JCI The Journal of Clinical Investigation

THE ANEMIA OF INFECTION. II. THE EXPERIMENTAL PRODUCTION OF HYPOFERREMIA AND ANEMIA IN DOGS

G. E. Cartwright, … , I. M. Merrill, M. M. Wintrobe

J Clin Invest. 1946;25(1):81-86. https://doi.org/10.1172/JCI101691.

Research Article

Find the latest version:

https://jci.me/101691/pdf

THE ANEMIA OF INFECTION. II. THE EXPERIMENTAL PRODUCTION OF HYPOFERREMIA AND ANEMIA IN DOGS [1]

By G. E. CARTWRIGHT, M. A. LAURITSEN, S. HUMPHREYS, P. J. JONES, I. M. MERRILL, AND M. M. WINTROBE

(From the Department of Medicine, University of Utah School of Medicine, Salt Lake City)

(Received for publication July 7, 1945)

In the preceding paper (1), it was reported that the anemia of infection in humans is associated with hypoferremia and is accompanied by an increase in the content of copper in the serum, as well as by coproporphyrinuria and an increase in erythrocyte protoporphyrin. The theory was offered that the anemia of infection is the consequence of an alteration in the intermediary metabolism of iron which diverts this metal to the tissues and makes it unavailable for hemoglobin synthesis.

In this paper, the results of the experimental production of inflammation in dogs are presented, as well as experiments devised to elucidate the pathogenesis of the hypoferremia and anemia which developed.

METHODS

Large mongrel dogs were used for these experiments. The strain of hemolytic *Staphylococcus aureus* used was isolated from the draining sinus of a patient with chronic osteomyelitis and anemia of long standing. Toxin was prepared by heating a 15-day broth culture at 70° C. for 2 hours in an oven at atmospheric pressure. Commercial triple typhoid vaccine was used as a source of typhoid toxin. For the production of sterile turpentine abscesses, oil of turpentine, rectified, U.S.P. was used. Sterile pleural effusions were produced by first anesthetizing the dogs with a 5 per cent solution of pentobarbital, given intravenously, and then injecting 1.5 ml. of turpentine intrapleurally under sterile precautions. The effusions were removed after 48 hours with an aspirating needle.

The volume of packed red cells was measured by the method of Wintrobe (2). Plasma iron was determined by using the procedure of Kitzes, Elvehjem, and Schutte (3). The erythrocyte protoporphyrin determinations were performed by the method of Grinstein and Watson (4).

[1] The work described in this paper was carried out under a contract, recommended by the Committee on Medical Research, between the Office of Scientific Research and Development and the University of Utah. The expenses of this investigation were also defrayed in part by grants from Parke, Davis and Company and the Upjohn Company.

EXPERIMENTAL DATA

The effects of the intramuscular injection of a pure growth of hemolytic staphylococcus aureus in 3 animals on the volume of packed red cells and on the plasma iron are shown in Figure 1. Following 5 initial observations under normal conditions in each animal, 2 ml. of the broth culture were injected, and the dose doubled each day thereafter for 4 days. A large abscess involving the entire leg developed in each case and 3 to 4 days later a profuse purulent exudate began to drain. Complete recovery took place only after 3 to 4 weeks. As can be seen from the figure, the plasma iron dropped precipitously to levels of 15 to 30 micrograms per cent in 48 hours. Maximal anemia did not develop until approximately 15 days later at which time the plasma iron had risen to slightly above the preinjection level. Following this rise, the volume of packed red cells and plasma iron returned simultaneously to the normal levels. Erythrocyte protoporphyrin was measured at intervals throughout the experiment, but the value did not rise above the normal range of 30 to 50 micrograms per 100 ml. RBC.

To eliminate the possibility that the changes observed might be due to the repeated withdrawal of blood, 3 control animals were subjected to the same procedures, except that infections were not produced in them. Neither anemia nor hypoferremia developed. The volume of packed red cells remained above 43 ml. per 100 ml. of blood, and the plasma iron values were never less than 100 micrograms per cent.

To ascertain whether or not the changes observed in association with infection (Figure 1) were due to the elaboration of bacterial toxins, staphylococcal toxin was injected intramuscularly into 3 animals for 4 consecutive days. The results are presented in Figure 2. Only a very questionable fall in plasma iron occurred and no anemia developed. The experiment was then repeated (Figure 3) using a larger initial dose of toxin, and a definite fall in plasma iron resulted. The plasma iron dropped, however, not to between 15 and 30 micrograms per cent as it did when an infection had been produced (Figure 1), but only to between 75 and 110 micrograms per cent. Moreover, the pre-injection plasma iron levels were higher than in the animals to which smaller doses of toxins had been given. There was a moderate local reaction at the sites of the injections, the dogs became lame, and there was a definite systemic reaction. Typhoid vaccine (Figure 4) in the doses used failed to produce hypoferremia. No local or systemic reactions were noted.

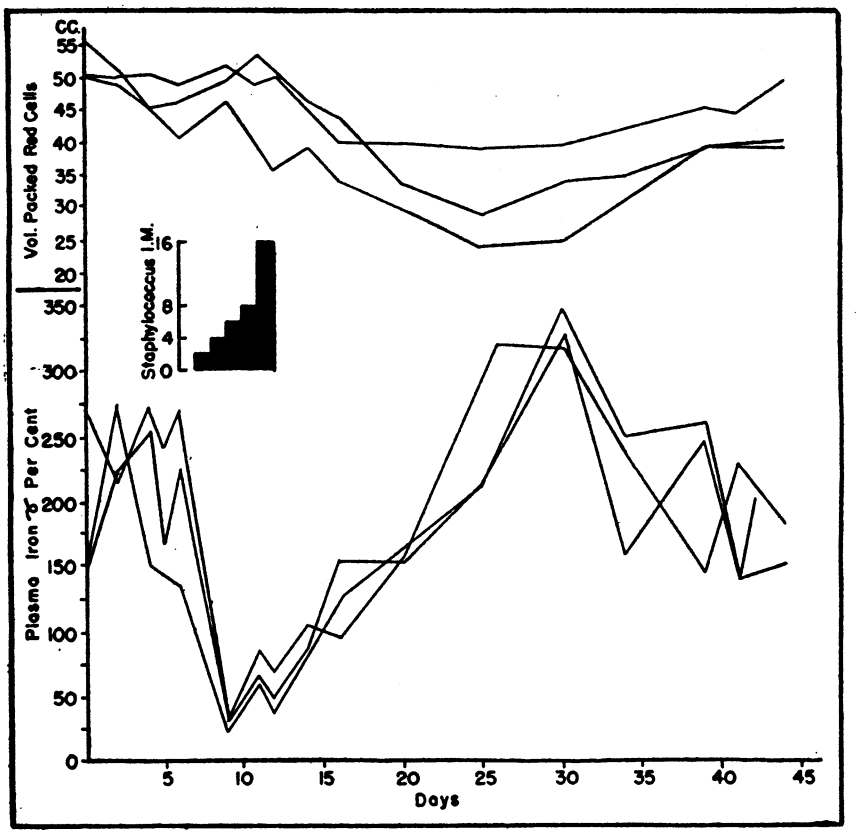

FIG. 1. HYPOFERREMIA AND ANEMIA DEVELOPING AS THE RESULT OF THE PRODUCTION OF STAPHYLOCOCCUS ABSCESSES IN 3 DOGS

The abscesses healed after 3 to 4 weeks.

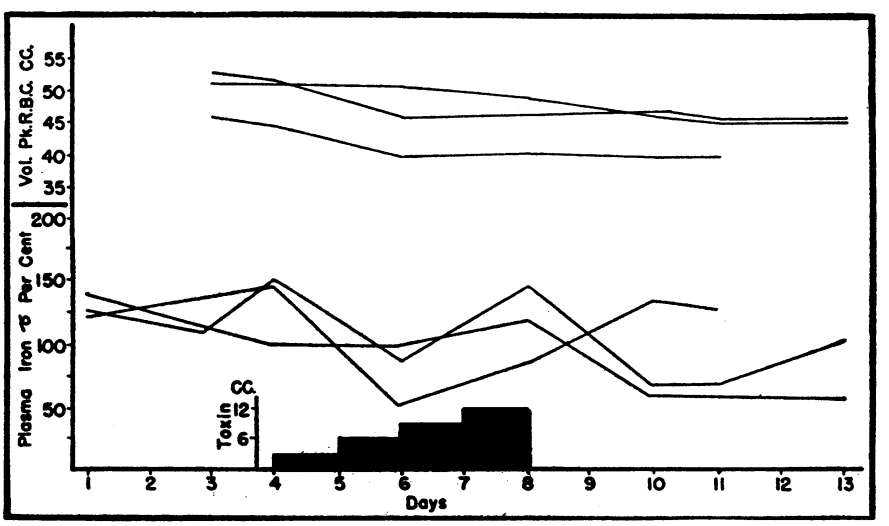

FIG. 2. THE EFFECT OF THE INTRAMUSCULAR INJECTION OF STAPHYLOCOCCUS TOXIN ON THE PLASMA IRON AND VOLUME OF PACKED RED CELLS OF 3 DOGS

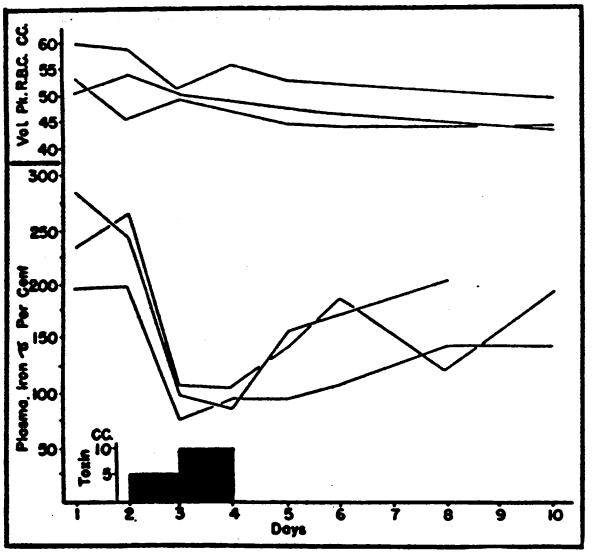

FIG. 3. THE EFFECT OF THE INTRAMUSCULAR INJECTION OF LARGER AMOUNTS OF STAPHYLOCOCCUS TOXIN IN 3 DOGS

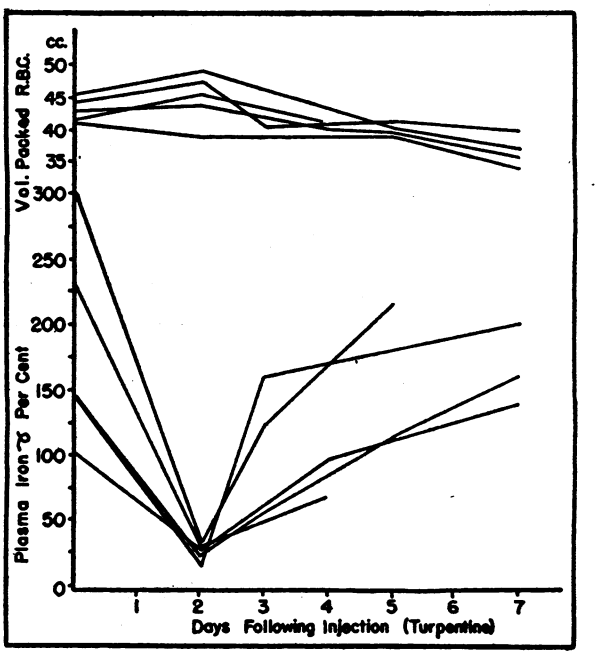

FIG. 5. RAPID DEVELOPMENT OF HYPOFERREMIA AND MODERATE ANEMIA FOLLOWING THE INTRAMUSCULAR INJECTION OF 10 ML. OF STERILE TURPENTINE IN 5 DOGS

It has been shown that a marked derangement in cellular metabolism occurs in inflammatory tissue and that specific substances are elaborated which cause fever, leukocytic migration, and leukocytosis and also affect the bone marrow (5). To investigate the possibility that inflammatory tissue might be capable of elaborating a substance which lowers plasma iron, sterile intramuscular abscesses were produced in 16 dogs with turpentine. Representative results in 5 animals are shown in Figure 5 where it can be seen that the plasma iron fell precipitously during the first 48 hours following injection of turpentine and rose quite rapidly thereafter. The volume of packed red cells declined slightly in 7 days. There was no change in the erythrocyte protoporphyrin content in this time. All of the dogs were acutely ill and incapacitated from the single injection of 10 ml. Marked edema involving the entire extremity developed in about 48 hours. A few of the abscesses broke down and exuded a thin, slightly hemorrhagic material. These dogs subsequently recovered without the development of definite anemia.

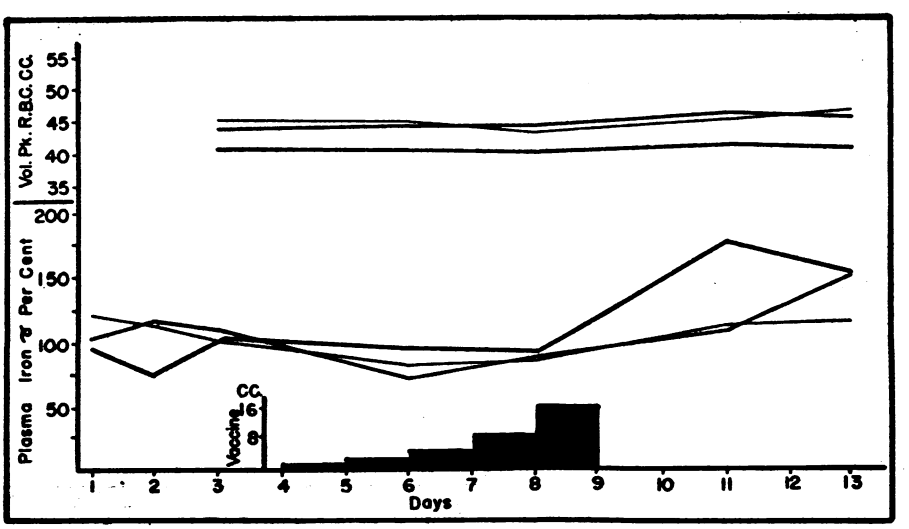

FIG. 4. THE EFFECT OF THE INTRAMUSCULAR INJECTION OF TYPHOID VACCINE ON THE PLASMA IRON AND VOLUME OF PACKED RED CELLS OF 3 DOGS

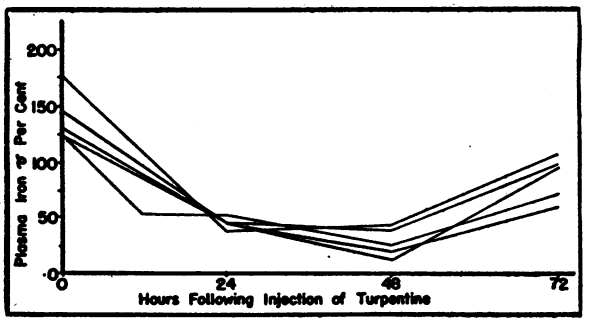

FIG. 6. HYPOFERREMIA DEVELOPING FOLLOWING THE INTRAMUSCULAR INJECTION OF 10 ML. OF STERILE TURPENTINE IN 5 DOGS

The rate of fall of the plasma iron is depicted in 5 additional animals in Figure 6. The iron was significantly lowered in 24 hours and reached the lowest values (12 to 40 micrograms per cent) in 48 hours. Thereafter, the level began to rise. In Figure 7, the results of a second intramuscular injection of turpentine in 3 dogs are shown. These dogs became definitely anemic, and 2 of them died on the sixteenth day of the experiment. The third survived, and in this dog the plasma iron rose.

The effects of the injection of sterile turpentine intrapleurally with the production of a pleural effusion in 3 animals are shown in Figure 8. This experiment was done in 7 additional animals with the same results. The

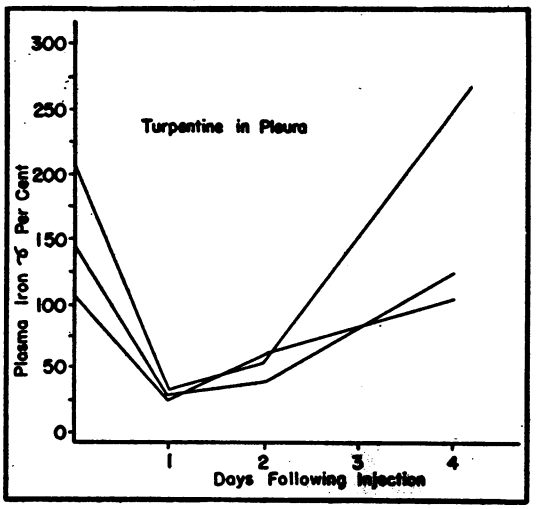

FIG. 8. THE EFFECT OF THE PRODUCTION OF A STERILE PLEURAL EFFUSION (TURPENTINE) ON THE PLASMA IRON LEVELS OF 3 DOGS

plasma iron fell somewhat more rapidly than following the intramuscular injections and reached the low level of 25 to 35 micrograms per cent in 24 hours.

With the object of demonstrating the presence of a hypoferremia-producing factor in inflammatory exudates, dogs were injected intrapleurally with turpentine, and 48

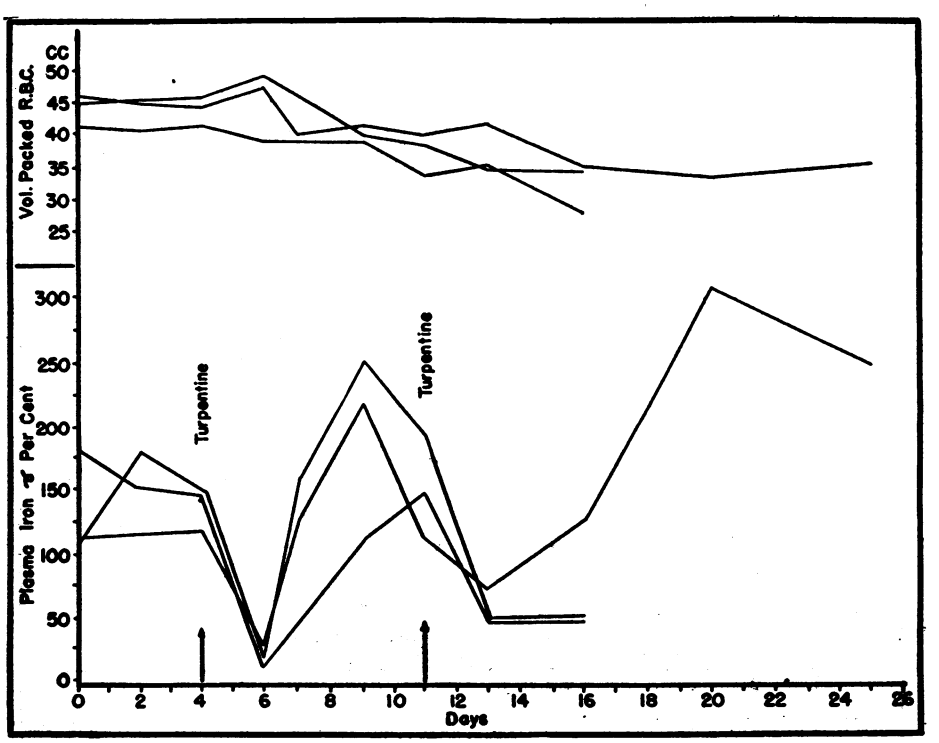

FIG. 7. HYPOFERREMIA AND MODERATE ANEMIA FOLLOWING 2 INTRAMUSCULAR INJECTIONS OF 10 ML. OF STERILE TURPENTINE IN 3 DOGS

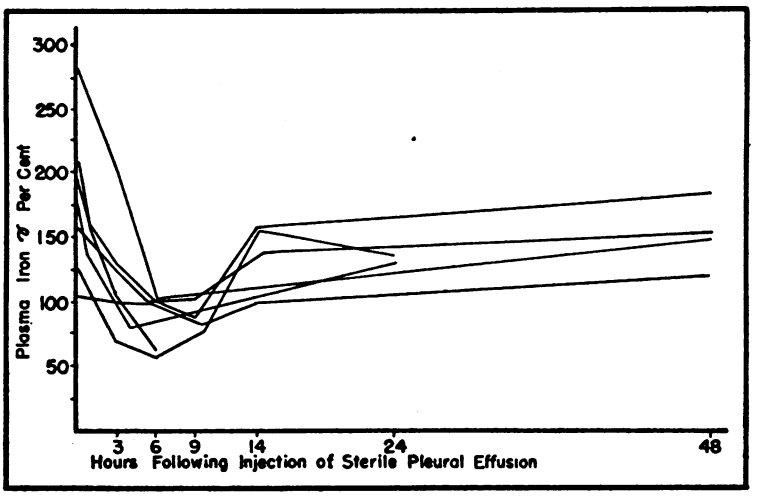

FIG. 9. HYPOFERREMIA DEVELOPING FOLLOWING THE INTRAVENOUS INJECTION OF 50 ML. OF STERILE PLEURAL EFFUSION FLUID IN 7 DOGS

hours later 50 ml. of the effusion was aspirated and immediately injected intravenously into each of a second group of 12 dogs. The results in 7 animals are shown in Figure 9. A fall in plasma iron occurred following injection of the sterile pleural exudates. This fall was not as marked as in the case of sterile turpentine given either intramuscularly or intrapleurally. In 5 control animals from which samples of blood were taken at intervals corresponding to those in the injected dogs, the plasma iron also fell, although not to a comparable degree.

DISCUSSION

It appears from these experiments that hypoferremia of pronounced degree develops in dogs following the production of an infected (staphylococcus) or a sterile (turpentine) abscess. This effect was marked 24 hours following the injection of bacteria or turpentine and was maximal at 48 hours. The intravenous injection of a sterile pleural effusion produced by the injection of turpentine, also was followed by a slight drop in plasma iron. The effects of the injection of staphylococcal toxin were less clear-cut.

Anemia developed following the injection of bacteria (Figure 1) and a moderate fall in volume of packed red cells occurred following the injection of turpentine (Figures 5 and 7). It is of interest that hyperferremia of brief duration appeared to develop following the hypoferremia and preceded the time when the volume of packed red cells commenced to return to normal.

It is to be noted that the experimental condition produced in these dogs differed from that seen in patients with anemia associated with chronic infection in that hypoferremia was of long duration in the latter. Furthermore, studies to date have revealed no increase of erythrocyte protoporphyrin in the dogs nor any significant alterations in the level of copper in the serum. These differences may possibly be due to the relatively acute character of the experimental disorder.

In the previous paper (1), evidence was presented which suggested that iron is rapidly removed from the blood stream in cases of chronic infection associated with anemia. The similarities between the anemias of infection and iron deficiency were pointed out. It was also reported that in the anemia of infection there is an increase in erythrocyte protoporphyrin and in serum copper.

In regard to these observations it is noteworthy that an accumulation and fixation of iron in inflammatory tissue has been reported (7, 8) and an increased iron retention has been found in febrile conditions in children (9). Moreover, it has been demonstrated (10, 11) that during mild infections in mice, iron accumulates in the reticuloendothelial system, a finding which was interpreted as indicating that this system is stimulated in infection to store iron.

On the basis of these observations, it now seems possible to outline a working hypothesis for the pathogenesis of the anemia of infection. According to this hypothesis, as a consequence of

inflammation, the iron is diverted to the tissues and is not available for hemoglobin synthesis. As a result of this demand of the tissues for iron, the removal of this element from the blood stream is hastened and possibly its absorption from the bowel is increased. Because iron is not available for use by the bone marrow, the reaction Protoporphyrin + Iron $\xrightarrow{Cu++}$ Heme cannot proceed. The protoporphyrin in the erythrocytes and the copper in the plasma cannot be utilized and are stored awaiting the time when iron is available. Whether a specific hypoferremia-producing factor is elaborated by inflammatory tissue, we are unable to state from the evidence which is now available. This possibility is being investigated further.

If under the conditions of infection the tissues have a high requirement for iron, questions of great interest are why they should need more iron, and what function this element performs in the inflammatory reaction. Little is known in regard to these questions, except for the observations (12, 13) which demonstrated that in tuberculous rabbits the repeated intravenous injection of ferric chloride results not only in an accumulation of iron in the caseous areas, but also in an increase in the survival time of the rabbits. Thus, it seems that the increase in tissue iron may exert an effect which is beneficial.

SUMMARY

1. Hypoferremia and anemia developed in dogs when abscesses were produced by the intramuscular injection of staphylococci. The hypoferremia preceded the development of anemia.

2. Staphylococcal toxins failed to cause anemia and produced only a moderate lowering of the plasma iron. Typhoid vaccine failed to produce either hypoferremia or anemia.

3. Dogs in which sterile turpentine abscesses were produced developed pronounced hypoferremia within 48 hours.

4. A working hypothesis for the pathogenesis of the anemia of infection has been outlined.

BIBLIOGRAPHY

1. Cartwright, G. E., Lauritsen, M. A., Jones, P. J., Merrill, I. M., and Wintrobe, M. M., The anemia of infection. I. Hypoferremia, hypercupremia, and alterations in porphyrin metabolism in patients. J. Clin. Invest., 1946, **25**, 65.
2. Wintrobe, M. M., Clinical Hematology. Lea and Febiger, Philadelphia, 1942.
3. Kitzes, G., Elvehjem, C. A., and Schutte, H. A., The determination of blood plasma iron. J. Biol. Chem., 1944, **155**, 653.
4. Grinstein, M., and Watson, C. J., Studies on protoporphyrin. III. Photoelectric and fluorophotometric methods for the quantitative determination of the protoporphyrin in blood. J. Biol. Chem., 1943, **147**, 675.
5. Menkin, V., Further studies on the leukocytosis promoting factor and on necrosin in inflammatory exudates. Am. J. Med. Sc., 1944, **208**, 290.
6. Willison, G. W., The effects of bacterial toxins on erythrogenesis. J. Lab. and Clin. Med., 1939, **24**, 283.
7. Menkin, V., Studies on inflammation. III. Fixation of metal in inflamed areas. J. Exp. Med., 1930, **51**, 879.
8. Menkin, V., and Menkin, M. F., The accumulation of iron in tuberculous areas. J. Exp. Med., 1931, **53**, 919.
9. Schäfer, K. H., Untersuchungen über den exogenen Eisenstoffwechsel bei fieberhaften Infekten im Kindesalter. Klin. Wchnschr., 1940, **19**, 979.
10. Schäfer, K. H., Gewebeeisenstoffwechsel und Hämoglobinbildung bei Infektionen. Klin. Wchnschr., 1943, **22**, 98.
11. Schäfer, K. H., The influence of infections and similar processes on the iron metabolism. Z. ges. exptl. Med., 1942, **110**, 678, 697, 713. In Chem. Abst., 1944, **38**, 2384.
12. Menkin, V., The accumulation of iron in tuberculous areas. II. The survival time of tuberculous rabbits injected with ferric chloride. J. Exp. Med., 1932, **55**, 101.
13. Menkin, V., The accumulation of iron in tuberculous areas. III. The effect of ferric chloride injections on the course of development of tuberculosis in rabbits. Am. J. Med. Sc., 1933, **185**, 40.