I. BARTONELLA INCIDENCE IN SPLENECTOMIZED BILE FISTULA DOGS

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PLATE 3

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This paper deals with the factors responsible for the pigmentary disturbances previously described (8) in the splenectomized bile fistula dog. In the absence of demonstrable infection in such animals it had been assumed that the periods of bile pigment surplus and anemia were of physiological origin related to a lack of spleen and bile constituents. The observations given below point to "Bartonella canis" as the probable cause. The bile fistula dog alone shows no such changes, and the splenectomized dogs in our kennels have not spontaneously developed an anemia. Dogs having a combination of splenectomy and a bile fistula, sooner or later develop the picture of anemia and pigment excess described below. This condition was first described by Hooper and Whipple (2), working with open bile fistula dogs subsequently splenectomized. These dogs always showed moderate infection of the bile fistula tract. The use of the closed sterile bile fistula (10) and the gall bladder-renal fistula (3) did away with bacterial infection of the biliary tree, yet such dogs when splenectomized showed the same periods of anemia and excess pigment output.

Other investigators have observed periods of anemia in splenectomized dogs. Krumbhaar (6) holds an initial anemia period to be a physiologic response to the loss of the spleen. Kikuth (4) and others (9) have demonstrated cyclical periods of anemia following splenectomy. They have shown the relationship of such periods to the presence in and on the red blood cells of their dogs, of bodies which they consider to belong to the Bartonella group, which they have called Bartonella canis. Kikuth has reported two types of the disease in dogs, an acute form which runs a comparatively short course and ends with death, and a chronic form which
persists over a long period of time. He has observed that the characteristic bodies appear in the blood in greatest numbers just before the greatest drops in the red count and hemoglobin occur. He has been able to reproduce the condition in other splenectomized dogs by inoculation of blood from infected animals. His attempts to cultivate the bodies were unsuccessful.

To determine whether *Bartonella canis* is associated with the pigment and blood phenomena observed in splenectomized bile fistula dogs, the following experiments have been carried out.¹

**Methods**

Blood smears from gall bladder-renal and sterile closed fistula dogs as well as normal and splenectomized dogs were made at frequent intervals. The fistula dogs were among those being used for pigment studies, and were kept under standard conditions as to diet, bile intake, and routine care prior to and during the course of the investigation. Paper II (5) of this series relates full details as to the care and details of study of the pigment metabolism of these dogs.

The blood smears were stained with Wright's stain by the usual method, and were examined under oil immersion for *Bartonella* bodies.

Frequent red cell counts were made in conjunction with hemoglobin and hematocrit estimations.

In transmission experiments, the necks of the dogs were shaved and cleansed using soap, alcohol, and iodine technique. The blood (5-10 cc.) was removed from the jugular vein of the donor in a sterile syringe, and introduced, without the addition of an anticoagulant, into the jugular vein of the recipient.

**EXPERIMENTAL OBSERVATIONS**

Four splenectomized gall bladder-renal fistula dogs were studied. Three of these were showing typical severe disturbances in pigment metabolism. The fourth (32-74) had never showed the marked degree of blood destruction that was observed in other animals. This dog will be described in some detail later. In the three former animals, bodies have been found associated with the red blood cells which are morphologically indistinguishable from *Bartonella canis*, according to descriptions and photographs of Kikuth (4) and Regendanz and Reichenow (9).

Fig. 1 shows the characteristic appearance of the bodies in the blood of a dog (29-329) in which they are quite numerous. They

¹ We are grateful to Dr. Cornelius P. Rhoads for suggesting the possible association of *Bartonella canis* with the phenomena observed in these animals.
appear as dark blue staining elongated beaded rod-like forms, and most frequently lie in a radial direction, on or in the cell. Pale vacuolated spaces in the affected red cell are commonly noted at one or both ends of the bodies. In addition to the beaded rod-like forms, of which 1–4 have been observed in association with one cell, there are short rods and small coccoid bodies, which may be present in the same cells with the rods, or many of this type alone may be numerous in other cells. Tiny ring forms such as those described by Regendanz and Reichenow are frequently present, and are most numerous early in the course of a typical period of blood destruction. Occasional narrow semilunar sickle-like forms have been observed. Such bodies have a granular appearance and sometimes extend, at the periphery of the cell, about two-thirds of the way around it.
Usually the more severe the involvement of the animal, as measured by the blood destruction, the more diverse are the types of bodies present. A short time after the initial appearance of such bodies in the blood, usually 3–5 days, every dog which has been observed has shown evidences of blood destruction. This reflects itself in a drop in the hemoglobin percentage, and in a rise in the bile pigment output. The bodies disappear quite abruptly from the blood, and intervals of a few days to a month may elapse before they reappear. Not one of the dogs has so far spontaneously recovered from the involvement.

Chart B shows an interval of about 2 months during the course of one of the splenectomized gall bladder-renal fistula dogs (29-329). It represents the daily bile pigment output, the weekly hemoglobin determination, and frequent examinations of blood smears. The periodic nature of the changes in these determinations
is readily seen. A few days before the rise of the bile pigments with the associated hemoglobin drop, characteristic bodies appeared in the blood, and reached their greatest numbers a day or two before the highest rise in bile pigment. A rough quantitative estimation of the bodies is designated by plus marks. One plus indicates infrequent bodies, less than one per oil immersion field; two plus, one or two cells containing bodies per oil immersion field; three plus, more than two; and four plus numerous bodies in every field. Fig. 1 would indicate a four plus reaction. Negative smears are shown by hollow blocks.

Such periods are characteristic of those observed in other animals. In one dog (31-359), never more than one plus smears have been observed except on a single occasion, when the smear showed numerous bodies. In another dog (32-74) a single one plus smear has been seen.

This dog (32-74) was operated upon under ether January 10, 1933. No pigment disturbances were noted until June 11, at which time a very slight period of pigment excess started. This lasted for 9 days. From this time, the pigments remained within control limits. On August 14, 1933, it was inoculated with blood from Dog 31-359, while the latter animal was at the height of a breakdown period. Two days after the inoculation, the bile pigment elimination increased, and there was a drop in the hemoglobin. From this time the dog continued to have regular, but slight periods of pigment overproduction. One smear out of many studied during this interval was found to be positive. A second inoculation of "infected" blood has not caused positive smears, although the animal has continued to have infrequent slight periods of pigment overproduction.

Transmission Experiments

Two splenectomized dogs (33-292, 32-105) were selected for transmission experiments.

One of these animals (32-105) had been operated upon 6 months previous to the inoculation, and its blood picture had been followed. At no time had there been evidence of an anemia. Blood smears were examined daily for a period of 28 days preceding the inoculation, and none showed Bartonella bodies. Chart B shows the hemoglobin, erythrocyte count, and result of blood smear examinations for a month subsequent to the intravenous injection into this dog of 10 cc. of blood from a splenectomized biliary-renal fistula dog (29-329), which on the day of inoculation showed a one plus blood smear. For 5 days subsequent to the inoculation there was no significant change in the erythrocyte count or in the hemoglobin determination. On the 6th day, the smear was positive for Bartonella bodies, and 2 days later was again positive. There was, at that time, a slight drop in the red cell count, and in the hemoglobin percentage. Nine days later, numbers of bodies appeared in the blood, and persisted for 8 days, following which there was a moderate, but definite drop in the hemoglobin and in the red count.
The course of the second splenectomized dog (33-292) is illustrated in Chart C. This dog had had a splenectomy 9 days previous to the inoculation. Blood smears had been studied daily for 28 days previous to the inoculation, and all had been negative. Ten cc. of blood from the same dog (29-329) were injected intravenously on the same day that the previous splenectomized animal was inoculated. Two days after inoculation, bodies were first noted in the blood, and the smears continued to be positive for 15 days. This was associated with a definite drop in the number of circulating red cells, and in the hemoglobin. The cyclical tendency after the initial positive period shows up to advantage in this chart.

The third animal to be inoculated was a splenectomized closed sterile fistula dog (33-51) which had been operated upon 9 days previously. Smears studied previous to the inoculation were negative. Four days after inoculation a four plus smear was observed, and the bile pigments showed a definite rise. This animal continued to have cyclical periods of blood destruction.

**CONTROL EXPERIMENTS**

Daily blood smears from a normal dog (31-271) were examined for 9 consecutive days. All were negative. On the 9th day the dog was inoculated with 10 cc. of
blood from a splenectomized gall bladder-renal fistula dog (29-329), which showed at the time a one plus smear. For a period of 31 days, daily smears were examined. Red blood counts were made tri-weekly, and hemoglobin and hematocrit estimations were carried out weekly. At no time was there any significant variation in the latter three determinations, and no Bartonella bodies could be found in any of the blood smears.

In addition, daily blood smears from four other normal dogs have been studied over a period of time, and no Bartonella bodies have been noted.

Cultural Attempts

So far, attempts to culture the bodies have been futile. The effort has not been undertaken on a large scale, but blood from two dogs showing characteristic bodies was introduced in various dilutions into leptospira media prepared according to the method of Noguchi (7). The cultures were incubated at 30°C. and were examined at intervals up to a month. A few tubes were contaminated with moulds, otherwise no growth was observed.

Therapeutic Measures

Both Kikuth and Regendanz and Reichenow have reported “sterilization” of the blood stream of animals bearing Bartonella canis by the use of neosalvarsan. We have carried out one preliminary test along these lines. A dog (29-329) which has shown regular periodic intervals of blood destruction associated with the presence of Bartonella bodies over a period of 8 months was given a single intravenous dose of neosalvarsan. 210 mg. or the equivalent of 15 mg. per kilo weight were injected on a day when the blood smear was three plus. The following day the smear was negative, and over a period of 7 weeks, frequent examinations have remained negative. In the meantime, no demonstrable periods of blood destruction have occurred; the hemoglobin has risen almost to the control level; and the bile pigments have remained low.

Before the association of Bartonella canis to the condition was recognized, spleen extracts were fed to a dog on the assumption that the periods of blood destruction might be related to the lack of some intrinsic spleen factor. Not until after the spleen feeding had been discontinued, was the relation of Bartonella canis demonstrated. At this time the animal was again showing typical cyclical periods of
anemia and pigment overproduction. *Bartonella* bodies were demonstrated in large numbers, in relation to the anemic periods. Since the results obtained were so clear-cut it seems worth while to include them in this report.

A dog (31-269) was splenectomized and a gall bladder-renal fistula made on March 28, 1932, with an uneventful recovery. It remained normal, as far as pigment metabolism was concerned, until September 22, when the first period of excess elimination of bile pigment began. During the following 3 months there were six distinct periods of bile pigment excess and anemia. At the height of the seventh cycle (December 25, 1932) spleen extracts were added to the diet in the following amounts: 3.6 gm. of No. 55 and 5 gm. of No. 343. Cycles of pigment overproduction continued so that on January 18, 1933, 24 days later, the amounts of extract were doubled. Thirteen days later, 11.25 gm. of liver extract No. 55 were added in addition to the spleen factor, and the dog was kept on this diet for 3 months. The cycles still persisted, so the No. 55 spleen extract was increased to 10.8 gm. and the No. 343 spleen extract to 15 gm. The liver extract was discontinued at this time. The above amounts of spleen extract are equivalent to about 700 gm. of fresh spleen.

Chart D shows what occurred after the extracts were increased to the above stated amounts. The periods of pigment overproduction are seen to stop abruptly, and the amounts of bile pigment eliminated thereafter are comparable to the basal control level. The hemoglobin rises and remains at about 100 per cent. On July 13, 1933, 24 months later, spleen feeding was discontinued, and 4 days later a typical period of pigment excess occurred. Similar periods, increasing in severity, followed rapidly over an interval of 5 months until December 12, 1933, when the dog was accidentally killed.

**DISCUSSION**

The results indicate that the hitherto inadequately explained periods of bile pigment overproduction and anemia which have occurred spontaneously in splenectomized bile fistula dogs under observation in this laboratory, are associated with the presence of *Bartonella* bodies in the blood. That bodies were demonstrated but once in one animal (32-74) and irregularly in another dog (31-359), does not exclude the

The spleen and liver extracts were prepared and furnished to us by Eli Lilly and Company. Spleen extract No. 343 is prepared according to the method of Cohn and Minot (1) which is used in making liver extract No. 343, a fraction potent in pernicious anemia. The preparation of liver extract No. 55, potent in secondary anemia, is described in a previous publication (11) and the spleen extract No. 55 is prepared according to this method.
possible relationship of *Bartonella* to the condition. That animals apparently have individual susceptibility which varies greatly has been pointed out by Kikuth, in descriptions of acute and chronic forms of *Bartonella* infection. The one dog (32-74) in which we failed to get a clear-cut picture of *Bartonella* bodies in the blood was probably highly resistant to the infective agent. As evidence for this is the fact that on two occasions it was inoculated with blood from another animal which showed *Bartonella* bodies, and on neither occasion was there nearly as marked a reaction in the form of anemia as was observed in the other animals.

It is of interest that spontaneous involvement with *Bartonella canis*, while apparently regularly occurring in splenectomized bile fistula dogs, has not occurred in dogs in this laboratory following a splenectomy alone. Other workers have observed such an event in simple splenectomized dogs. Numerous splenectomized dogs have been studied over long periods of time in our anemia colony, and in no instance has there been any evidence pointing to blood destruction by *Bartonella* or by any other agent. The anemia colony, however, consists of dogs raised in the kennels, and at all times completely isolated from contact with any other dogs. This fact may well explain freedom from infection. In our dogs with splenectomies alone, the post-inoculation anemia has not attained the severity of that observed in the splenectomized animals with bile fistulas. The rôle played by the bile fistulas in the determination of the spontaneity and severity of the condition is not understood. Possibly the lack of certain bile constituents plays a part in the lowered resistance of the animals.

Our single test with neosalvarsan agrees with the work of Kikuth, who tested this drug in five dogs.

Our limited experience with the use of spleen extracts suggests that they may have an inhibiting effect on the condition. Further work along this line is in progress.

**SUMMARY AND CONCLUSIONS**

Splenectomized bile fistula dogs in this laboratory have regularly exhibited spontaneous periods of anemia, which are associated with excessive bile pigment production.

In three out of four dogs, such periods have been shown to be as-
associated with the presence in the blood of bodies morphologically indistinguishable from descriptions of *Bartonella canis*.

Simple splenectomized dogs have not shown such periods of anemia arising spontaneously. Inoculations of blood containing *Bartonella* bodies into two splenectomized dogs have resulted in intervals of blood destruction associated with the presence in their blood of bodies similar to those in the inoculated blood. Injection of such blood into a splenectomized biliary-renal fistula dog has resulted in a similar picture.

Efforts to cultivate *Bartonella* bodies in artificial culture media have so far been unsuccessful.

Neosalvarsan appears to have a specific sterilizing effect on the condition.

Spleen extract feeding appears to have an inhibiting effect upon the periods of anemia and bile pigment overproduction.

**BIBLIOGRAPHY**


**EXPLANATION OF PLATE 3**

Fig. 1. *Bartonella* bodies in blood of a splenectomized bile fistula dog (29-329).
Fig. 1

(Knuttli and Hawkins: I. Splenectomized bile fistula dogs)