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The Functional Development of the Reticulo-Endothelial System

III. THE BACTERICIDAL CAPACITY OF FIXED MACROPHAGES OF FOETAL AND NEONATAL CHICKS AND RATS

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Summary. The bactericidal capacity of the fixed macrophages of foetal and neonatal rats and chicks was investigated. It has been shown that as a population the phagocytic cells of these embryos lack a bactericidal mechanism for certain strains of Gram-negative bacteria, even in the presence of specific antibody. Neonates, however, were shown to have developed such a mechanism soon after birth. The implication of these findings is discussed in relation to antibody formation.

INTRODUCTION

The existence of a functional reticulo-endothelial system has been demonstrated in foetal chicks (Karthigasu and Jenkin, 1963) and in foetal rats (Reade and Jenkin, 1965). It has been shown that the elements of this system are able to phagocytose foreign particles such as bacteria, carbon and ultramicroscopic sols. There is evidence which indicates that bacteria in particular are phagocytosed by cells of the reticulo-endothelial system only if specific opsonins are present (Karthigasu and Jenkin, 1963), a phenomenon which has been noted frequently in adult animals of various species (Wardlaw and Howard, 1959; Benacerraf, Sebestyen and Schlossman, 1959; Jenkin and Rowley, 1961; Biozzi and Stiffel, 1962).

While opsonins have been implicated also in the intracellular killing of bacteria by these phagocytic cells little is known of the mechanism by which killing takes place or of the development of this capacity as the animal matures (Jenkin, 1963).

It is the purpose of this paper to describe studies which have been made on the changes in the bactericidal potential of the fixed macrophages of the reticulo-endothelial system during the development of chicks and rats from foetuses to neonates.

MATERIALS AND METHODS

Strains of bacteria and their cultivation

The bacteria used in these studies were *Escherichia coli* (Lilly), *Salmonella gallinarum* 9S and *Salmonella typhimurium* C5. These organisms have been described previously (Karthigasu and Jenkin, 1963; Furness and Rowley, 1956). They were used as log phase cultures grown in nutrient broth.

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Opsonization of bacteria

This procedure was as described by Jenkin and Rowley (1961) and was carried out using serum from adult chickens and adult rats.

Operative procedures on foetal and neonatal chicks

Eggs were obtained from a strain of White Leghorn fowls. The eggs incubated for 12, 14, 16, 18 and 20 days were prepared and injected with approximately 10^4 bacteria according to the method of Karthigasu and Jenkin (1963). Following the aspiration of blood at times 0, 15, 30, 45 and 60 minutes the chicks were perfused with 0·15 M NaCl at 37° until the effluent fluid from an incised vein was colourless. The liver, spleen and foetal membranes were excised and macerated in sterile tissue grinders. The homogenates were assayed for viable bacterial content by plating duplicate 0·1 ml samples onto nutrient agar plates. At least two eggs were used for each time period tested. Injection into the neonatal animals was by a femoral vein, and blood samples were collected at determined time intervals by cardiac puncture. The bacterial content of the aspirated blood and of the macerated liver and spleen was assayed as above.

Operative procedures on foetal and neonatal rats

Pregnant Hooded Wistar rats and their foetuses were prepared according to the method described by Reade and Jenkin (1965). The foetuses aged 16, 18, 20 and 22 days were injected via the orbital branch of the anterior facial vein with approximately 10^4 bacteria in 0·05 ml of 0·15 M NaCl. At times 0, 15, 30, 60, 90, 120, 150 and 180 minutes 0·01 ml of blood was aspirated by cardiac puncture and the bacterial population determined by direct plating onto nutrient agar. At these times the liver and placenta of each conceptus was macerated and the bacterial content estimated. At least two foetuses were used for any one time. Neonatal rats aged 10 and 21 days were injected via a femoral vein and blood samples collected by cardiac puncture. Samples of blood and of the homogenized livers of these animals were assayed for bacterial content. The results of these experiments were expressed graphically by plotting $\log_{10}$ of the concentration of bacteria in the various samples against the time after injection.

Virulence tests in foetal and neonatal chicks

Various concentrations of bacteria in 0·1 ml of 0·15 M NaCl were injected into the chorio-allantoic vein of incubating chicks of various ages. The chicks serving as controls were injected with a similar volume of 0·15 M NaCl. After injection the deficiencies in the shells were sealed with Norbecutaine (Evans Medical Ltd, Speke, Liverpool) and the eggs returned to an incubator. Viability of the foetal chicks was assessed at daily intervals by candling and examining for movement. The neonatal chicks were injected via a femoral vein and then housed in compartments in a brooder. The method of Reed and Muench (1938) was used to determine the LD_{50} for the organisms tested.

RESULTS

AGE CHANGES IN THE BACTERICIDAL CAPACITY OF THE FIXED MACROPHAGES OF FOETAL CHICKS TOWARDS *Escherichia coli* (LILLY)

Foetal chicks at various stages in their development were injected intravenously with *Escherichia coli* (Lilly) and the fate of the bacteria followed as described. The results of these

experiments are illustrated in Fig. 1 from which it is apparent that the bactericidal potential of the phagocytic cells of the foetus towards the strain of bacteria increases with increasing age.

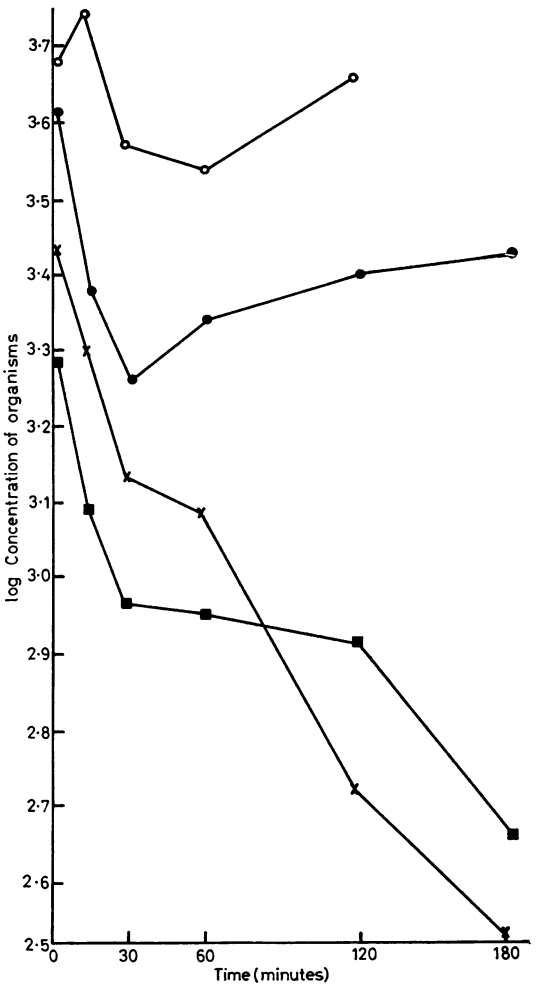

FIG. 1. Survival of *E. coli* (Lilly) in chick embryos of various ages: ○, 11 days; ●, 13 days; ■, 15 days; ×, 17 days.

AGE CHANGES IN THE BACTERICIDAL CAPACITY OF THE FIXED MACROPHAGES OF FOETAL AND NEONATAL CHICKS TOWARDS *Salmonella gallinarum* 9S

Previous studies (Karthigasu and Jenkin, 1963) have shown that foetal chicks lacked opsonins against *Salmonella gallinarum* 9S. Since the foetal chick is deficient in opsonins for *Salmonella gallinarum* 9S, a situation which is reflected in the very low phagocytic index for this organism, adult chicken serum was used as a source of opsonins. From the results of these experiments which are shown in Fig. 2 it can be seen that, even when antibody is

not a limiting factor, these bacteria survive within the phagocytes. It is obvious from Fig. 2, however, that neonatal chicks have developed a bactericidal mechanism within the cells

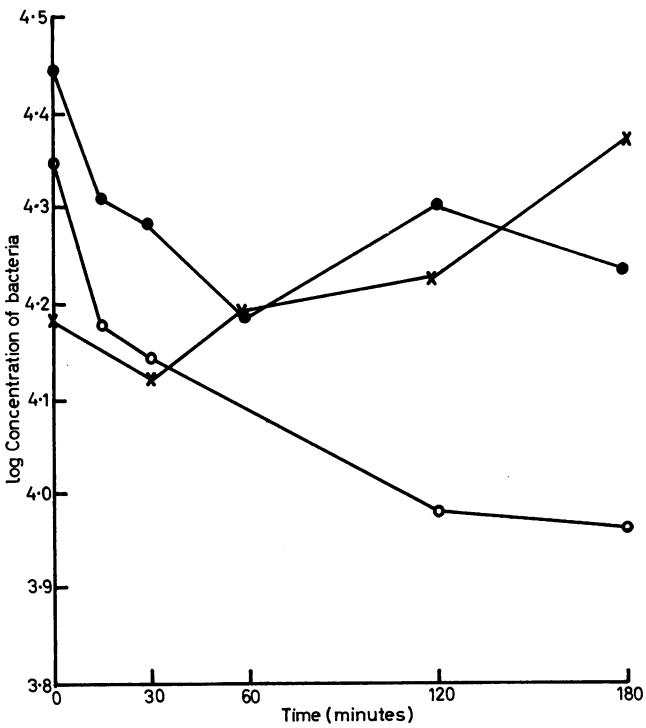

FIG. 2. Survival of opsonized *S. gallinarum* in the chick embryo and natal chick. ×, 17-day-old embryo; ●, 1-day-old chick; ○, 7-day-old chick.

of their reticulo-endothelial systems. Soon after hatching this dramatic change is also reflected in LD_{50} studies which demonstrate a rapid increase in resistance as the chicks mature from foetus to neonate (Table 1).

TABLE 1

THE LD_{50} OF TWO STRAINS OF GRAM-NEGATIVE BACTERIA FOR CHICK EMBRYOS OF DIFFERENT AGES

Days of incubation	LD_{50} *E. coli* (Lilly)	LD_{50} *S. gallinarum* (9S)
11	890	<20
13	2500	<20
14	4200	<20
15	10000	<20
18	60000	<20
1 day after hatching	—	2×10^4
7 days after hatching	—	2×10^7

AGE CHANGES IN THE BACTERICIDAL CAPACITY OF THE FIXED MACROPHAGES OF FOETAL AND NEONATAL RATS TOWARDS *Salmonella typhimurium* C5

Previous studies (Reade and Jenkin, 1965) have shown that this organism is cleared from the blood of foetal and neonatal rats with an increasing rate until birth, from which time the rate remains about the same, until it begins to increase again from the time of weaning. It was also demonstrated in this study that the rates of clearance were not limited by a deficiency of opsonins.

The bactericidal capacity of the phagocytes of the liver of all the ages of foetal rats examined were similar and it will, therefore, suffice to describe the results obtained with 22-day foetal rats. As these foetuses were not perfused, a proportion of the bacteria recovered from each homogenate was attributed to residual blood and when allowance was made for this it was considered that the placenta contained few organisms associated with cells. Fig. 3(a) shows the number of organisms in the circulating blood and in the

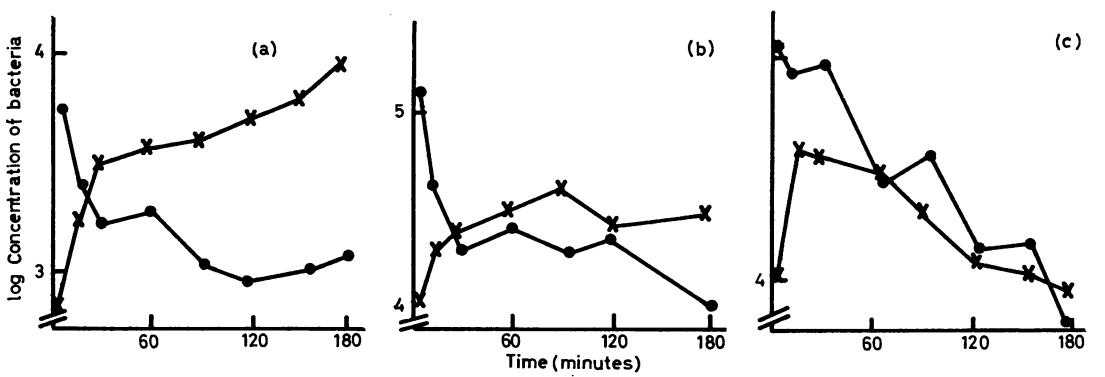

FIG. 3. Survival curves of *S. typhimurium* (C5) in 22-day foetal rats (a), 10-day neonatal rats (b), and 21-day neonatal rats (c). ×, Bacteria associated with the liver; ●, bacteria circulating in the blood.

liver over a period of 180 minutes and this illustrates the elimination of the bacteria from the blood and their accumulation in the liver. The results shown in this figure also indicate an inability on the part of the foetal phagocytic cells to kill the salmonellae which they have ingested. In fact after 60 minutes it appears as if the organisms have commenced to multiply.

Since the phagocytic cells of foetal rats were unable to kill *Salmonella typhimurium* C5 it was of interest to compare the fate of these bacteria with that of an organism susceptible to the bactericidal mechanism of the phagocytic cells of foetal chicks. The experiment was consequently repeated using *Escherichia coli* (Lilly) and the results obtained from 22-day foetal rats are shown in Fig. 4(c). It is apparent that this organism is treated in a similar manner to the salmonellae.

Opsonization of *Salmonella typhimurium* C5 with adult rat serum did not result in any enhancement of bactericidal activity by the foetal phagocytic cells (Fig. 4b).

Adult rats of the strain used are highly resistant to this organism, and experiments were performed on neonatal rats in an attempt to establish the time at which the cells of the reticulo-endothelial system acquired bactericidal properties. Fig. 3(b and c) show the results of experiments on 10- and 21-day neonatal rats. There is evidence of bacteriostasis

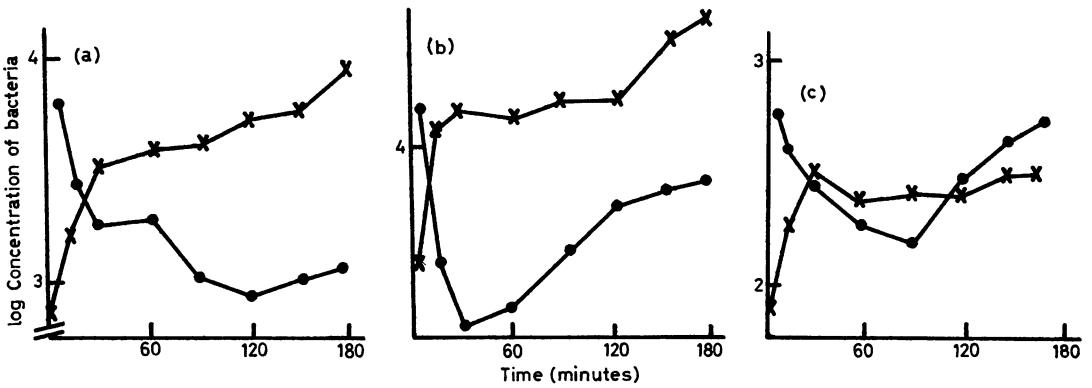

FIG. 4. Survival curves of unopsonized (a) and opsonized (b) *S. typhimurium* C5 and of *E. coli* (Lilly) (c) in 22-day foetal rats. ×, Bacteria associated with the liver; ●, bacteria circulating in the blood.

in the 10-day neonates and killing in the 21-day weanling rats. It appeared, therefore, that the bactericidal properties of the phagocytic cells of the liver of the rat developed to a demonstrable level during the latter part of the suckling period.

DISCUSSION

Recently emphasis has been placed on the role of the macrophage in antibody production. Certain results indicate that the function of the macrophage is to process the antigen and to pass on information, possibly in the form of messenger RNA, to certain primitive lymphoid cells which, following a maturation process, produce the specific antibody (Fishman, 1961; Nossal, Ada and Austin, 1964). Studies on antibody production in various foetal and neonatal animals have shown that the existence of the postulated null period in immunogenesis is dependent both on the type and dose of antigen used to initiate the immune response. It would appear that many embryos and neonates are capable of an immune response providing the right stimulus is given. Indeed in many instances the foetus or neonate may respond as vigorously to an antigenic stimulus as the adult animal (Schinkel and Ferguson, 1953; Smith, 1960; Howard and Michie, 1962; Uhr, Dancis, Franklin, Finkelstein and Lewis, 1962; Silverstein, Uhr, Kraner and Lukes, 1963). The failure of certain antigens to stimulate antibody production may lie in the way the antigen is treated after entering the phagocytic cell. In this connection whilst the present study has not been directly concerned with antibody production, it has revealed that the macrophage population as a whole deals with bacteria differently according to the age of the animal. Furthermore, it would appear from the results with the chick embryo that the capacity of the macrophages at one particular age to deal with ingested particles may vary according to the type of particle used. These results suggest that a study of the rate of degradation of various antigens within phagocytic cells may well throw some light on the phenomenon of antigenicity.

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