Phagocytosis of *Salmonellae* by Reticulo-endothelial Cells of New-born Piglets lacking Natural Antibody

It is a well-established fact that the uptake of bacteria by phagocytes is enhanced by serum factors (opsonins) acting on bacterial surface. The importance of serum opsonins in the phagocytosis of bacteria by fixed macrophages of the reticulo-endothelial system has been demonstrated by organ perfusion technique. It has been shown that serum factors playing a part in bacterial opsonization include immune or natural antibody, complement and possibly a third unidentified factor^{1,2}.

ance of labelled bacteria was established by measuring the radioactivity of blood samples removed from the opposite ear vein. When about 90 per cent of the radioactive bacteria were cleared from the blood the animals were killed and the radioactivity of different organs determined.

In another group of piglets the phagocytic activity of the reticulo-endothelial system was established by measuring the blood clearance and organ distribution of intravenously injected colloidal carbon using a technique previously described¹⁰.

The results obtained in these experiments are summarized in Fig. 1.

It appears that *Salmonellae* are removed very slowly

from the blood with a *K* value of 0.007. The distribution of the radioactivity in the organs shows that the majority of the bacteria are fixed in the spleen. The spleen, which weighed only about 1 g. accumulated 44 per cent of the injected bacteria while the whole liver (about 33 g) contained only 17 per cent. The number of bacteria per g of tissue was 80 times higher in the spleen than in the liver and this latter organ contained about the same amount of radioactivity as the lungs. It is important to note that the organ distribution of *Salmonellae* was very different in adult animals containing natural or immune antibodies. In adult animals the liver fixed 50–80 per cent and the spleen 5–25 per cent of the injected number of bacteria^{3,4,7}.

Examination of the phagocytic activity of the reticulo-endothelial cells with colloidal carbon in new-born piglets shows that the lack of bacterial fixation in the liver is not due to the inefficiency of the phagocytic function of the Kupffer's cells in the early stage of life, since these cells phagocytize 75 per cent of the injected amount of carbon. The phagocytic index *K* and the carbon distribution in the organs of new-born piglets are similar to those of adult animals¹¹. Šterzl *et al.* have also shown that the reticulo-endothelial cells of new-born piglets deprived of colostrum phagocytize carbon particles at the

same rate as piglets fed with colostrum¹².

These results indicate that, in new-born piglets, the absence of natural antibody inhibits the uptake of bacteria by Kupffer's cells, while the phagocytes of the spleen are capable of vigorous phagocytosis. It is interesting to emphasize the striking difference in the part played by opsonins in phagocytosis of bacteria by the Kupffer's cells and by the reticulo-endothelial cells of the spleen.

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Fig. 1. Rate of blood clearance (*K*) and organs distribution of *S. typhimurium* labelled with iodine-131 and colloidal carbon in new-born piglets. The figures represent the mean value of three animals. The *K* value is determined by the formula: $K = \frac{\log C_t - \log C_f}{t - t_f}$. *C*, blood concentration; *t*, time expressed in min.

The use of radiolabelled bacteria has made possible quantitative measurement of opsonins in the phagocytosis of bacteria by the reticulo-endothelial system *in vivo*³⁻⁵. The importance of antibody and complement in the phenomenon of bacterial opsonization has therefore been demonstrated in the intact animal^{6,7}. It has also been suggested that the rate of phagocytosis of bacteria in different animal species was related to the amount of natural antibodies present in their serum⁸. In germ-free mice which are supposed to contain a lower amount of natural antibodies, the blood clearance of *E. coli* is slightly decreased. Nevertheless the bacterial repartition in the phagocytes of liver and spleen is similar in germ-free and control mice⁸.

Recently, Šterzl *et al.* have shown that serum from new-born piglets deprived of colostrum was totally lacking in natural antibodies but contained 1–3 units of complement per ml.⁹. New-born piglets offer, therefore, an exceptional opportunity to investigate whether phagocytosis of bacteria by reticulo-endothelial cells *in vivo* occurs even in the absence of natural antibodies.

Investigation of phagocytosis of *S. typhimurium* by the reticulo-endothelial system in new-born piglets deprived of colostrum are reported here. These experiments were performed in fasting piglets 3–5 h after birth. The smooth phase of *S. typhimurium* 34/59 trace labelled with iodine-131 (ref. 4) was injected in an ear vein. The rate of blood clear-