THE INFLUENCE OF ANEMIA ON PHAGOCYTIC FUNCTIONS
AND RESISTANCE TO INFECTION IN MICE

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INTRODUCTION

A

N INCREASE in the phagocytic activity of granulocytes in anemic human
blood, as measured in vitro, was found by Berry, Davis, and Spies\(^1\) to occur
in all classes of anemia tested. The increase was maintained when washed
"anemic" leukocytes were tested in the presence of either normal serum or the
homologous anemic serum. Berry, Leyendecker, and Spies\(^2\) interpreted this as
indicating a fundamental change in the phagocyte rather than in some property
of the serum. In order to pursue these interesting observations further under more
rigidly controlled conditions, Berry and Haller\(^3\) subjected male albino rats to
periodic bleeding and followed the changes in phagocytic function that accom-
panied the development and remission of the anemia. They found that phagocytosis
became more active as the anemia became more severe and then fell to slightly
subnormal levels with recovery of a normal blood picture. They also demonstrated
an increase in the phagocytic activity of the macrophages from a peritoneal exudate
during the time of severest anemia and found that it was essentially equivalent to
the increase observed for blood taken at the same time. The macrophages had
normal phagocytic activity when tested again after the animals had recovered
for four weeks.

In spite of the similarity between the results with human and with rat blood,
it was considered desirable to attempt to duplicate these observations in still
another species and to supplement the in vitro measurements with tests in vivo.
Accordingly, the albino mouse was chosen, since it could be studied in sufficient
numbers for the results to be statistically significant. The general plan was,
therefore, to bleed the mice until an anemia developed and to test the phagocytic
activity of the neutrophiles at intervals during this period. When the anemia
was pronounced and phagocytosis showed an appreciable increase above normal
levels, the death rate of the bled animals infected with Salmonella typhimurium
was compared to that of controls similarly infected. The anemic mice were found
to be two or three times as resistant to the infection as the control mice.

METHOD

Young adult male albino mice of the CF-1 strain obtained from Carworth Farms
were maintained on a commercial diet of "rabbit pellets." They were given both

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food and water ad libitum. Two lots of animals were used in two series of experiments, and some modifications, which will be indicated, were made in the procedure with the second group.

In order to induce anemia, the mice were bled according to the method of Tabor, Kabat, and Rosenthal. The animals were sorted according to body weight, to the nearest gram, for each bleeding, and a volume of blood equal to 2 per cent of total body weight was removed. The 2 per cent volume was used because Tabor et al. have shown that a very low mortality results from this volume of blood loss.

In the first series, they were bled twice weekly for four and one-half weeks, and in the second series they were bled every third day for nine bleedings.

In the first series only, phagocytic activity was measured in vitro according to the method of Cottingham and Mills, with a few minor modifications previously described by Berry, Leyendecker, and Spies. The volume of whole blood used for phagocytic measurements was reduced from 0.5 ml. to 0.25 ml. which was diluted in an equal volume of heparinized physiological solution of sodium chloride.

For these determinations, the mice were bled by cardiac puncture under light ether anesthesia using a tuberculin syringe and a 27 gage needle. Sufficient blood was removed and discharged into a paraffined watch glass so that total erythrocyte and total leukocyte counts could also be made with this blood, using Bureau of Standards pipettes and cover glasses for the counting chambers. Hemoglobin was determined colorimetrically. The same data were collected on control animals for comparison. Complete determinations were made on the sixth, eighth, and ninth bleedings of the first series. Bleeding by cardiac puncture resulted in the death of a majority of the animals. For this reason, the blood counts that were made ten days after the last bleeding were from tail blood. In the second series, no phagocytic measurements were attempted and blood counts were taken with tail blood on the third and ninth bleedings.

Each series was begun with 108 animals being bled. In the first series 63 survived at the time of infection. Only 1 death was thought to be due to loss of blood. Most of them died as a result of injury received while being bled from the heart. Ninety-four mice survived the last bleeding in the second experiment. One half of the survivors in this series were bled, after being infected, every third day for three additional bleedings.

The mice were infected intraperitoneally with a suspension of Salmonella typhimurium† in physiological saline. This route of infection was selected with the hope that the cellular defense of the animal would be as rigidly tested as possible.

The density of the suspension was measured in a Coleman spectrophotometer after washing the cells through three changes of saline. A reading of 75 per cent transmission at 550 mµ was used. The suspension was then diluted 1:10 and a volume of 0.1 cc., measured in a tuberculin syringe, was injected. This amount of inoculum was determined by a series of preliminary injections into control mice.

* The heparin was supplied through the courtesy of Dr. Leo A. Pirk of Roche-Organon, Inc., Nutley, N. J.
† The culture was obtained from Dr. James A. Harrison, Department of Biology, Temple University, Philadelphia, Pa.
Postmortem examinations were made each day on some of the mice that died of the infection. Histological slides were prepared, at intervals, of the liver, spleen, ileum, large intestine, and lungs as a check on the pathology of the organs.

RESULTS

First Series. In this set of experiments, the aim was to subject the mice to sufficient blood loss to produce anemia and to measure the phagocytic function of the leukocytes during this period. The results presented in table 1A give the date of the bleeding in column 1 with the number of the bleeding in parenthesis. The first blood counts were made at the third bleeding and the total erythrocytes (column 2) and hemoglobin value in grams per 100 ml. of blood (column 4) are within normal limits, according to the values given by Fekete. Each value entered in the table is the arithmetic mean of from 5 to 10 individual determinations. The hemoglobin concentration in grams on July 6 shows a slight decline, but it was again above eleven grams on July 9. However, on this same date the erythrocyte total was down to 5.33 millions. The following entry, on July 12, shows, in addition to the red cell count and hemoglobin level, the leukocyte total in column 6 and the phagocytic activity count of 10.7 bacteria per cell in column 8. These may be compared with the control values entered in columns 3, 5, 7 and 9. Phagocytosis by the cells of the bled animals is 147 per cent of normal on this date, and the increase is statistically highly significant, as shown in column 11. Similar data are given in the table for both July 19 and July 23, the eighth and ninth bleedings, respectively. The total erythrocyte count was reduced to less than five million and the hemoglobin concentration was more than 2
grams below the value for control animals. It is particularly interesting to note that the total leukocyte counts on July 23 are essentially the same for both the bled group of mice and for the controls. On this same date, phagocytosis was 161 per cent of normal, column 10, with great statistical significance. Phagocytosis was also measured using a culture of Salmonella typhimurium in vitro with the result, not shown in the table, that the bled animals had an activity 250 per cent of normal for this organism.

On the following date, July 24, all 63 surviving bled animals were infected, as previously described, along with the same number of control mice. The percentage survival curves for each group are shown in figure 1. Exactly twice as many bled animals as controls survived the infection—28 versus 14. It should be observed that most of the protection came in the first three days postinfection and that thereafter the two curves follow essentially parallel courses. The death rate, therefore, from the third day on was the same in the two groups, and the difference in number of survivors remained practically constant.

Daily observation of the appearance and behavior of the mice was equally important in conveying the impression of differential susceptibility to disease in the two groups. From the first day after infection, the majority of the control animals lost their activity and sat hunched in a corner of the cage, usually with
their heads bent down. The fur was ruffled and dull. Anorexia was pronounced and conjunctivitis resulting in closure of the eyelids was frequent. In the bled group, normal activity was noticeable in the majority of the mice, especially during the first few days postinfection. They maintained the usual interest in food, and even during the period of maximum fatality some members of the group seemed free of disease. It would have been perfectly apparent to any casual observer that these mice were in better condition than the control animals.

![Graph](image_url)

**Fig. 1.** Same as figure 1. The arrows indicate the day that the mice in the bled anemic group had blood equal to 2 per cent of total body weight removed.

Postmortem examinations were also made on mice dying each day after infection. The findings were in agreement with those described by Dingle for acute infections with Salmonella typhimurium following massive doses. There was congestion in all blood vessels and viscera, and the liver and spleen were usually enlarged and dark red in color. Microscopically, the organs were hyperemic and fatty degeneration was found in the liver. After the fourth day, small, yellow, pin-head lesions were visible in the livers of some of the mice and sections of the spleen showed marked infiltration with inflammatory cells. Bacteriological tests for members of the Salmonella group were made each day by culturing the heart’s blood, spleen, liver, lungs, and intestinal contents of a mouse that had...
recently died. Standard methods were employed and positive tests for Salmonella were always obtained in at least one of the cultures. From the combined results, it seemed safe to attribute at least the majority of deaths to infection with mouse typhoid.

Second Series. A repetition of the first experiment was considered advisable for two reasons. In the first place, the experimental protection of animals against disease by bleeding to an anemic level before infection was, in itself, sufficiently new to justify a duplicate experiment for purposes of verification. And secondly, the observation that most of the protection came in the first three days after infection suggested that another bleeding at this time might extend the protective period. In the second series, therefore, one half of the bled animals (47) were bled every third day for three additional bleedings in order to test this possibility. The bled animals at the time of infection were known to be anemic, as shown in table 1B. Measurements of phagocytic functions were omitted in this series, since the results in table 1A were confirmation enough of the in vitro increase in phagocytosis that accompanies anemia.

The survival curves obtained with this group of animals are shown in figure 2. The mortality of the control mice was higher than in the first series, while the anemic group, which is to be compared with that in figure 1, shows the same percentage survival but an even greater relative resistance. No deaths occurred during the first three days postinfection, and a lower death rate, compared to the controls, was obtained between the third and fourth days. Beyond that time, the two curves are in agreement. The anemic group that was bled after the infection gave a survival curve intermediate between that of the controls and of the other experimental group, but more closely approximating the former. It is perfectly clear that the additional bleedings increased the death rate of these mice. Whether this is due to the bleeding alone or to a diminished bodily resistance is a question which still remains to be answered.

DISCUSSION

The present study shows that the neutrophiles of anemic mice, studied in vitro, have an increased phagocytic activity when compared to those of normal animals. These results are in agreement with those previously described by Berry, Davis, and Spies for anemic human beings and by Berry and Haller for anemic rats. In vitro measurements of phagocytosis, however, are subject to certain unpredictable errors, as shown by Berry, Leyendecker, and Spies, and drawing conclusions from the number of phagocytic cells included in the phagocytic counts for the rats and mice is open to the same criticisms as have been leveled at the work of Cottingham and Mills by Harmon, Zarafoitis, and Clark.

Since the mice having the elevated phagocytic functions were also more resistant to infection than control mice, there is good reason to believe that the in vitro measurements have validity. It must be recognized, even in the face of this evidence, that these experiments merely suggest that the protection against mouse typhoid that is associated with anemia is directly attributable to an increase in phagocytosis. Other defense mechanisms which have not been subjected to measurement could be involved, but at least the cellular defense is elevated. The possible cor-
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relation between in vitro phagocytosis and resistance to infection should yield valuable information on this point. If a transfer of knowledge gained from the rat to a species as closely related as the mouse is permissible, it might be argued that this cellular defense applies to both microphages and macrophages, since Berry and Haller found the phagocytic function of both cellular types increased during anemia in the rat.

The fundamental question that must ultimately be answered in this work is the question of how the phagocyte becomes more active during the development of anemia. There is good reason to believe it is due to a fundamental change in the phagocyte (Berry, Leyendecker, and Spies) but there are no clues regarding the nature of this change. Only one attempt has been made to obtain a cell-free extract from leukocytes in anemic blood, and in this attempt no increase in the phagocytosis of normal human neutrophiles, measured in vitro, was produced by the extract. Should some means of enhancing the cellular defense mechanism prove applicable, however, a new therapeutic tool of tremendous value would become available. It is not inconceivable that the sulfonamides and antibiotics combating the infectious organisms might be supplemented by artificially enhanced phagocytosis. With this as the primary justification for the work, additional experiments are in progress to analyze further phagocytic functions.

SUMMARY

The increase in phagocytic function of neutrophiles of mice was measured during the progressive development of anemia due to blood loss. The animals were bled from the tail, 2 per cent of total body weight being removed twice weekly for nine bleedings. Phagocytosis was 161 per cent of normal at the end of this period, and the erythrocyte totals and hemoglobin values were approximately two thirds of normal. The anemic mice were infected intraperitoneally with a suspension of Salmonella typhimurium and their survival rate was compared with normal mice similarly infected. Twenty-eight of 63 anemic mice survived and 14 of 63 normal mice survived.

In a second experiment, mice were subjected to bleeding every third day for nine bleedings, and were then infected along with a control group. One half of the anemic animals were bled for three additional bleedings after the infection. Two of 48 control animals survived, 5 of 47 anemic mice, additionally bled, survived, and 19 of 47 anemic mice, not bled after infection, survived. This suggests that the increase in phagocytosis that accompanies anemia may be, at least in part, responsible for the increased resistance to infection.

REFERENCES


* This sample of anemic blood was obtained from Max Strumia, M.D., of the Bryn Mawr Hospital, and the test was performed by one of us (LJB) with the assistance of Miss Ruth Leyendecker.
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