# THE SPLANCHNIC REMOVAL OF BACTERIA FROM THE BLOOD STREAM OF LEUKOPENIC RABBITS*

By GRACE P. KERBY, M.D., AND SAMUEL P. MARTIN, M.D.

*(From the Department of Medicine and the Department of Bacteriology, Duke University School of Medicine, Durham)*

(Received for publication, October 6, 1950)

Leukopenic states are complicated frequently by bacteriemia. The number of bacteria present in the blood stream is determined by the rate at which local tissue defenses permit their entry, by the species of bacteria, and by the rate at which the bacteria are removed from the blood stream. It is well known that the absence of leukocytes interferes with the destruction of the bacteria in the tissues so that large numbers of organisms may enter the blood stream in severe leukopenic states. The quantitative removal of bacteria from the blood stream has been studied previously by a method of continuous intravenous infusion of bacteria combined with a technique of venous catheterization for obtaining repeated samples of blood for culture from the various organs of the intact animal (1). The results showed that bacteria are removed from the blood stream predominantly during their circulation through the organs drained by the hepatic vein (the splanchnic area). The effect of leukopenia on the splanchnic removal of bacteria which reach the blood stream has not been studied. The data presented here demonstrate that a profound leukopenia does not interfere with the efficiency of the splanchnic removal of bacteria from the circulating blood of the intact rabbit.

## EXPERIMENTAL

Healthy rabbits of mixed stock were used. Bone marrow damage was induced by the use of benzene and mechlorethamine hydrochloride. In each instance an attempt was made to lower the peripheral white blood cell count to less than 400 cells per c.mm. before studying the animal for the efficiency of splanchnic removal of bacteria from the blood stream. A high mortality rate was encountered in the course of preparation of the animals for this study.

The organism used was a hemolytic *Micrococcus aureus* of human origin, coagulase-positive. A bacteriemia was induced, as in previous studies (1), by the continuous infusion of bacteria into a marginal ear vein. Blood samples were obtained for culture by means of repeated cardiac punctures or catheterization of the inferior vena cava and of the hepatic vein. Anesthesia was maintained throughout each experiment by local metycaine infiltration.

*Group A.*—Each animal received a daily subcutaneous injection of 2 ml. per kilo of a mixture in equal parts of benzene and olive oil (2). White blood cell counts on peripheral

* This work was supported by the Anna H. Hanes Research Fund.

venous blood were recorded frequently. There was noted considerable variation in the rapidity with which the white blood cell count decreased, and in most instances there were obvious weight loss and diarrhea during the period of observation. Sixteen rabbits died with peripheral white blood cell counts of 8160 to 200 per c.mm. after one to eleven injections of benzene. One animal with a peripheral white blood cell count of 10,080 was sacrificed after twenty-two injections of benzene. The following three rabbits survived the period of preparation and a subsequently induced bacteriemia:—

*No. 4.*—Initial weight 2.5 kilos. Splanchnic removal of bacteria was studied after eight injections of benzene over a 9 day period, after which time the peripheral white blood cell count was 400 per c.mm.

TABLE I

*Per Cent Splanchnic Removal of M. aureus in Groups A and B*

| Rabbit | Drug | WBC per c.mm. at time of induced bacteriemia | Average per cent E. |
|---|---|---|---|
| | | | *per cent* |
| 10 controls (3) | | | 62 ± 20 (S.D.) |
| *Group A* | | | |
| No. 4 | Benzene | 440 | 72 |
| No. 12 | Benzene | 40 | 69 |
| No. 16 | Benzene | 65 | 73 |
| *Group B* | | | |
| No. 25 | Mechlorethamine HCl | 204 | 82 |
| No. 33 | Mechlorethamine HCl | 144 | 73 |
| No. 36 | Mechlorethamine HCl | 336 | 85 |

*No. 12.*—Initial weight 2.5 kilos. The animal was studied after seven injections of benzene in 7 days, after which time the peripheral white blood cell count was 40 per c.mm.

*No. 16.*—Initial weight 2.7 kilos. A bacteriemia was induced after nine injections of benzene in 9 days, after which time the peripheral white blood cell count was 65 per c.mm.

After a series of blood samples had been obtained for culture, each of the animals in group A received 7 ml. of thorotrast[1] intravenously, by a single injection in rabbit 4, divided into three injections at 5 minute intervals in rabbit 12, and divided into five injections at 5 minute intervals in rabbit 16. Repeated samples of blood for culture were obtained from rabbits 4 and 16 at intervals after the administration of thorotrast (47 to 105 minutes and 31 to 52 minutes respectively).

The clearance data on these animals are combined with the group B data and presented in Table I.

*Group B.*—Each animal received intravenously 10 mg. of mechlorethamine hydrochloride. White blood cell counts on peripheral venous blood were recorded daily thereafter. There

[1] A stable colloidal thorium dioxide contrast medium prepared by Heyden Chemical Corporation, New York.

was no obvious weight loss or diarrhea in these animals, but four rabbits died 6 to 8 days after the injection, and one rabbit died 1 day after a second injection of 10 mg. of mechlorethamine hydrochloride given on the 4th day after the first injection. Peripheral white blood cell counts in these five animals were 1660 to 168 per c.mm. terminally. The following three animals survived the period of preparation and a subsequently induced bacteriemia:—

*No. 25.*—Initial weight 2.5 kilos. This animal received a second intravenous injection of 10 mg. of mechlorethamine hydrochloride 4 days after the first injection, and splanchnic removal of bacteria was studied on the day following the second injection, at which time the peripheral white blood cell count was 204 per c.mm.

*No. 33.*—Initial weight 2.5 kilos. Splanchnic removal was studied 4 days after the injection of mechlorethamine hydrochloride, at which time the peripheral white blood cell count was 144 per c.mm.

*No. 36.*—Initial weight 2.5 kilos. A bacteriemia was induced 2 days after the injection of mechlorethamine hydrochloride, at which time the peripheral white blood cell count was 336 per c.mm.

After a series of blood samples had been obtained for culture from the group B animals, each rabbit received intravenously 10 ml. of 10 per cent Higgins India ink in physiologic saline, immediately after which the animal was sacrificed by means of a lethal intravenous dose of nembutal.

In every instance in groups A and B, blocks of liver, spleen, and femoral bone marrow were obtained at the close of the experiment for histologic examination.

Histologically the liver, spleen, and femoral bone marrow of five of the six rabbits of groups A and B showed virtually total destruction of the blood-forming tissues. One rabbit (No. 33) of group B showed severe damage in these areas, but there remained some erythropoietic and granulopoietic foci in the femoral bone marrow. In all six animals there was no histologic evidence of damage to the Kupffer cells, and ample phagocytosis of thorotrast and India ink by the Kupffer cells of the liver and by the macrophages of the spleen could be demonstrated.

The bacteriologic findings in these six animals in which blood-forming tissues had been severely damaged and bacteriemia induced are summarized in Table I. There is no impairment of the efficiency with which *M. aureus* is removed from the splanchnic circulating blood of these animals, as compared with a group of ten normal animals drawn from a previous study (3).

In rabbits 4 and 16, after thorotrast was given intravenously, the average per cent removal rates were 24 per cent (No. 4) and 58 per cent (No. 16). On the basis of extensive studies on thorotrast blockade to be published from this laboratory, these bacterial removal rates, while variable, are interpreted as being suggestive of early blocking.

*Group C.*—Each animal received intravenously 10 mg. of mechlorethamine hydrochloride. White blood cell counts were recorded daily thereafter. There was no obvious weight loss or diarrhea in these animals.

*Group C-1.*—On the 3rd day after the injection, each of four rabbits (Nos. 39, 40, 43, 45) with white blood cell counts of 572, 352, 374, and 594 per c.mm., respectively, were paired with normal control rabbits, and each pair of animals received an intramuscular injection of

large numbers of *M. aureus* in 3 ml. total volume, divided between two sites. The inoculum titre was $66 \times 10^7$ *M. aureus* per 3 ml. for each of two pair (Nos. 39 and 41, Nos. 40 and 42) and $86 \times 10^7$ *M. aureus* per 3 ml. for each of the two remaining pairs (Nos. 43 and 44, Nos. 45 and 46). Blood for culture was obtained by cardiac puncture from the first two pairs of animals at intervals of 30 minutes, 1 hour, 2 hours, 4 hours, 8 hours, and 12 hours after the bacterial injection. Blood samples were obtained similarly from the second two pairs prior to bacterial injection and at intervals of 1 hour, 4 hours, and 5 hours thereafter.

The four control animals and two of the four leukopenic animals (Nos. 43 and 45) of this group had negative blood cultures during the 12 hour period of observation following the injection. One leukopenic animal (No. 39) showed a progressive *Pseudomonas aeruginosa* bacteriemia, the first blood sample obtained 30 minutes after injection of *M. aureus* being positive for *Ps. aeruginosa*. One leukopenic animal (No. 40) developed blood cultures positive for *M. aureus* 8 hours after the injection of *M. aureus*, and the bacteriemia was progressive.

*Group C-2.*—Four additional rabbits (Nos. 47, 48, 49, 50) received intramuscular injections of *M. aureus* of the same order of magnitude as above, on the 3rd day after receiving mechlorethamine hydrochloride. At the time of the bacterial injection, the white blood cell counts were 165, 187, 550, and 682 per c.mm., respectively. All these animals were catheterized 16 to 22 hours after the bacterial injection, and hepatic venous blood samples were obtained for culture comparison with peripheral venous blood cultures.

Three of the four leukopenic animals that were catheterized did not show a bacteriemia when studied. One animal (No. 47) had a secondary *M. aureus* bacteriemia, and three paired samples of blood for culture showed an average splanchnic removal rate of 63 per cent, as compared to $62 \pm 20$ per cent (S.D.) in the control rabbits (3).

*Group C-3.*—Two additional rabbits (Nos. 35 and 34) with white blood cell counts of 650 and 1050 respectively were paired each with a control animal, and each received subcutaneous injections of *M. aureus* of the same order of magnitude as above, 2 and 3 days, respectively, after the test animals had received mechlorethamine hydrochloride. Blood samples were obtained for culture thereafter at intervals of 4 to 26 hours.

Blood cultures were negative for *M. aureus* in one leukopenic and two control rabbits of this group. The second leukopenic rabbit (No. 35) showed a few colonies of *M. aureus* per ml. of blood 24 hours after injection. There was a striking difference in the gross appearance of the local lesion in both of the leukopenic animals as compared with the control rabbits. Large, flat, sharply marginated, purpuric lesions without any erythema or edema developed within 5 hours around the injection site in the leukopenic animals, and the lesions began to show central fading within 24 hours. The control animals showed a small purpuric area surrounded by a large zone of intense erythema within 5 hours at the injection site, and within 24 hours the purpuric element faded while the erythema became more intense and, in one instance, edema became

marked. Histologically the difference in the skin lesions as observed in sections obtained from one pair of animals (Nos. 34 and 37) was equally striking. The lesion from the control animal showed a zone of necrosis in the cutis surrounded by a thick ring of neutrophils with some necrosis also in this cellular zone. The lesion from the leukopenic animal showed absolutely no cellular reaction, but all of the tissue including not only the skin but the underlying fat and muscle was dead, having the appearance of tissue allowed to autolyze before fixation. Great numbers of cocci and colonies of proliferating organisms could be seen even in the preparation stained with hematoxylin. There was necrosis of blood vessels with hemorrhage.

## DISCUSSION

It is evident from the data presented here that the rabbit with histologically demonstrable, severe damage to the blood-forming tissues retains a highly efficient splanchnic mechanism for the removal of bacteria from the blood stream. This is in complete accord with previous observations made in dogs by the venous catheterization technique (1) in which no consistent ability to remove organisms from the circulating blood outside the splanchnic area could be demonstrated. The present data make possible also an evaluation of the relative importance of macrophagic and leukocytic activity as observed histologically in the splanchnic area by Cannon and his coworkers (4) and by Steffee (5). Although these investigators could demonstrate histologically the phagocytic activity of both macrophages and leukocytes in the splanchnic area during the clearing of injected bacteria from the blood stream of the rabbit and the rooster, the present studies suggest that the over-all role of the leukocytes in this situation is quantitatively unimportant. As pointed out by Rich (2), the macrophages of the splanchnic area are very resistant to benzene intoxication, and these cells may be presumed to be the active phagocytes in the present studies in the absence of histologic evidence of their damage and in the presence of histologic and hematologic evidence of profound damage to the blood-forming elements. Thus while the leukocytes undoubtedly play a role in the control of infection in local situations, as confirmed by Wood (6) and many others, the importance of their role in significantly affecting the clearance of bacteria from the splanchnic circulating blood appears to be minimal. This is in accord with Wright's observation (6*a*) that a very considerable reduction in leukocytes may be made without seriously affecting the capacity of the rabbit to clear its blood of bacteria following a single intravenous injection of virulent pneumococci.

As previously noted (7, 8, 1) all present evidence indicates that a bacteriemia cannot be maintained unless the organisms are introduced constantly into the blood stream from some source. Rich and McKee (9, 2) have demonstrated that the leukocyte is not an essential agent in the immobilization of bacteria

at the site of local injection, provided the animal possesses an active or passive immunity to the infecting organism. However, even in the immune animal, immobilization of bacteria at the site did not prevent the progressive growth of bacteria in the absence of leukocytes, and a delayed but progressive bacteriemia invariably followed. The failure of development of a secondary bacteriemia following the intramuscular and subcutaneous injection of organisms into leukopenic animals in the present study must be assumed to be due to the short period of observation following the injection of the organisms. In the one instance in which a leukopenic rabbit survived to develop a secondary bacteriemia which was studied by the venous catheterization technique, there was no evidence of impaired function of the splanchnic mechanism for removing the bacteria from the blood stream. This continued function of the splanchnic removal mechanism during the course of a bacteriemia secondary to local infection has been noted previously (3).

The present study demonstrates again the remarkable and persistent efficiency of the splanchnic removal mechanism in clearing the blood stream of bacteria and reemphasizes the apparent necessity of a constant source of organisms for the maintenance of a bacteriemia. It is toward the identification and eradication of the local source that attention should be directed particularly in the leukopenic state. The leukopenia appears to impose no hardship in so far as the splanchnic clearing of the blood stream of bacteria is concerned.

## SUMMARY

In rabbits subjected to profound damage of the blood-forming tissues by the use of benzene and mechlorethamine hydrochloride, no impairment of efficiency in the splanchnic removal of *M. aureus* from the blood stream could be demonstrated by a method involving catheterization of the hepatic vein.

All evidence continues to indicate that a bacteriemia cannot be maintained unless the organisms are introduced constantly into the blood stream from some source.

The importance of the leukocyte in the establishment and clearing of the bacteriemias appears to lie in its phagocytic activities at the local source of the bacteriemia rather than in any quantitatively significant phagocytosis of organisms circulating in the blood stream.

The present studies suggest that in the study of the leukopenic state attention should be directed more especially toward the identification and eradication of the local source of organisms.

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