THE ASSOCIATION OF BARTONELLA BODIES WITH INDUCED ANEMIA IN THE DOG

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PLATE 4

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The great value of the dog in experiments involving the effect of various procedures on hematopoiesis makes important any pathological condition of that animal which has a bearing on blood formation. In the course of experiments designed to produce anemia in dogs by the feeding of diets which are productive of characteristic mucous membrane changes, the spleens of certain of the experimental animals were extirpated. A number of the animals subjected to this procedure developed an anemia of severe grade, associated with the presence of small coccobacilliform bodies in or on the erythrocytes and free in the plasma. The general appearance of these organisms was strikingly, though not wholly, similar to the appearance of the Bartonella bodies which are associated with anemia in the human being and in certain lower animals, notably the rat. The severity of the anemia, its characteristic nature, and the resemblance of the bodies to the Bartonella bodies, prompted a more detailed study of the phenomenon.

The etiologic rôle of the minute microorganisms associated with Peruvian Oroya fever of human beings was first suggested by Barton (1) in 1909. Strong (2) and his coworkers considered them to be of protozoan nature and suggested the term "Bartonella." It remained for Noguchi (3), however, to grow these organisms in pure culture from a fatal case of Oroya fever, and to produce with the cultures disease manifestations in animals. Noguchi (3) also isolated similar organisms from the blood of a splenectomized rat. Lwoff and Vaucel (4) were able to infect mice with intact spleens with Bartonella. Lauda (5) studied the anemia of splenectomized rats in which similar inclusion bodies were present in the erythrocytes. Ford and Eliot (6) were able to carry on the condition in successive animal passages. Studies of Bartonella bodies in animals have been greatly
extended by Marmorston-Gottesman and Perla (7). They were able to isolate *Bartonella* bodies in pure culture from the blood of splenectomized rats with *Bartonella muris* anemia, and could cause anemia in other animals by infection with these cultures. They concluded that the adult rat is frequently a carrier of the bodies, although they only appear following splenectomy. These same workers showed that autoplastic splenic transplants protected against the development of anemia, and that lipoid extracts of the spleen were also effective.

Kikuth (8) gave the first description of *Bartonella* infection in dogs. In a splenectomized dog infected with *Piroplasma canis*, *Bartonella*-like bodies appeared in the blood stream after 6 days. The infection could be transferred to uninfected splenectomized dogs. A chronic type of disease with sharp increases in the number of organisms at regular intervals was described, as well as an acute form followed by death in about 2 weeks. The organisms could not be cultured, but the infection could be cured with salvarsan.

**Appearance of Bartonella Bodies in Splenectomized Dogs Fed a Diet Producing Black Tongue**

The *Bartonella* bodies of the present observations were first seen in a female terrier weighing 13.7 kilos, which had been splenectomized and fed the modified Goldberger black tongue-producing diet described by Rhoads and Miller (9). During 6 months, a slowly progressive anemia developed. At the end of that period anemia of severe degree appeared suddenly, the number of erythrocytes dropped to below 1,000,000 per c.mm., and the hemoglobin to 15 per cent. Examination of Wright-stained smears of the blood showed a large number of oval to round basophilic bodies present in and on the erythrocytes and free in the plasma. These bodies were morphologically strikingly similar to the *Bartonella* bodies occurring in splenectomized rats. When 200 gm. of lean beef were fed daily, a treatment known to be prophylactic against the effect of the diet in producing black tongue, an increase in reticulocytes occurred followed by a marked rise in the numbers of erythrocytes and in the hemoglobin values. During this remission the *Bartonella* bodies were only rarely demonstrable in the circulating blood. When the meat supplement to the diet was discontinued, however, a marked exacerbation of the anemia followed, during which the *Bartonella* bodies were once more present in great profusion. In Text-fig. 1 are shown graphically the exacerbations of the anemia followed by remissions induced by meat feeding and associated with sharp increases in the number of reticulocytes.
Text-FIG. 1. Changes in blood levels of Dog 1. Splenectomy and the black tongue-producing diet were combined in this instance. *Bartonella* bodies were uniformly present when the anemia was pronounced. C.I., color index. M.C.V., mean corpuscular volume.
The disease course of this animal was typical of that of four others splenectomized and fed the diet producing black tongue. A long period of diet feeding was followed by a precipitous drop in blood levels with the appearance of Bartonella bodies in the blood stream. Remissions, preceded by reticulocyte crisis, and followed by a marked decrease of the numbers of the Bartonella bodies, followed the feeding of lean beef as a supplement to the diet.

**Transmission of Bartonella Bodies to Splenectomized Dogs Fed the Black Tongue Diet**

The association of the presence of the Bartonella bodies with anemia in Experiment 1 suggested an attempt to transmit the conditions to other dogs. Two animals were splenectomized and fed the diet producing black tongue. They were immediately injected intravenously with 3 cc. of citrated anemic blood containing Bartonella bodies. The inoculation was repeated three times at 3 day intervals. The changes in the blood levels of one such dog are presented in Table I. A progressive fall in the number of erythrocytes and the percentage of hemoglobin resulted. When the animal was treated with lean beef there was a rise in the number of reticulocytes and an increase of the blood values. Bartonella-like bodies were present in large numbers at the height of the anemia and became fewer as improvement took place. They eventually disappeared entirely. The course of the disease in these two dogs was similar in all respects to that observed in Experiment 1, and resembled in many details the anemia associated with Bartonella bodies in other animal species. The rapidity of the fall of blood levels precludes the possibility that the anemia resulted from the splenectomy and black tongue diet alone. Furthermore, the results of the next experiment serve as controls on the spontaneous appearance of the Bartonella bodies.

**Transmission of the Bartonella Bodies to Splenectomized Dogs Fed a Normal Diet**

Since in Experiments 1 and 2 variables were present, one an inadequate diet and the other the removal of the spleen, it was deemed desirable to ascertain which factor allowed the Bartonella bodies to develop. It was found possible to cause a profound and fatal anemia
irregularly in splenectomized dogs fed a normal diet and injected with blood from Dog 1 containing *Bartonella* bodies. In Table II are presented the hematological findings in one such animal, typical of the four dogs injected.

From these results it is apparent that splenectomy is a major factor in allowing the development of the *Bartonella*-like bodies in the bloodstream of the dog once they have been artificially introduced. Krumbhaar (10), in an extensive series of studies of the hematologic effect of splenectomy in the dog, had failed to note any considerable degree of anemia or the presence of *Bartonella* bodies. Moreover, in our experiments the bodies were never observed in instances in which splenectomy was not combined with the feeding of an inadequate diet. In view of this fact, it was inferred that the diet in our experiments must also have an effect in the production of anemia, an inference which was borne out by the following experiment.

**Attempted Transmission of Bartonella Bodies to Non-Splenectomized Dogs Fed the Black Tongue Diet**

In Experiment 2 it was shown that by the injection of blood containing *Bartonella*-like bodies it was possible to cause the appearance...
of similar bodies, and, also, anemia in splenectomized dogs fed a diet causative of black tongue. Moreover, the previous experiment was evidence that the diet feeding was not necessary to allow the development of the transmitted disease, since splenectomy alone was sufficient to allow the effect to occur. It was desirable to ascertain next whether the diet, without splenectomy, was sufficient to allow the proliferation of the bodies in the blood stream. To answer this question, two dogs which were fed the diet producing black tongue, but which had not

### TABLE II

**Dog 3**

<table>
<thead>
<tr>
<th>Date</th>
<th>Erythrocytes per c.mm.</th>
<th>Hemoglobin per cent</th>
<th>Leukocytes per c.mm.</th>
<th>Bartonella bodies</th>
</tr>
</thead>
<tbody>
<tr>
<td>1933</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Mar. 9</td>
<td>6.83</td>
<td>95</td>
<td>6,800</td>
<td>None</td>
</tr>
<tr>
<td>Mar. 20</td>
<td>5.18</td>
<td>81</td>
<td>29,200</td>
<td>None</td>
</tr>
<tr>
<td>Mar. 27</td>
<td>6.07</td>
<td>81</td>
<td>11,650</td>
<td>None</td>
</tr>
<tr>
<td>Apr. 4</td>
<td>3.60</td>
<td>54</td>
<td>13,250</td>
<td>Few</td>
</tr>
<tr>
<td>Apr. 10</td>
<td>1.02</td>
<td>13</td>
<td>22,350</td>
<td>Enormous number</td>
</tr>
<tr>
<td>Apr. 13</td>
<td>1.17</td>
<td>22</td>
<td>24,900</td>
<td>Enormous number</td>
</tr>
<tr>
<td>Apr. 16</td>
<td>0.64</td>
<td>10</td>
<td>45,900</td>
<td>Enormous number</td>
</tr>
<tr>
<td>Apr. 21</td>
<td>0.70</td>
<td>11</td>
<td>30,100</td>
<td>Enormous number</td>
</tr>
</tbody>
</table>

### TABLE III

**Dog 4**

<table>
<thead>
<tr>
<th>Date</th>
<th>Erythrocytes per c.mm.</th>
<th>Hemoglobin per cent</th>
<th>Leukocytes per c.mm.</th>
<th>Bartonella bodies</th>
</tr>
</thead>
<tbody>
<tr>
<td>1933</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Feb. 22</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>3 cc. of blood from Dog 1 containing many <em>Bartonella</em>-like bodies injected intravenously</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>493</td>
<td>70</td>
<td>22,900</td>
<td>None</td>
</tr>
<tr>
<td>Mar. 6</td>
<td>474</td>
<td>71</td>
<td>10,400</td>
<td>None</td>
</tr>
<tr>
<td>Mar. 13</td>
<td>437</td>
<td>65</td>
<td>—</td>
<td>None</td>
</tr>
<tr>
<td>Mar. 21</td>
<td>481</td>
<td>61</td>
<td>14,000</td>
<td>None</td>
</tr>
<tr>
<td>Mar. 27</td>
<td>417</td>
<td>59</td>
<td>13,850</td>
<td>None</td>
</tr>
<tr>
<td>Apr. 4</td>
<td>371</td>
<td>50</td>
<td>20,800</td>
<td>None</td>
</tr>
<tr>
<td>Apr. 10</td>
<td>329</td>
<td>44</td>
<td>14,700</td>
<td>None</td>
</tr>
<tr>
<td>Apr. 13</td>
<td>292</td>
<td>44</td>
<td>13,400</td>
<td>None</td>
</tr>
<tr>
<td>Apr. 17</td>
<td>277</td>
<td>46</td>
<td>13,700</td>
<td>None</td>
</tr>
</tbody>
</table>
been splenectomized, were injected with blood of Dog 1 containing large numbers of the bodies. The results are shown in Table III. There occurred a progressive decrease in blood levels, described by Rhoads and Miller (9) as an effect of the diet feeding alone, if persisted in for a sufficiently long time to cause chronic stomatitis to appear. At no time, however, were any Bartonella bodies to be found in the blood stream, a fact in striking contrast to the results of the two previous experiments. It was concluded, therefore, that although the diet feeding alone would cause anemia, it did not suffice of itself to allow proliferation of the Bartonella-like bodies. The course of the changes of the blood of one such animal is presented in Table III.

**Attempted Transmission of Bartonella Bodies to Non-Splenectomized Dogs Fed a Normal Diet**

Two normal dogs fed a diet used in this laboratory for many years and known to be adequate to maintain dogs in good health for an indefinite period, were injected intravenously with 20 cc. of blood from Dog 1 containing many Bartonella bodies. At no time did those bodies appear in the blood of the recipient animal, nor did any decrease of blood levels take place.

**Attempted Transmission of Bartonella Bodies to Other Animals**

Marmorston-Gottesman reported that she was able to cause anemia and the appearance of Bartonella muris bodies in the blood of a variety of animals by the injection of cultures of that organism.

Mice, white rats averaging 30 gm. in weight, young rabbits, puppies, and young guinea pigs were all injected intraperitoneally with dog blood containing the Bartonella bodies. In no instance did symptoms of disease result or anemia appear, nor were the bodies found in the blood stream. So far as the evidence goes, it favors the view that the bodies seen in the dog, though morphologically similar to those occurring in the splenectomized rat, differ radically from them in that they cannot infect other animals.

**Morphology of the Bodies**

In smears of blood stained by Wright's method, the bodies are mostly present in or on the red corpuscles, but are occasionally found
BARTONELLA BODIES AND INDUCED ANEMIA

free. They are round to oval in shape and measure from 0.2 to 0.4 micron in width. The long forms may attain a length of 6 microns, but it is impossible to state whether these are single organisms or chains of smaller forms. Bent forms are frequently present. The longer bodies frequently show a regular indentation of the lateral border, giving the appearance of chains of smaller bodies. This is not always the case, however, since perfectly straight sided forms have been observed. The bodies do not stain with gentian violet when stained by Gram's method and when studied by dark-field illumination they are actively motile.

DISCUSSION

The experiments reported suggest the conclusion that both the diet producing black tongue and splenectomy favor the appearance of anemia together with Bartonella bodies in the blood of the dog. Neither procedure alone has resulted in this association. If blood containing the bodies is injected into splenectomized animals fed either a good or a deficient diet, anemia develops and the bodies appear in great number. This did not occur in any dog from which the spleen had not been removed. It is conceivable that the black tongue diet reduced the natural defense, which had already been lowered by splenectomy, almost to the threshold at which the Bartonella bodies appear.

From the morphologic resemblance between the bodies seen in the blood of the animals reported in this communication, and those reported by Kikuth in the dog and termed Bartonella canis, the conclusion is unavoidable that they are the same. This is borne out by the similarity of the associated blood dyscrasia.

The fact that the administration of lean beef, a substance known to be preventive of the symptoms which result from the diet feeding, was followed by increases in the number of reticulocytes and improvement of the blood levels, is of particular interest, since it provides evidence that the diet feeding was in part, at least, causative of the hematological disorder. From the studies of Goldberger and his coworkers, it has been assumed that beef is potent in preventing the pathological manifestations resulting from the diet feeding because of its content of vitamin B₃ (G)—a vitamin which may be defined as that heat-stable
fraction of the vitamin B complex which is required to allow growth in rats. But experiments performed in this laboratory have shown clearly that the diet is wholly adequate for rat growth when fed alone, while, furthermore, it has been impossible to cause black tongue in dogs by feeding diets lacking various parts of the vitamin B complex. It seems probable, then, that the effect of the diet is not due to a lack of the rat growth-promoting vitamin, although positive proof is lacking of the way in which it produces disease manifestations.

Proof of the etiologic relationship of the bodies to the anemia has not been presented. The association of the two is suggestive, but some unrecognized anemia-producing agent may have been introduced with them, while in the transfer experiments conceivably the anemic state may have favored multiplication of the bodies. The similarity of the pathological picture with that following Bartonella infection in other animals, however, suggests strongly that the bodies caused the anemia when the ground had been suitably prepared by splenectomy.

SUMMARY AND CONCLUSIONS

On feeding to splenectomized dogs a diet producing black tongue, severe anemia developed associated with the presence of small bodies in or on the erythrocytes. The bodies were morphologically similar to Bartonella muris and Bartonella canis. The addition of lean beef to the diet, a material prophylactic against its effects, was followed by improvement of the blood levels, the presence of increased numbers of reticulocytes in the circulating blood, and the disappearance of the bodies.

When the blood containing Bartonella-like bodies was injected into other splenectomized dogs fed a normal or a black tongue-producing diet, anemia developed in them and the bodies appeared in large numbers. Similar injections into non-splenectomized animals fed in the same way had anemia alone as its result. Injections into normal animals fed a normal diet caused not even anemia.

BIBLIOGRAPHY

BARTONELLA BODIES AND INDUCED ANEMIA


EXPLANATION OF PLATE 4

Fig. 1. Photomicrograph of a smear of the blood of Dog 1 taken at the height of the anemia. Many Bartonella bodies are present. The large dark areas are masses of platelets. Wright's stain. × 1500.
Fig. 1

(Rhoads and Miller: Bartonella bodies and induced anemia)