STUDIES ON BARTONELLA MURIS ANEMIA OF ALBINO RATS

I. Trypanosoma lewisi Infection in Normal Albino Rats Associated with Bartonella muris Anemia

II. Latent Infection in Adult Normal Rats

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It has been observed (1, 2) that removal of the spleen in adult albino rats is followed by a severe anemia within a few days to 3 weeks. This anemia is characterized by a marked drop in the normal red cell count from 8 to 10 million cells per cubic millimeter to 1 million or less. There is a corresponding drop in the hemoglobin and the appearance in the red blood cells of inclusion bodies known as Bartonella muris bodies. These bodies appear as diplococci and rods on the surface of the cell. They occur in very large numbers (20 to 30 per cell) at the height of the anemia. Poikilocytes, anisocytosis, polychromasia and normoblasts are present in the circulating blood. Erythrocytosis by large circulating mononuclears is occasionally observed. Noguchi has succeeded in growing a bacillus for one generation from the blood of infected rats on a special Leptospira medium, to which blood has been added (3). The failure of other investigators to isolate these organisms has cast some doubt on the etiological rôle played by these bodies in the anemia (4). Nevertheless their persistent appearance coincidental with the anemia is striking.

All the rats used in our studies have been raised in our laboratory from original Wistar Institute stock. The anemia develops in the adult rats of this stock following splenectomy in all instances. About 20 per cent of the rats die within 8 to 10

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days from anemia. The anemia begins as early as the 3rd day and as late as the 16th day. It is accompanied by a severe hematuria. The disease is rapidly progressive, reaches its peak about the 8th to the 12th day and then diminishes. In those recovering from the disease it disappears after 3 to 5 weeks, the count returning to the normal level. Recurrences occasionally occur, but if the rat survives the height of the first attack it survives subsequent attacks. At times the onset is precipitous, the number of red blood cells per cubic millimeter dropping to 2 or 3 million within 24 hours after the onset. Males develop the anemia more rapidly than females. It has been observed that some strains of rats do not develop the anemia (5).

Autopsy reveals a profound anemia of the organs and marked icteric tint to the subcutaneous fat. There is generally free fluid in the serous cavities, the blood in the heart is unclotted and watery. The liver, kidneys and heart show marked fatty changes. The bone marrow is red. Microscopically there are focal areas of necrosis in the liver. The Kupfer cells are markedly distended with engulfed red blood cells, hemosiderin pigment and *Bartonella muris* bodies. In general the reticulo-endothelial system is severely damaged. The kidneys reveal an extensive nephrosis and in some instances embolic hemorrhagic glomerular nephritis. The glomeruli may show necrotic changes, the tubules show marked degeneration and their lumina are filled with albuminuous material and iron containing pigment and occasional casts of red blood cells. There may be small areas of infarction in the kidneys without any surrounding inflammatory response. This is most striking in young rats with a fatal infection. In very anemic young rats with intact spleens, the pulp cells show extensive degenerative changes and there are areas of necrosis.

From the work of Lauda (1) and Mayer (2) the infectious nature of the anemia had been suspected, but the anemia could not be transmitted from the splenectomized rat to any adult normal animal. Ford and Eliot (4) were able to transmit this anemia through many passages in immature normal rats under 40 gm. in weight and immature normal rabbits by direct injection of whole blood of a splenectomized anemic rat. This would indicate a difference in the functional activity of the spleen in the young and adult animal. It would suggest that the adult normal rat is resistant to the anemia due to a permanent immunity from an infection with the "virus" of *Bartonella muris* anemia in early life. This immunity is broken down in the adult rat by the removal of the spleen and a second invasion with the virus occurs. It is possible, however, that the virus remains latent in the adult rat following an early infection and that the adult normal rat though immune is a "carrier."
An effort was made to determine experimentally (a) whether the adult normal rat is a "carrier" of the virus of the anemia; (b) whether injury to the spleen by a protozoan infection permits the occurrence of an anemia and if so, whether such an anemia is identical with the Bartonella muris anemia.

1. The Transmission of a Strain of the Bartonella muris from Splenectomized Adult Rats to Normal Young Rats and Rabbits

The anemia of splenectomized rats may be transmitted to young rats under 40 gm. and to 3 week old rabbits. This observation made by Ford and Eliot has been confirmed by us.

The injection of 0.5 cc. of whole blood from a splenectomized rat intravenously into 3 week old rabbits is followed by a profound anemia within 10 days which persists for several days and within 3 weeks the blood count returns to normal. The anemia can then be transmitted to baby rats for many transfers.

Sample Protocol.—Rabbit 4 injected intravenously with 0.5 cc. of whole blood from a splenectomized rat at height of the anemia.

| Blood count | Hbg. (Dare) | Bart. bodies |
|-------------|-------------|--------------|
| 6,350,000   | 105%        | Numerous rods and diplococci |
| 6,600,000   | 90%         | " |
| 3,900,000   | 37%         | " |
| 1,800,000   | 10%         | Occasional rods and diplococci |
| 1,850,000   | 50%         | " |
| 3,500,000   | 60%         | " |
| 5,310,000   | 90%         | " |

* Injected same day with blood of anemic rat.

The hemoglobin drops more rapidly than the red cell count and returns more rapidly to normal.

2. Trypanosoma lewisi Infection Associated with Bartonella muris Anemia in Normal Albino Rats

Mayer (7) noted the occasional appearance of Bartonella muris rods in the cells of mice and rats infected with trypanosomes.

In studies on Trypanosoma lewisi infection in adult albino rats we observed a moderate anemia during the first week of infection, with
the appearance in small numbers of rods and diplococci of the *Bartonella muris* bodies in the red cells. An effort was made to determine whether this anemia found in trypanosome infected rats is identical with the *Bartonella muris* anemia of splenectomized rats.

0.5 cc. of blood of an albino rat infected with *Trypanosoma lewisi* was injected into 3 week old rabbits. The trypanosomes died in the rabbit but a moderate anemia occurred within 10 days with the appearance of rods in the red cells. A small quantity of the blood of the anemic rabbit was injected into 30 gm. rats and an anemia developed in these rats with the appearance of the rods and cocci in the red cells. The virus of the anemia was thus transmitted through 30 gm. rats for several transfers. In this manner a strain of the virus of *Bartonella muris* anemia was isolated from adult nonsplenectomized albino rats infected with *Trypanosoma lewisi*.

*Trypanosoma lewisi* infection in adult albino rats causes a profound disturbance in the function of the spleen and lowers the resistance of the rat to the virus of the *Bartonella muris* anemia. The deaths sometimes observed in young rats from *Trypanosoma lewisi* are due to the concomitant *Bartonella muris* anemia which in young rats is so often fatal. *Trypanosoma lewisi* infection causes a marked increase in the size of the spleen. The pulp cells become engorged with engulfed red blood cells and a marked disturbance in the function of these cellular elements is present. The effect is similar to that of splenectomy in the adult rat.

**Latent Bartonella muris Anemia in the Adult Albino Rat**

It has been observed that the *Bartonella muris* anemia cannot be transmitted to adult animals with intact spleens by direct injection of blood from an infected animal. This infection can be transmitted, however, to normal young rats with intact spleens. This would indicate the probability of an infection early in life which results in a permanent immunity to the infection in the rat. This immunity is broken down with removal of the spleen and permits a second invasion with the virus. The rat often recovers, but repeated attacks may be produced either by the injection of whole blood from other anemic splenectomized rats or may occur spontaneously. It is possible however that the virus of anemia is present in the system from an infection in early life, and remains latent in the adult albino rat. An effort was
The Isolation of Bartonella muris Virus from Trypanosome Infected Unoperated Adult Rats and from Normal Unoperated Adult Rats (Sample Protocols)

The letter "T" is used in place of the last three zeros in the red cell count. The hemoglobin is expressed in percentages as calculated from Dare hemoglobinometer readings.

| Adult rats | 3 week rabbits | 30 gm. rats |
|------------|----------------|-------------|
| Unoperated Normal Rat → Rabbit 2 |
| 8,000 T | 5,600 T | 95% | 18R 5,400 T 90% — 31R (Strain NS) 6,400 T 95% |
| 3,600 T 50% → 11E 6,300 T | 90% | 19R 5,750 T 95% |
| 4,000 T 60% | | 4,000 T 60% |
| 17N 8,000 T | 2,200 T | |

Unoperated Normal Rat with Trypanosoma lewisi Infection → Rabbit 1

| 9,300 T | 5,700 T 90% |
| 4,450 T | 3,400 T 50% |
| 2,450 T 50% | 6C 5,400 T 85% |
| 3,810 T 60% |

Splenectomised Rat → Rabbit 4

| 9,700 T 100% | 6,600 T 100% |
| 965 T 10% |

25R 5,400 T — 32R (Strain SP4) 6,400 T |

1,800 T 10% | 2,400 T |

3,150 T |

All the young rats showed Bartonella muris bodies in the red cells after injection with blood of the other animals.

* Only the red blood cell count before injection and lowest count after injection indicated.
made to determine whether or not a latent infection with the *Bartonella muris* virus is present in the normal adult unoperated rat.

Repeated examination of smears of the blood of normal rats failed to reveal the presence of the *Bartonella muris* bodies in the red cells. It was found that intravenous injection of 0.5 cc. of the blood of a normal adult rat into young rabbits produced anemia with the appearance of the *Bartonella muris* bodies in the red cells. The transfer of 0.5 cc. of the blood of anemic young rabbits intraperitoneally into young rats is followed by a severe anemia in these rats with the appearance of *Bartonella bodies* in the red cells. The anemia can be passed through successive transfers in young rats. The blood of a normal rabbit produces no effect when injected into young rats. The blood either of young or of mature normal rats produces no effect when injected into other young rats.

By the passage of whole blood of normal adult rats through young immature rabbits the virulence of the virus of the latent infection with the *Bartonella* anemia is sufficiently enhanced to produce the infectious anemia in young rats. It may be that the virus increases in quantity in the rabbit sufficiently to produce an anemia when the blood is subsequently injected into the rat.

The Effect of Splenectomy in Young Rats

The adult normal rat is a carrier of the *Bartonella muris*. Further efforts were made to determine at what time in the life cycle of the rat it becomes a carrier of the virus of the *Bartonella muris* anemia.

Six 3 week old suckling rats were splenectomized. These young rats were placed in sterilized cages, completely isolated from the stock rats. None of these rats developed *Bartonella muris* anemia. It is evident then that during the first weeks of life the rat does not harbor the virus of *Bartonella muris* anemia, and therefore removal of its spleen is not followed by the infectious anemia. Since this period corresponds to the only period of life during which the *Bartonella muris* anemia may be transmitted in normal young rats by the injection of blood from an anemic splenectomized adult rat, it is not probable that the failure of splenectomized young rats to develop spontaneously the *Bartonella muris* anemia is due to a temporary protective immunity conferred from the mother. It would further suggest that the transmission of the virus from rat to rat is not necessarily through an insect host.
These experiments indicate that the *Bartonella muris* infection is latent in the adult normal rat. These rats are carriers of the virus and from their blood the virus can be isolated by passage through young rabbits. The invasion of the rat with this virus must occur after the suckling period, since suckling rats do not harbor the virus. The experiments further suggest a difference in the function of the spleen in young and adult rats.

**DISCUSSION**

From the data presented it can be concluded that the adult rat is a carrier of the virus of *Bartonella muris* anemia. The failure to produce the picture of *Bartonella muris* anemia in other adult animals by removal of the spleen may be due to the fact that these animals are not carriers of the virus. The normal adult rat cannot be given the anemia by direct injection of blood from an anemic splenectomized rat, since the adult rat possesses a high degree of resistance, presumably from a transient infection in early life. During the suckling period the young rat does not harbor the virus of the anemia. Therefore removal of the spleen in these rats is not followed by *Bartonella muris* infection.

The spleen is physiologically and anatomically injured in many protozoan infections as in trypanosomiasis, leishmaniasis, spirochiosis and piroplasmic diseases and malaria. The spleen plays an important rôle in resistance of the organism to *Bartonella* infections. Severe injury to the spleen may bring about the same condition as splenectomy. In many mammals *Bartonella* and *Grahamella* infections accompany protozoan infections. It has been suggested by Bayon (8) that the *Grahamella* is capable of invading the organism as a result of injury to the spleen by a hematozoic infection. *Grahamella* infections are not observed in rodents under other conditions. From our work on *Bartonella muris* infection in rats, however, it is evident that the *Bartonella* anemia in the adult rat occurs only after an immunity to this infection acquired in early life is broken down by injury to or removal of the spleen. The spleen is the important factor in the maintenance of the resistance. No doubt *Grahamella* infections occur under similar circumstances.

*Bartonella bacilliformis* infection in man is a disease resembling the *Bartonella* and *Grahamella* infections of rodents. Oroya fever is a systemic infection charac-
terized by profound anemia and the occurrence of *Bartonella* bodies in the red cells. Verruga peruana is a skin infection without anemia and in this no *Bartonella* bodies occur in the red cells but they are found in the endothelial cells in the skin lesion. Noguchi (9) and Mayer and Kikuth (10) have demonstrated the common etiological factor in these two human diseases. It is of importance to determine what factors in the individual are responsible for the occurrence of one type or the other. From the observations in the literature in analogous infections and from our own work with *Bartonella muris*, an explanation presents itself. Verruga peruana may be a first infection in an individual with a normal spleen. Oroya fever may be a recurrence of an early infection or a flaring up of a latent infection in an individual with an injured spleen. Chronic malaria is a very common concomitant infection in individuals with Oroya fever. Such a chronic protozoan infection causes profound physiological injury to the spleen and may thus be responsible for the breaking down of the resistance of the individual to the *Bartonella* infection. Noguchi (11) made an effort to demonstrate the effect, if any, of malaria and of splenectomy on the course of infection with *Bartonella bacilliformis* in monkeys. From several observations he concluded that both malaria and *Bartonella bacilliformis* may coexist without unfavorable effect of one disease upon the course of the other, that splenectomy led to no appreciable aggravation of the *Bartonella* infection and that both malaria and splenectomy in the same animal had no appreciable effect on experimental verruga infection. The experiments, however, were not comprehensive enough to establish the relationship of chronic malarial infection to Oroya fever, nor do they disprove the conception that the spleen plays an important rôle in the latter disease.

Suggestive is the single observation made by Noguchi on a chimpanzee infected with *Bartonella bacilliformis*. A severe anemia developed in this animal only after an accidental infection with Rocky Mountain fever by the bite of the *Dermacentra andersonii* and disappeared only when the blood became negative on culture.

Kikuth (12) reports an increase in the number of “takes” of verruga peruana from 50 to 100 per cent following splenectomy in monkeys inoculated with material from human verruga papulae. In one instance typical Oroya fever with severe anemia developed in one of the monkeys that previously had been injected with verruga material but that had failed to develop lesions and subsequently was reinjected with such material.

These observations and our own in *Bartonella* infections in rats offer a possible explanation of the modus operandi of *Bartonella* infections in human beings. A first infection with *Bartonella bacilliformis* may result in verruga peruana or may have no clinical manifestations. Subsequent injury to the spleen by a chronic protozoan infection breaks down the protective mechanism of immunity to this organism with the occurrence of a systemic infection with the *Bartonella bacilliformis* in Oroya fever.
SUMMARY

1. The virus of *Bartonella muris* anemia of splenectomized rats may be transmitted to normal young unoperated rats and rabbits. This confirms the observations of Ford and Eliot.

2. *Trypanosoma lewisi* infections in normal adult rats are accompanied by an anemia most marked at the height of the infection and the appearance of *Bartonella muris* bodies in the red blood cells.

3. In young rats *Trypanosoma lewisi* may produce death from the severity of the anemia, complicating the disease. The anemic virus may be separated from the *Trypanosoma lewisi* infected blood by passage through young rabbits with subsequent maintenance of the strain in immature rats.

4. A strain of the virus of *Bartonella muris* anemia capable of producing an anemia in young rabbits and young rats for successive transfers has been isolated from the blood of normal adult unoperated rats by passage through young rabbits.

5. The adult normal rat is a carrier of the *Bartonella muris* virus.

6. Splenectomy in young suckling rats separated from the mother is not followed by a *Bartonella muris* anemia. The young suckling rat is not a carrier of the infection.

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