BARTONELLA BODIES IN THE BLOOD OF A NON-SPLENECTOMIZED DOG

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In the course of a series of plasmapheresis experiments on dogs, a 10.5 kg. male mongrel poodle developed an uncontrollable anemia with hemolytic and icteric plasma and urine and with many round and rod-shaped bodies in or on the erythrocytes identical to those described and named *Bartonella canis*. Inasmuch as the literature states that the spontaneous appearance of *Bartonella canis* and the successful transmission of the infection has been only in splenectomized dogs, the finding of *Bartonella* bodies in the blood of our non-splenectomized dog seems worthy of recording.

Kikuth (2, 3) and others (6, 8) demonstrated cyclical periods of anemia in splenectomized dogs associated with the presence of bodies in the erythrocytes belonging to the *Bartonella* group. These bodies showed great similarity to *Bartonella bacilliformis*, the etiological agent in Oroya fever of human beings (10) and to the *Bartonella muris* of splenectomized rats (5) and were named *Bartonella canis*. The characteristic microorganisms appeared in the blood in greatest numbers just prior to the greatest drops in the erythrocyte count and hemoglobin. The infection could be transferred to uninfected splenectomized dogs. They could not culture the microorganisms. Neosalvarsan exercised a specific influence on the infection. In this laboratory, Knutti and Hawkins (4) found *Bartonella* bodies in the blood of splenectomized gall bladder-renal fistula dogs which were exhibiting spontaneous periods of anemia associated with excessive bile pigment production. Their simple splenectomized dogs did not spontaneously show such periods of anemia but when inoculated with blood from the infected dogs showed intervals of blood destruction associated with the presence of *Bartonella* bodies. Rhoads and Miller (9), feeding a deficient black tongue-producing diet, demonstrated severe anemia in splenectomized dogs associated with the presence of *Bartonella* bodies in the blood. They were able to transmit the infection to other

splenectomized dogs fed either a normal or a deficient diet but not to non-splenectomized dogs on similar diets.

Methods

Briefly, our experiments dealt with the systematic standardization of food proteins for potency in plasma protein regeneration, as previously described (1, 7). The general plan of these experiments was that blood plasma proteins were depleted by practically daily bleeding with the return of washed red cells obtained from healthy donors (plasmapheresis), so that the dogs were brought to a steady state of low plasma protein (3.6 to 4.0 per cent) and uniform plasma protein production on a given basal diet. Such dogs were excellent test subjects by which the potency of various diet factors for plasma protein regeneration could be measured by adding them to the basal diet.

On Oct. 2, 1934, Dog 34-53 was placed on a basal diet consisting of soy bean meal, Karo corn syrup, canned tomatoes, cod liver oil, cottonseed oil and a salt mixture. This diet included all the essential vitamins, furnished 75–80 calories per kilo, was readily consumed and maintained the animal at a constant weight and in good clinical condition for about 13 weeks. The initial normal plasma protein level of 7.3 per cent was lowered to 5.4 per cent by 9 weeks of this diet without plasmapheresis. Plasmapheresis was begun on Dec. 1, 1934, but after 4 weeks, it was obvious that soy bean meal was too efficient as a protein builder to make a satisfactory basal diet. The blood volume of this dog was 890 ml. and an average of 1050 ml. of blood were being removed each week without lowering the plasma protein level to the desired base line. Potatoes and bran were substituted for soy bean in the diet on Dec. 28. The red cell hematocrit had been maintained at 45–50 per cent throughout the first 4 weeks of plasmapheresis but at the close of the 5th week (Jan. 4, 1935) it had dropped to 41.3 per cent in spite of replacement of red cells ordinarily adequate to maintain the normal level. The next day the plasma showed rather marked hemolysis and the animal vomited bloody mucus. Following each subsequent exchange of blood the plasma and urine grew progressively more jaundiced, the mucous membranes became pale and yellow, the diet was only partially consumed and vomiting was frequent. Occult blood was found in the urine. The hematocrit was 33.4 per cent on Jan. 3. Plasmapheresis was discontinued, due to the unfavorable reactions; and kidney, liver and Lextron (primary and secondary anemia liver fractions plus iron) were incorporated in the diet to aid in hemoglobin regeneration. During the 40 days of plasmapheresis, 5354 ml. of whole blood were removed from the dog. This contained 3214 ml. of plasma bearing 143.3 gm. of protein. The plasma protein level was lowered to an average of 4.14 per cent for the last week.

On Jan. 14 the plasma protein level had climbed to 5.1 per cent but the hemato-

crit had dropped to 19.4 per cent. The leucocyte count was 27,600 with 80 per cent polymorphonuclears. Twenty normoblasts, several megaloblasts and many large polychromatophilic erythrocytes, possibly reticulocytes, were seen in counting 100 leucocytes. A striking finding in the blood smears was many small coccoid bodies and beaded rods of varying lengths staining blue by Wright's method in or possibly on the erythrocytes, sometimes singly but often several to a cell. These bodies were morphologically indistinguishable from those described by various investigators as *Bartonella canis*. They were found on 3 consecutive days. No blood exchanges had been made for 6 days due to the reaction following each but with the hematocrit at 18.5 per cent on Jan. 15, a whole blood transfusion was considered imperative and 198 ml. were given. The hematocrit was 23.4 per cent on the following day and the *Bartonella* bodies were still present. On Jan. 16, 165 mg. of neoarsphenamine or the equivalent of 15 mg. per kilo weight were injected intravenously. The smears were negative the following day and have remained so over a period of 15 weeks. 3 days after the apparent sterilization the hematocrit had climbed to 32.5 per cent and the plasma was only slightly icteric. 25 ml. of washed red cells from a donor dog were injected to observe the reaction. The animal vomited, occult blood appeared in the urine and the hematocrit dropped to 30.6 per cent showing that there still remained an unfavorable reaction towards the injection of erythrocytes even though the *Bartonella* infection had apparently disappeared. Subsequently the hematocrit has climbed to 42 per cent, the dog appears to be in perfect health and is in the animal colony awaiting further work on the hemolytic problem. An exploratory laparotomy performed on Apr. 26 showed normal appearing abdominal viscera including a spleen measuring 14 x 5 x 1–2 cm.

Just prior to the neoarsphenamine sterilization on Jan. 16, 10 ml. of blood from Dog 34-53 was injected intravenously into splenectomized Dog 33-353 whose blood smears were negative for *Bartonella* bodies and whose red cell hematocrit was 40.4 per cent with water clear plasma. 3 days after the injection the hematocrit was 35 per cent, the plasma was distinctly icteric and occasional coccoid bodies and short, slender rods were seen in the erythrocytes stained by Wright's method. By the 9th day the infection was heavy, with *Bartonella* bodies present in practically every red cell and the hematocrit was 32 per cent. The following day (Jan. 25) the hematocrit was 27 per cent. The bodies could only be found after careful searching and remained scarce for 3 days. On Jan. 28, the microorganisms appeared in great numbers in the erythrocytes and the hematocrit stood at 30 per cent. 3 days later the hematocrit dropped to 24 per cent with increased icterus of the plasma and the bodies were again difficult to find. Having satisfactorily demonstrated *Bartonella* infection in splenectomized Dog 33-353, a single dose of neoarsphenamine (15 mg. per kilo) was injected intravenously with apparent sterilization. The hematocrit climbed back to 38.5 per cent in 3 weeks, the plasma lost its icteric color and no *Bartonella* bodies were demonstrated in multiple examinations. Howell-Jolly bodies were frequently encountered in the erythrocytes

during the anemic periods. The dog was in good physical condition throughout this period of transmitted infection and is still in our animal colony.

DISCUSSION

Inasmuch as *Bartonella* infection has been reported only in splenectomized dogs and with but few exceptions in splenectomized rats, the spleen has naturally been assumed to exert a protective action. Kikuth thinks that a latent infection may be held in check by the reticulo-endothelial system so that if this system is interfered with by the loss of a great portion of it, such as the spleen, the power is lost. Wills and Mehta (11) produced severe anemia in intact rats by feeding diets deficient in vitamins A and C and demonstrated *Bartonella muris* in their red cells in great numbers. They raised the question as to the possibility of the deficient diet having a specific action on the reticulo-endothelial system rendering the animal peculiarly liable to infections. We are at a loss to suggest a plausible explanation for the infection in our dog since the spleen was intact and all essential vitamins were adequately furnished in the diet. It is possible that the spleen elaborates some substance which is liberated into the circulating blood which has an inhibitory effect upon the *Bartonella* infection. Knutti and Hawkins (4) have shown that spleen extract feeding in *Bartonella* infected, splenectomized, bile fistula dogs appeared to have an inhibiting effect upon the periods of anemia and bile pigment overproduction. Our Dog 34-53 may have carried a latent infection of *Bartonella* which was activated by the steady loss of large amounts of blood with the depletion of plasma protein. Since this animal was losing more blood each week than its total blood volume, it may well have been losing some protective substance elaborated in the spleen. It may also have been inoculated with the infection from one of the donor dogs furnishing red cells for the exchanges. However, these same donor dogs have been in use in our plasmapheresis colony for several years without the previous appearance of *Bartonella* bodies.

The cyclical periods of anemia with the appearance of the *Bartonella* bodies in greatest numbers just prior to the greatest drops in the hematocrit are well illustrated in the inoculated splenectomized Dog 33-353. The association of the rapidly developing anemia and *Bartonella* bodies in non-splenectomized Dog 34-53 is also suggestive

but not conclusive evidence of their etiological relationship. This becomes more doubtful as one considers the hemolysis and fall in the hematocrit following the injection of only 25 ml. of washed red cells after the apparent sterilization with neoarsphenamine. Since the hemolytic episode with this dog, two other dogs, after several weeks of plasmapheresis, suddenly developed severe bouts of hemolysis, jaundice and anemia with hematocrits as low as 7 per cent, but *Bartonella* bodies were not demonstrated. Neoarsphenamine did not alter the course in one of these dogs and was not tried on the other. Both animals have recovered but are no longer suitable for plasmapheresis, since this hemolytic phenomenon has been repeatedly observed in them following the injection of washed erythrocytes. Other dogs tolerate plasmapheresis over long periods. One animal was exchanged for 41 weeks with 55,580 ml. of blood removed in 202 exchanges (7).

SUMMARY

A non-splenectomized dog, on a vitamin-adequate basal diet, in the course of a plasmapheresis experiment, developed an uncontrollable anemia associated with the presence of bodies in or on the erythrocytes, indistinguishable from the descriptions of *Bartonella canis*. The normal plasma protein level of 7.3 per cent was reduced to 4.1 per cent by diet and the removal of 5354 ml. of whole blood in 33 bleedings. The *Bartonella* infection was transferred to a splenectomized dog by an intravenous injection of whole blood. Each animal was apparently sterilized by one injection of neoarsphenamine equivalent to 15 mg. per kilo weight. It is possible that the spleen liberates some substance into the blood stream which has an inhibitory effect upon a latent *Bartonella* infection and that this protective substance was diminished by the many bleedings associated with the lowering of plasma proteins in the non-splenectomized dog and was lacking in the inoculated splenectomized dog.

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