THE SPLANCHNIC REMOVAL IN RABBITS DURING FATAL BACTERIEMIAS OF THE CIRCULATING ORGANISMS AND OF SUPERIMPOSED NON-PATHOGENIC BACTERIA*

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There is a considerable difference in the degree of efficiency with which various organisms in the blood are removed in the splanchnic area (1, 2). After lethal intravenous injections of bacteria the initial bacteriemia disappears, but is followed by an increasing bacteriemia later which persists until death (3–5). Hopkins and Parker (4) enumerate as hypotheses which might account for this (*a*) an exhaustion of the removal mechanism, (*b*) the subsidence of an initial stimulation of the removal mechanism, (*c*) the acquisition by the bacteria of a resistance to removal, and (*d*) the continued introduction of bacteria from infected tissues at a rate more rapid than the removal rate. Their studies and those of Bull (3) showed that the repeated introduction of excesses of the same organism during the period of increasing bacteriemia from the initial infection was followed by a rapid removal of the excess bacteria. Organisms which multiplied in the mouse peritoneum and which were resistant *in vitro* to agglutination and phagocytosis were removed with equal rapidity when introduced into the blood stream of the rabbit. Thus their work suggested that the septicemia was maintained by the washing out of organisms from infected tissues into the blood.

The present study was undertaken to compare the efficiency of the splanchnic removal of bacteria from the blood stream in bacteriemias derived from a local dermal infection and in bacteriemias induced by a continuous bacterial infusion. The study was designed further to test quantitatively whether the ability of the splanchnic area to remove a second species of bacteria from the blood was altered by an overwhelming bacteriemia from dermal infection with a first species. The venous catheterization method of investigating the splanchnic removal of bacteria from the blood stream was employed (2).

Methods

Healthy 2.0 to 3.8 kilo albino rabbits were used. Anesthesia was maintained throughout each experiment by local procaine infiltration and by supplementary intravenous injections of sodium pentobarbital as necessary.

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The organisms used were a Type I pneumococcus obtained from Dr. Maclyn McCarty and a hemolytic *Micrococcus aureus* of human origin, coagulase-positive. Pneumococcal bacteriemias were obtained by the dermal infection method of Goodner (6). A superimposed *M. aureus* bacteriemia was induced, as in previous studies (2), by a continuous infusion of bacteria into the superior vena cava. Blood samples were obtained for culture by means of repeated cardiac punctures or catheterization of the inferior vena cava and of the hepatic vein. Blood samples were diluted with beef infusion broth as necessary to obtain countable plates. Differential plate counts were feasible because of the marked difference in the gross appearance of the colonies of the two organisms used. In some instances the induced level of the *M. aureus* count was far below that of the pneumococcus, so that a dilution resulting in countable plates offered the *M. aureus* colonies as discrete and well spaced against a background solid with pneumococcal growth. Suitable control plates showed that this did not introduce an artifact. Plates with and without a heavy pneumococcal inoculum were inoculated further with equal numbers of *M. aureus*. The resultant *M. aureus* counts were essentially the same in the two sets of plates. In other instances the concentration of infusion of the *M. aureus* was increased until the counts were comparable to those of the usual pneumococcal level in bacteriemias of infection.

Four groups of animals were studied:—

Group A.—Seven rabbits received a continuous infusion of Type I pneumococci into the superior vena cava. The inoculum consisted of organisms suspended in 20 per cent human albumin in distilled water. The pneumococci were obtained from blood broth cultures after 8 to 12 hours of incubation at 37°C. The titres of the inoculum ranged from 5×10^8 to 9×10^6 organisms per ml. As in previous studies (2) about 1 ml. of inoculum was infused per minute.

One additional rabbit was infused similarly throughout the first half of the experiment with *M. aureus* suspended in 20 per cent human albumin in saline. The infusion was then discontinued, and, after a brief period of no bacterial infusion, *M. aureus* suspended in physiological saline was substituted throughout the latter half of the experiment. This experiment was designed to control the possible effect of 20 per cent human albumin as the suspending medium on the removal rate. The removal rate was essentially the same during the two infusions.

Group B.—Nine rabbits received intracutaneously 0.2 ml. of a dextrose blood beef infusion pH 7.8 broth culture of a Type I pneumococcus. The cultures were used after 6 to 14 hours of incubation at 37°C. At intervals of 10 to 24 hours after the injection, when the typical dermal lesion and secondary bacteriemia described by Goodner (6) had developed, the animals were studied as outlined above for their ability to remove bacteria in the splanchnic circulation.

Group C.—Ten rabbits received a continuous infusion of *M. aureus* into the superior vena cava. The inoculum consisted of a suitably diluted saline suspension of organisms obtained from a plain agar slant after 6 to 24 hours of incubation at 37°C. The titres of the inoculum ranged from 17×10^3 to 77×10^3 bacteria per ml. As in previous studies (2) about 1 ml. of inoculum was infused per minute.

Group D.—Eight rabbits received Type I pneumococcus intracutaneously, by the same method used in group A. At intervals of 24 to 52 hours from the time of injection, and after a series of hepatic and intracardiac or inferior vena caval blood samples had been obtained for culture, a superimposed *M. aureus* bacteriemia was induced, as in group B, and further blood samples were obtained to determine the percentage removal of *M. aureus* during the course of an overwhelming pneumococcal bacteriemia produced by the dermal infection.

RESULTS

The percentage removal of organisms by way of the splanchnic circulation was calculated from the hepatic venous bacterial counts of blood from the

hepatic vein as compared with counts either of intracardiac blood or of that from the inferior vena cava (2). Previous studies have shown no significant difference between the intracardiac bacterial level and that of the inferior cava (7). In the first three groups of animals the rates of removal in the splanchnic area were as follows:—

Group A (induced pneumococcal bacteriemia): 9 ± 15 per cent (S.D.).

Group B (pneumococcal bacteriemia of infection): 8 ± 14 per cent (S.D.).

Group C (induced *M. aureus* bacteriemia): 62 ± 20 per cent (S.D.).

The wide variation in individual determinations may be in part a reflection of the technical difficulties inherent in any method which attempts the counting of such large numbers of bacteria as were dealt with in the present experiments. Representative bacterial levels are noted in Table I. Despite the wide variation

TABLE I

Group D

Rabbit No.	Duration of dermal infection	Average bacterial count (Bacteria/ml. of intracardiac or inferior vena caval blood)		Per cent splanchnic removal of bacteria	
		Pneumococcal bacteriemia of infection	Induced and superimposed *M. aureus* bacteriemia	Pneumococcal bacteriemia of infection	Induced and superimposed *M. aureus* bacteriemia
	hrs.				
R58	24	70×10^1	26×10^1	1 ± 8 (S.D.)	76 ± 5 (S.D.)
R62	26	1×10^4	25×10^1	5 ± 10	55 ± 18
R64	51	112×10^4	17×10^1	10 ± 14	78 ± 6
R65	51	362×10^4	26×10^1	10 ± 6	71 ± 7
R66	50	2972×10^4	29×10^4	4 ± 6	83 ± 5
R69	52	15×10^4	130×10^1	12 ± 20	57 ± 9
R70	24	3×10^1	8×10^4	16 ± 38	60 ± 9

encountered, the data are useful in showing the relative inefficiency of the mechanism of splanchnic removal for the Type I pneumococcus as contrasted with the significantly greater splanchnic removal of *M. aureus*.

Group D.—Table I presents the bacterial counts per milliliter of intracardiac or inferior caval blood and the per cent splanchnic removal of Type I pneumococcus and of *M. aureus* when an induced *M. aureus* bacteriemia was superimposed upon an overwhelming Type I pneumococcal bacteriemia due to infection. The per cent removal of *M. aureus* in the splanchnic area is significantly greater than that of Type I pneumococcus. Despite the wide variation in the individual determinations, it is evident that the removal of *M. aureus* is of the same order of magnitude in the present group of animals as in the normal control rabbits of group C.

DISCUSSION

The experiments show that *M. aureus* is removed from the splanchnic circulating blood of the normal rabbit much more efficiently than is the pneumococ-

cus. Of the various organisms which have been studied by the present method, the lowest splanchnic removal rate is that for the pneumococcus. The pneumococcus is also the only one of the group of organisms tested with which we have been able consistently to establish a bacteriemia secondary to an experimentally induced local dermal infection. The low removal rate must certainly be a factor in determining whether or not a bacteriemia can become established secondary to a local infection.

The splanchnic removal rate of pneumococci is the same in normal rabbits and in rabbits with bacteriemias secondary to dermal infections. This supports the observations of Hopkins and Parker (4) that the bacteria do not acquire a resistance to removal during the course of fatal infection.

In the rabbit the secondary pneumococcal bacteriemias progress uniformly to a fatal outcome. However, *M. aureus* is removed from the blood stream with the same high degree of efficiency in normal rabbits and in rabbits with an overwhelming pneumococcal bacteriemia secondary to dermal infection. Thus no final exhaustion of the splanchnic removal mechanism can be demonstrated.

The question of multiplication of the bacteria in the blood stream or in the splanchnic area cannot be answered at present. However, the failure to observe a diminution in the degree of splanchnic removal during the course of an experiment suggests that the organisms are not multiplying in the splanchnic area, unless the efficiency of splanchnic removal increases in exact proportion to the rate of multiplication.

Hopkins and Parker (4) concluded that septicemias are maintained by the washing out of organisms from infected tissues. Observations to date support this view. The splanchnic removal rate is a determining factor in the number of organisms which must be introduced into the blood stream per unit of time, and must therefore have considerable influence on the establishment and maintenance of a bacteriemia. Despite this fact, there is no evidence at present to indicate that a bacteriemia can be maintained without a constant introduction into the blood stream of organisms from some source.

SUMMARY AND CONCLUSIONS

1. By a method of hepatic venous catheterization previously described, comparative data have been obtained concerning the removal of pneumococci from the splanchnic circulating blood of the intact rabbit in bacteriemias secondary to a dermal infection and in bacteriemias induced by a continuous infusion of organisms into the blood stream. The average splanchnic removal in secondary pneumococcal bacteriemias was 8 ± 14 per cent (S.D.), in induced bacteriemias 9 ± 15 per cent (S.D.).

2. Similar data were obtained in induced *M. aureus* bacteriemias in normal rabbits and in induced *M. aureus* bacteriemias superimposed upon pneumococcal bacteriemias secondary to dermal infection. The average splanchnic removal

of *M. aureus* in normal rabbits was 62 ± 20 per cent (S.D.). The splanchnic removal of *M. aureus* in rabbits with a simultaneous pneumococcal bacteriemia of infection was of the same order of magnitude as in normal rabbits.

3. The efficiency of the mechanism of splanchnic removal for a given organism is an important factor in the establishment and maintenance of a bacteriemia. Present evidence indicates that the maintenance of a bacteriemia requires a constant introduction into the blood stream of organisms from some source.

4. Pneumococci do not acquire a resistance to removal from the splanchnic circulating blood during the course of fatal infection in rabbits.

5. No final exhaustion of the mechanism of splanchnic removal can be demonstrated in fatal pneumococcal bacteriemias in rabbits.

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