy and the Reticuloendothelial System*

By

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INTRODUCTION

It is well known that the reticuloendothelial system holds an important position in the mechanism of biological protection against infections, but the marked development of chemotherapy in recent years has produced a tendency to ignore the factors of a living organism in the therapy of infections. On the other hand, wide-spread use of chemotherapeutics has produced problems of resistance and cross-infections. It has become possible to make quantitative examination of the functions of reticuloendothelial system by the development of colloid chemistry and utilization of radioisotopes. It seemed necessary, at this time, to recognize again the role of the reticuloendothelial system in connection with infection, especially on its treatment, and the following examinations were made from such a standpoint.

1) Fundamental examination of the test method for reticuloendothelial functions.
2) Effect of infection on the function of the reticuloendothelial system.
3) Effect of reticuloendothelial functions on infection.
4) Effect of infection therapy on the functions of the reticuloendothelial system.

1) Fundamental Examination of the Test Method for Reticuloendothelial functions

There are various methods for testing the functions of the reticuloendothelial system but the carbon-particles method, using Pelikan Ink, proposed by Halpern and others1-3, was used in the present experiments. First, the size distribution of Pelikan Ink particles was examined under an electron microscope.
and it was found that the particle size was fairly homogeneous, the sizes being 20–35mμ. The presence of shellac, which was pointed out by Halpern3) as the reason for toxicity of the ink, was examined by infrared spectral analysis and its presence in Pelikan Ink was confirmed.

For experimental animal, rabbits weighing around 2 kg. were used. For the measurement, a definite quantity of Pelikan Ink per kg. of body weight was injected intravenously, blood was collected from the aural vein every 2–5 minutes, and concentration of carbon was measured by photoelectric colorimeter, from which disappearance curve was plotted. Within a definite range of the quantity of carbon loaded, disappearance of the carbon from blood can be represented as the exponential function of time, \( C = C_0 \times 10^{-KT} \). This \( K \), which Halpern and others designated as the phagocytic index, was taken as the index of the functions of reticuloendothelial system.

The quantity of carbon to be loaded was set at 4mg./100g. body weight, considering the presence of shellac as stated above and from the result of perfusion experiment to be described later. Distribution of \( K \) by loading 4mg./

![Fig. 1. Distribution of the phagocytic index K by loading 4 mg of carbon per 100g body weight in 100 normal rabbits.](image-url)
Fig. 2. Variation of K in the days following single loading of 4 mg carbon/100 g in normal rabbits.

100 g. in 100 normal rabbits gave a mean value of 0.0291±0.0107, indicating that there is a fairly great individual difference (Fig. 1). It seemed more appropriate, therefore, to observe the course in one individual when testing the variation of K. In such a case, the problem is the effect of the measurement itself on the reticuloendothelial system. For that reason, measurement was carried out on one individual twice, with different intervals, and it was found that the value of K was low for the first 1–2 days but returned approximately to the original value after the third day (Fig. 2). From this result, it was decided to carry out the second measurement, after some kind of a treatment, with an interval of three days after the first measurement.

Examination was next made on the blocking effect. Using one rabbit, 4 mg./100 g. of carbon loading per day was continued for some time and measurement of the functions was carried out at the same time. In general, the value of K became lower for the first two days but the value tended to recover from the third day, and the value returned to normal or showed acceleration of phagocytic activity, in spite of continued loading (Fig. 3). This result shows that the blocking effect is more marked on short-term loading than by a long-term loading and that the effect is of short duration. It is assumed from this result that the reticuloendothelial system, even if blocked once, shows a fair capacity by taking a kind of reaction state after a definite period. An experiment was therefore
Fig. 3. Variation of K in rabbits loaded with 4 mg of carbon/100g/day continuously.

Fig. 4. Disappearance curves of carbon particles in the perfused rabbit liver.
carried out by continuous loading of 4 mg./100g. for two days as one blockade and then K became less than 60%.

For observation of the acceleration of phagocytic activity, effect of albumin-globulin complex, reported by Benacerraf and others,\(^4\) was examined. After heating 5% solution of dried human plasma at 60°C for 30 minutes, its 5 cc. was injected intravenously into a rabbit for four days. The marked elevation of K value was found 3-4 days after the termination of the injection.

Halpern and others reported that 80-90% of the carbon particles was ingested by the liver, so the phagocytic activity of the liver alone was examined by the use of liver perfusion apparatus. The perfusion liquid was the blood of the rabbit tested, the same concentration of the carbon as in vivo loading was mixed in this blood, and concentration of the carbon in the out-flowing blood from hepatic vein was examined periodically. Compared to the in vivo loading, the disappearance curve in perfusion tended to give two components and the concentration was found to decrease rapidly by initial passage through the liver. In a concentration corresponding to 2 mg/100g. the carbon was found to disappear almost completely after one passage through the liver (Fig. 4). In order to see whether this rapid lowering of the concentration was due to phagocytosis or not, perfused liver was examined under electron microscope. After 10 minutes of perfusion, carbon particles were adhered to the surface of Kupffer cells and part of the carbon particles seemed to be already incorporated by the cells. After 30 minutes of perfusion, the carbon particles were clearly incorporated by Kupffer cells. Histologically, therefore, the liver Kupffer cells seem to take in the carbon particles fairly rapidly.

2) Effect of Infection on the Function of Reticuloendothelial system

Pneumonia was caused in a rabbit by tracheal injection of about \(2 \times 10^6\) Pneumococci type I, according to the method of Robertson, and variation of K values was observed. The variation of K value was not so marked immediately after injection but a marked elevation of K value was observed 2-3 days after the infection and this elevation of K value was found to continue for a fairly long period after pneumonia was cured (Fig. 5). When the same quantity of pneumococci was injected intravenously or subcutaneously, similar marked elevation of K value was found 3-4 days after the infection. The same elevation of K value was observed after daily intravenous injection of about \(7 \times 10^4\) pneumococci killed with formaldehyde but the value tended to return to the original value in a comparatively short period, differing from the case of infection with live bacteria (Fig. 6).

As a state corresponding to infection, intravenous injection of 0.1 cc. of typhoid vaccine was given five times consecutively. Changes of K value were not so marked immediately after the injection but the value increased markedly
from the third day (Fig. 7). Continuous injection of 0.1 cc. of typhoid vaccine immediately after blocking was found to cause lowering of the K value (Fig. 8). These experiments seem to indicate that the same infectious stimulation given to an organism results in different reaction according to the state of functions of the reticuloendothelial system.

Continuous daily intravenous injection of 2γ of dysentery toxin did not cause such a marked elevation of the K value.

The foregoing experiments show that a continuous infectious stimulation of a suitable degree for a certain period causes acceleration of phagocytic activity of the reticuloendothelial system. However, this acceleration differs by the mutual relationship between the strength of infectious stimulation (kind, amount and toxicity of the bacteria, speed of infectious progress, etc.) and the state of reticuloendothelial functions of the organism. If the infectious stimulation is relatively strong, there may be inhibition of the phagocytic activity.
Fig. 6. Effect of intravenous injections of killed pneumococci on clearance rate of carbon particles.

Fig. 7. Effect of continuous injections of typhoid vaccine on the phagocytic index.
This acceleration of phagocytic activity during injection may be caused by various factors, such as the accelerated functions of the cells, activation of cells in stationary phase, increased blood flow, or mobilization and proliferation of reticuloendothelial cells. In order to elucidate part of this mechanism, relationship between variation of hepatic blood flow and phagocytic activity during infection was examined, using Hensel's calorimeter probe. Intravenous injection of 0.2 cc. of typhoid vaccine in a rabbit results in gradual elevation of body temperature 30 minutes after the injection, with concurrent increase of hepatic blood flow and this increased flow was found to rise in parallel with elevation of body temperature. However, the increase in blood flow was only about 10% and this did not seem to have a great effect on the phagocytic activity. In fact, there was no marked change in the K value immediately after intravenous injection of 0.2 cc. of typhoid vaccine. These experiments seem to indicate that the increase in blood flow is not an important factor for the acceleration of phagocytic activity during infection. Since the acceleration of phagocytic activity was found to require a certain period, in the experiment with various infections, the principle of acceleration of phagocytic activity during infection seems to lie in
the mobilization and proliferation of cells in the reticuloendothelial system, rather than acceleration of this function at the cell level.

3) Effect of Reticuloendothelial Functions on Infection

In order to find the effect of depression or stimulation of the functions on the course of infection, survival period was compared in groups of rabbits infected with pneumococci; control group, blockaded group, and group with stimulated functions by administration of typhoid vaccine or albumin-globulin complex. The blockaded groups, both by intratracheal infection and intravenous infection, showed shortening of survival days and the groups with stimulated function showed prolongation (Figs. 9 & 10). These results seem to indicate that there is some kind of relation between the functions of reticuloendothelial system and biological defence mechanism against infection.

The foregoing experiment suggests, naturally, some correlation between phagocytic activity against carbon particles and bacterioplastic activity. Therefore, disappearance of bacteria from the blood after intravenous injection of pneumococci in rabbits was compared with the control group, blockaded group, and a group with stimulated functions. Intravenous injection of $10^7$ bacteria did not show any significant difference among the three groups (Fig. 11). Intravenous injection of $10^8$ bacteria showed some tendency for delayed and accelerated clearance of bacteria from the blood between the blockaded and stimulated groups respectively, but there was a distinct individual difference, even in the control group, that conclusive proof could not be obtained (Fig. 12). These results

![Graph illustrating the survival period of rabbits infected intratracheally with about $2\sim5\times10^7$ pneumococci.](image)

**Fig. 9.** Survival period of rabbits infected intratracheally with about $2\sim5\times10^7$ pneumococci. TV; Typhoid vaccine.
Fig. 10. Survival period of rabbits infected intravenously with about $2 \sim 5 \times 10^7$ pneumococci. CAG; Albumin-Globulin Complex, TV; Typhoid Vaccine

indicate that there is no marked parallelism between the granulopectic and bacteriopectic activities, as would be suggested from the result of infection experiments.
It should be remembered here that Benacerraf and others\textsuperscript{5,6}) stated that the rate of disappearance of bacteria from the blood is affected by antibody and other humoral factors. Therefore, effect of blocking or stimulating functions of the reticuloendothelial system on the elevation of antibody value was examined by repeated intravenous injection of dead dysentery bacillus in rabbits. The stimulated function group did not show any significant difference from the control group, but the elevation of antibody value in the blockaded group was found to be inhibited to some extent (Fig. 13). Of course, this experiment alone cannot be generalized but at least, the bacteriopexy differs from phagocytosis of
foreign matter like carbon particles and it seems necessary to consider the effect of antibody and other humoral factors as a preliminary to bacteriopexy, so that it is far more complicated. It may therefore be considered that the functions of a reticuloendothelial system, represented by the ability to dispose of foreign matter, cannot necessarily be taken as the mechanism for biological defense against infection.

4) Effect of Infection Therapy on Reticuloendothelial System

As has been stated above, there is variation in the functions of the reticuloendothelial system during infection and the depression or acceleration of the functions of the reticuloendothelial system affects in some way the course of infection. Consequently, effect of the treatment for infection on the reticuloendothelial system should naturally be taken into consideration.

First, the effect of various therapeutics on the normal reticuloendothelial system was examined (Table I). Continuous administration of $10^6$ units of penicillin G-K and 40 mg. of chloramphenicol did not cause any difference in the K value before and after administration. Snell and others⁷,⁸) had reported that tetracycline group antibiotics had affinity to the reticuloendothelial system but continuous administration of 40 mg. of oxytetracycline did not give much effect in general. Continuous administration of 4 mg. of prednisolone also did not affect the K value but administration of this drug immediately after blockade showed a slight lowering of the K value. The same tendency was also observed with nitrogen mustard N-oxide (HN$_2$-NO), showing no effect on continuous administration of 10 mg. of HN$_2$-NO in normal rabbits but a slight lowering of the K value when administered immediately after blockade. It may be assumed from these

| Table I. Effect of Intravenous Administration of Various Therapeutics on the Functions of the Normal Reticuloendothelial System. |
|---|---|---|---|---|---|
| | Penicillin $10^6 u \times 6$ | Chloramphenicol 40 mg $\times 7$ | Oxytetracycline 40 mg $\times 5$ | Prednisolone 4 mg $\times 5$ | HN$_2$-NO 10 mg $\times 6$ |
| | Control group | Blockaded group | Control group | Blockaded group |
| K(%)* | | | | | |
| 101.5 | 75.1 | 86.7 | 90.0 | 58.7 | 94.3 | 43.3 |
| 102.5 | 82.3 | 98.7 | 92.5 | 74.5 | 100.0 | 76.3 |
| 126.3 | 98.7 | 103.3 | 102.2 | 84.3 | 112.0 | 90.3 |
| 103.3 | 106.0 | 103.3 | 114.5 | 107.5 | 100.0 | 100.0 |
| 114.5 | 161.8 | 132.0 | 166.2 | 116.6 $\pm$ 28.1 | 102.1 $\pm$ 7.4 | 77.5 $\pm$ 21.5 |
| Mean | 108.2 $\pm$ 5.3 | 100.1 $\pm$ 17.7 | 94.9 $\pm$ 4.6 | 72.5 $\pm$ 10.5 | 102.1 $\pm$ 7.4 | 77.5 $\pm$ 21.5 |

$*$ K before administration of therapeutics $= 100$
experiments that prednisolone and HN$_2$-NO do not affect the reticuloendothelial system in normal state but act inhibitively against recovery of functions from the blockaded state.

The foregoing experiments have shown that various drugs do not greatly affect the normal reticuloendothelial system but the problem in actual treatment is the effect of these drugs on the reticuloendothelial system during infection. So, the effect of administration of various drugs was tested on accelerated phagocytic activity by the continuous administration of typhoid vaccine as a state corresponding to infection (Table II). Rabbits were administered with 0.1 cc. of typhoid vaccine and one of various therapeutic agents at the same time for the first 5 days, drugs alone for the next 2 days, and measurement of K value was carried out on the 8th day. The amount of each therapeutic agent administered was the same as that in single administration. Groups administered with typhoid vaccine and penicillin and with typhoid vaccine and chloramphenicol showed some inhibition of the elevation of K value compared to a group given typhoid vaccine alone but no marked difference was found as a whole. These results seem to suggest that neither penicillin nor chloramphenicol give much effect on the reticuloendothelial system during infection. Combined use of typhoid vaccine and oxytetracycline showed a slight inhibition of the elevation of the K value and this might be related to the affinity of this antibiotic to the reticuloendothelial system.

In contrast, combined use of typhoid vaccine with prednisolone clearly indicated inhibition of the elevation of K value by typhoid vaccine and the same inhibition was very maked in the use of HN$_2$-NO. Prednisolone and HN$_2$-NO are considered to act inhibitively against the reaction of reticuloendothelial system.

**Table II. Effect of Intravenous Administration of Various Therapeutics on the Accelerated Phagocytic Activity by Typhoid Vaccine**

<table>
<thead>
<tr>
<th></th>
<th>TV 0.1cc x 5 intravenously</th>
<th>Penicillin 10$^5$ u x 7 + TV 0.1cc x 5</th>
<th>Chloramphenicol 40 mg x 7 + TV 0.1cc x 5</th>
<th>Oxy-tetracycline 40 mg x 7 + TV 0.1cc x 5</th>
<th>Prednisolone 4 mg x 7 + TV 0.1cc x 5</th>
<th>HN$_2$-NO 10 mg x 7 + TV 0.1cc x 5</th>
</tr>
</thead>
<tbody>
<tr>
<td>K (%)*</td>
<td>167.3</td>
<td>86.2</td>
<td>90.9</td>
<td>117.2</td>
<td>92.0</td>
<td>89.7</td>
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<tr>
<td></td>
<td>169.0</td>
<td>95.0</td>
<td>101.5</td>
<td>141.5</td>
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</tr>
<tr>
<td></td>
<td>202.5</td>
<td>117.5</td>
<td>149.8</td>
<td>166.0</td>
<td>101.7</td>
<td>108.2</td>
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<tr>
<td></td>
<td>263.0</td>
<td>219.0</td>
<td>183.0</td>
<td>178.5</td>
<td>125.7</td>
<td>169.3</td>
</tr>
<tr>
<td></td>
<td></td>
<td>251.5</td>
<td>234.0</td>
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<tr>
<td>Mean</td>
<td>200.5 ± 38.7</td>
<td>153.8 ± 68.0</td>
<td>151.8 ± 52.8</td>
<td>150.8 ± 23.6</td>
<td>110.9 ± 17.1</td>
<td>116.8 ± 31.0</td>
</tr>
</tbody>
</table>

* K before administration of therapeutics and typhoid vaccine = 100

TV = typhoid vaccine
to infectious stimulation. It is not necessarily possible to weaken resistance against infection but the fact should be kept in mind when using these drugs during infection.

CONCLUSION

1) Functions of the reticuloendothelial system as represented by the carbon-particles method vary greatly according to individuals, even among normal animals, and it is more appropriate to observe variation of reticuloendothelial functions in one individual. The blocking effect is more marked by a short-term loading and the effect decreases by a long-term loading.

2) Continuation of a suitable infectious stimulation for a definite period stimulates functions of the reticuloendothelial system, but this is thought to differ according to correlation between the intensity of infectious stimulation and the state of reticuloendothelial functions. It should be noted here that a definite period is required before such functions show acceleration. This is thought to show that a certain period is required for the reticuloendothelial system to establish a kind of reactive state. It is interesting that this period corresponds approximately to the period for restoration after blockade. As for the principle of functional acceleration during infection, mobilization and proliferation of the cells of reticuloendothelial system are thought to constitute the principal mechanism from the result of time factor and the amount of blood flow.

3) Experiments with pneumococcal infection in rabbits have clearly indicated that there is some kind of a relationship between phagocytic activity and infection resistance, but there is no clear parallelism between bacterioplectic activity and phagocytic activity against carbon particles. It is assumed that bacterioplectic activity is more complicated by participation of an antibody and other humoral factors.

4) It should be emphasized that the effect of therapeutics on the reticuloendothelial system during infection must be taken into consideration. While prednisolone and nitrogen mustard N-oxide had no effect on the functions of normal reticuloendothelial system, they tended to inhibit the reaction of the reticuloendothelial system during infection. Although the effect of antibiotics was not so marked, this problem of the effect of therapeutics should be considered carefully, with the likelihood for appearance of more powerful drugs or possibility of an administration of larger doses.
References

1) Halpern, B-N. et al., Ann. Inst. Pasteur, 1951, 80, 582.