THE TRANSFER OF RAT ANEMIA TO NORMAL ANIMALS.

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PLATE 11.

(Received for publication, June 16, 1928.)

It has previously been shown by Lauda (1) that following extirpation of the spleen rats develop a severe anemia, characterized by destruction of red blood cells, hemoglobinurea, leucocytosis and an increase of blood platelets. This condition Lauda regarded as infectious and due to some type of virus, which might be vegetating on the mucous membrane of the stomach or intestine and be held in abeyance by the spleen. When the spleen is removed the virus gains entrance into the body and after an incubation period of a few days attacks the blood corpuscles and produces the anemia, the leucocytosis being a response to the infection. Lauda was unable to reproduce this anemia by the transfer of blood or organs to normal animals (except in one instance) but was able to produce it in rats from which the spleen had been removed and which had not yet developed the anemia or had recovered from it. Lauda's observations on rat anemia were subsequently confirmed by Mayer (2) and his associates who further noted that the red blood corpuscles of the anemic splenectomized rat contain small bacilliform bodies which resemble the bodies previously seen by Mayer (3) in the blood of rats with experimental trypanosomiasis and treated with Bayer 205. These bodies Mayer had named *Bartonella muris ratti* because of their resemblance to *Bartonella bacilliformis*, the etiological agent of Oroya fever. Mayer was unable to produce anemia in normal rats with blood containing *Bartonella muris* from anemic rats or to demonstrate *Bartonella muris* in the blood of the injected normal animals. Mayer (4) then observed that *Bartonella muris* could be eliminated from the blood of splenectomized rats by treatment with various compounds of arsenic. Injections of bartonella-containing blood produce anemia in such treated animals, with a multiplication of the bodies on the red corpuscles. Blood without the bartonella fails to produce the anemia. On the basis of these transfers Mayer concluded that *Bartonella muris ratti* is a living organism and the etiological agent in rat anemia after splenectomy.

The presence of *Bartonella muris ratti* in the blood of anemic splenectomized rats has subsequently been confirmed by Lauda and Marcus (5) and similar findings have been reported by Bayon (6) in England, de Faria and Cruz (7) in South America and by Jaffé and Willis (8), Noguchi (9) and Cannon, Taliaferro and
Dragstedt (10) in North America. Bodies resembling *Bartonella muris ratti* have been found in the blood of other species of normal animals and named *Grahamella*, but whether they are living organisms and have any relation to pathologic conditions has not yet been determined. *Bartonella muris ratti* has occasionally been observed in normal rats, notably by Lauda (5) and by Jaffé (8).

Bartonella-like organisms from rats have been cultivated for brief periods on artificial media by several observers, notably Mayer (11), Bayon (6) and Noguchi (9), but none of these cultivated organisms have been identified as the cause of the anemia owing to the lack of susceptible animals upon which to test them.

As a result of these investigations it may be regarded as established that in many strains of tame and wild rats splenectomy is followed by anemia and that this anemic blood always shows *Bartonella muris ratti* on the red cells. Thus far the anemia has not been reproduced in normal animals (*i.e.* those still possessing the spleen) nor has *Bartonella muris* been transferred successfully to normal animals.

During the past 2 years we have splenectomized a series of 66 rats with the following problems in mind:

1. To determine whether white rats in Baltimore develop anemia after splenectomy with *Bartonella muris ratti* on the red blood corpuscles.
2. To find some normal animal in which anemia can be produced by transfer of material (blood or organs) from anemic splenectomized rats.
3. To cultivate *Bartonella muris ratti* on artificial media.
4. To determine the relationship of *Bartonella muris ratti* to anemia.

**A. The Anemia of Splenectomized Rats.**

In two strains of rats available for this work, removal of the spleen is followed by an anemia like that described by Lauda and *Bartonella muris ratti* appears in the blood.

One is a strain of white rats purchased from a Baltimore dealer, the other a strain of hooded rats raised in the School of Hygiene. In a series of 58 rats some grade of anemia has appeared in all instances. In some cases the red cell count drops to between 2 and 3 million cells per c. mm. from an original count of 10–11 millions. The hemoglobin drops to 20–30 per cent (estimated by the Sahli hemoglobinimeter) and hemoglobin appears in the urine. The white blood corpuscles rise markedly, the count increasing from an original count of about 16,000 to perhaps 35,000 per c. mm., and sometimes to 65 or 70,000. The increase is
largest in the polymorphonuclear neutrophils but the mononuclears also show a relative and absolute leucocytosis. At the same time there is apparently an increase of blood platelets, anisocytosis is often marked, there is polychromatophilia and a shower of normoblasts follows the destruction of red cells. The phagocytosis of red blood cells by the circulating mononuclears is characteristic. There may be as many as a dozen red cells in a single mononuclear. In all instances the blood of these anemic splenectomized rats contains *Bartonella muris ratti*. These structures appear on the 2nd to the 3rd day after splenectomy and their number corresponds to the grade of the anemia which subsequently develops.

Of the 58 rats splenectomized in the first series all developed bartonella anemia and 12 died of anemia from 7–12 days following the operation. 17 others died following bleeding from the heart for transfer of the virus to other animals. All of these showed a rapidly falling hemoglobin and many bartonellas on the red cells, and gave every promise of developing a severe and perhaps fatal anemia. 30 rats recovered from the anemia completely and showed a normal blood picture within about 4 weeks. 8 of these underwent a spontaneous or induced relapse 4–8 weeks later and in every case died of the anemia.

In addition 8 Littlestown rats were splenectomized which did not develop anemia nor show bartonellas except after exposure to infected rats.

The following protocol shows the blood changes in a splenectomized hooded rat which succumbed to the anemia.

**Protocol for Rat 21.**

*Hooded Rat (School of Hygiene Strain).*

<table>
<thead>
<tr>
<th>Date</th>
<th>Hemoglobin</th>
<th>Red cells</th>
<th>White cells</th>
<th>Observations and operations</th>
</tr>
</thead>
<tbody>
<tr>
<td>Jan. 7</td>
<td>93</td>
<td>10,730,000</td>
<td>15,000</td>
<td>Splenectomized</td>
</tr>
<tr>
<td>&quot; 9</td>
<td>91</td>
<td>10,770,000</td>
<td></td>
<td></td>
</tr>
<tr>
<td>&quot; 10</td>
<td>80</td>
<td>8,780,000</td>
<td>15,800</td>
<td></td>
</tr>
<tr>
<td>&quot; 11</td>
<td>82</td>
<td>9,760,000</td>
<td>17,100</td>
<td>Occasional rods</td>
</tr>
<tr>
<td>&quot; 12</td>
<td>80</td>
<td>9,870,000</td>
<td>15,400</td>
<td>Few rods</td>
</tr>
<tr>
<td>&quot; 13</td>
<td>75</td>
<td>8,740,000</td>
<td>20,900</td>
<td>Many &quot;</td>
</tr>
<tr>
<td>&quot; 14</td>
<td>43</td>
<td>5,040,000</td>
<td>30,200</td>
<td>&quot; &quot;</td>
</tr>
<tr>
<td>&quot; 15</td>
<td>21</td>
<td>2,160,000</td>
<td>19,700</td>
<td>&quot; polychromatophilia, anisocytosis</td>
</tr>
<tr>
<td>&quot; 16</td>
<td>22</td>
<td>2,220,000</td>
<td>14,700</td>
<td>Fewer &quot;</td>
</tr>
<tr>
<td>&quot; 17</td>
<td>27</td>
<td>2,110,000</td>
<td>7,450</td>
<td>&quot;</td>
</tr>
<tr>
<td>&quot; 18</td>
<td>27</td>
<td>2,690,000</td>
<td>6,950</td>
<td>&quot;</td>
</tr>
<tr>
<td>&quot; 19</td>
<td></td>
<td></td>
<td></td>
<td>Found dead</td>
</tr>
</tbody>
</table>

Text-fig. 1 shows the characteristic blood changes in a splenectomized rat which developed a severe anemia, recovered and subsequently had a relapse.
B. Production of Anemia in Normal Animals.

Transfer to Young Rats.

Intraperitoneal or intravenous transfer of blood from anemic splenectomized rats has been without effect on normal adult rats (with certain exceptions to be noted later), normal adult guinea pigs, normal adult rabbits and half grown dogs. Anemia did not develop after the transfers nor did the blood show Bartonella muris. Intraperitoneal injection of young rats about 3 weeks old, however, gave very different results. The blood of anemic splenectomized rats, taken 3–6 days after removal of the spleen, will produce anemia in young normal rats with intact spleen, provided that these rats are not too old or too large.
Rats 20–30 gm. in weight give the best takes, rats 40 gm. in weight are usually less satisfactory and with 60 gm. rats inoculation is frequently without effect. The disease develops in these young normal animals in much the same way that it does in adult rats after splenectomy. Bartonella muris appears in the blood in 1–3 days and the blood count begins to drop. On the 4th or 5th day the count may reach a minimum of about 3,000,000 cells per c. mm. The hemoglobin drops correspondingly, often to 30–40 per cent from 70–80 per cent and hemoglobin may appear in the urine. The leucocyte count does not change. In normal young rats this count is usually about 8,000 per c. mm. and the count is not appreciably increased at any time after inoculation with anemic blood, nor is there any definite increase of blood platelets. This failure of increase of leucocytes and of blood platelets is the only difference which has been noted between the blood picture in the adult anemic splenectomized rats and the young rats which develop anemia after transfer of bartonella-containing blood. In severe cases the animals may die in 5–8 days and at autopsy the mucous membranes and the organs are very pale. There is some icterus of the skin, the liver looks fatty and hemoglobin may be present in the urine in the bladder. The spleen is often notably increased in size.

The following protocols show the effect of the inoculation of normal animals with blood from anemic splenectomized animals.

Protocol of Rat N143.

Young normal rat weighing 35 gm. Injected April 17 intraperitoneally with 0.3 cc. whole blood from Rat N142 (third transfer from original splenectomized Rat 57).

<table>
<thead>
<tr>
<th>Date</th>
<th>Hemoglobin</th>
<th>Red cells</th>
<th>White cells</th>
<th>Operation and observations</th>
</tr>
</thead>
<tbody>
<tr>
<td>Apr. 17</td>
<td>70</td>
<td>7,780,000</td>
<td>8,500</td>
<td>Injected 0.3 cc. blood</td>
</tr>
<tr>
<td>&quot; 18</td>
<td>62</td>
<td>6,490,000</td>
<td>8,600</td>
<td>Occasional rod</td>
</tr>
<tr>
<td>&quot; 19</td>
<td>54</td>
<td>5,525,000</td>
<td>8,370</td>
<td>Few rods</td>
</tr>
<tr>
<td>&quot; 20</td>
<td>52</td>
<td>4,160,000</td>
<td>9,300</td>
<td>Rods, polychromatophilia, anisocytosis</td>
</tr>
<tr>
<td>&quot; 21</td>
<td>44</td>
<td>3,850,000</td>
<td>Occasional rod polychromatophilia, anisocytosis</td>
<td></td>
</tr>
<tr>
<td>&quot; 24</td>
<td>64</td>
<td>5,720,000</td>
<td>8,250</td>
<td>Occasional rod</td>
</tr>
</tbody>
</table>
TRANSFER OF RAT ANEMIA TO NORMAL ANIMALS

Protocol of Rat III 14–1.

Young rat, 35 gm., injected intraperitoneally with 0.5 cc. blood from splenectomized Rat 23.

<table>
<thead>
<tr>
<th>Date</th>
<th>Weight gm.</th>
<th>Hemoglobin</th>
<th>Red cells</th>
<th>White cells</th>
<th>Operations and observations</th>
</tr>
</thead>
<tbody>
<tr>
<td>Jan. 16</td>
<td>35.0</td>
<td>75</td>
<td>7,000,000</td>
<td></td>
<td>Injection 0.5 cc. blood</td>
</tr>
<tr>
<td>&quot; 17</td>
<td>37.0</td>
<td>48</td>
<td>3,230,000</td>
<td></td>
<td>Many rods</td>
</tr>
<tr>
<td>&quot; 18</td>
<td>39.0</td>
<td>28</td>
<td>2,160,000</td>
<td></td>
<td>Occasional rod</td>
</tr>
<tr>
<td>&quot; 19</td>
<td>39.0</td>
<td>28</td>
<td>2,160,000</td>
<td></td>
<td>Rods</td>
</tr>
<tr>
<td>&quot; 20</td>
<td>26</td>
<td>3,300,000</td>
<td>3,300</td>
<td></td>
<td>Few rods. Died few hours later</td>
</tr>
</tbody>
</table>

Autopsy.—Liver yellowish, spleen mottled yellow and red. Bladder filled with blood-tinged urine, slight icterus of skin. Cells in liver and spleen show phagocytosed red blood cells.

Success in the transfer of the virus of rat anemia to normal animals depends on a number of factors. The best results have been obtained with well nourished rats on a good balanced diet which reach a weight of 30–40 gm. in about 3 weeks. Ill nourished rats which are older at 30–40 gm. weight are not so satisfactory as younger rats. Rats over 60 gm. are about as resistant as normal adult rats, at times however, a few bartonellas may appear and a light anemia develop. Moreover, the virus in the original splenectomized rats varies greatly in its potency. In some instances it seems to have little effect on the normal animals while in other cases it may give excellent takes. Blood in the early stages of the anemia, 2–4 days after splenectomy, contains more potent virus than after the peak of the anemia is reached, and during or after recovery when bartonellas have disappeared from the circulation, the blood in our experience invariably loses its ability to produce anemia.

Blood from these normal animals in which anemia has thus been produced contains the virus of the disease. On transfer to other animals anemia develops and Bartonella muris appears in the blood. Blood for transfer gives the best takes when the red blood cells show the heaviest infection with bartonellas. This is usually from 2–5 days after injection. This passage of the virus can be kept up for succes-
sive generations of normal animals. Nine different strains of the rat anemia virus have been obtained from time to time and carried through at least three generations. Of these one was carried through five generations, one through six generations, two through seven generations, one through nine generations and one through thirty generations.

After several passages the virus tends to become less potent so that a more moderate anemia develops, usually a hemoglobin reading of 40–50 per cent being the lowest. The bartonellas are less numerous in the blood, that is, fewer cells are infected and there are fewer bodies in each infected cell. These are usually the large deeply staining pleomorphic forms that appear after active multiplication has ceased, and which seem to be a resting or resistant phase of the bartonella. The virus which we have carried through thirty generations went through several periods of this apparently inactive phase in which little or no anemia is produced. The bartonellas are carried along from transfer to transfer, undergo multiplication for a period of perhaps 24 hours and then recede promptly. By making several successive transfers at 24 hour intervals into younger and smaller rats we were able to bring this virus back into a state of active multiplication on two different occasions. Whether our more rapid transfer and choice of young rats were really the deciding factors in reviving the bartonella anemia is impossible to say. It is theoretically possible that the virus must go through some sexual or symplastic cycle outside of the blood stream at certain intervals, and so renew its vegetative energy. At all events its virulence and the morphology of *Bartonella muris ratti* seem to vary from time to time. The smallest injection with which we have produced a good take in a young rat has been 1/20 cc. of infected blood, but 1/100 cc. is sufficient to cause the appearance of bartonellas in the blood stream after 24 hours, and 1/200 cc. after 48 hours. These small injections, however, are apparently easily handled by the host. The bartonella is destroyed or its multiplication inhibited, and no anemia develops. Our usual infecting dose is 0.2–0.5 cc. injected intraperitoneally.

In all, 212 young rats have been injected with blood from other anemic rats. Of these, eight have died with a fatal anemia in 5–8 days, while fifteen others died, probably of anemia, but the terminal records were not complete enough to be absolutely convincing.
Forty-one died after bleeding from the heart for transfers. As in the cases of the splenectomized rats, the best and most promising cases were always selected for transfer, so that many of these, killed in the early stages of the disease, might have gone on to a fatal issue, had they not been killed. Thirty-nine of the young rats died from causes which could not be directly connected with the anemia.

Transfer to Young Rabbits.

In addition to young rats, young rabbits have also been found susceptible to the virus of rat anemia. On three occasions young rabbits about 3 weeks old have developed characteristic anemia after intravenous inoculation of $\frac{1}{2}$ to 1 cc. of bartonella-containing rat blood. The blood count dropped to about 3,000,000 from 6,000,000 and the hemoglobin to about 20 per cent (Sahli). Bartonellas appeared on the blood corpuscles in 5–12 days and the height of the anemia was reached 3–7 days later. In none of these animals did the infection proceed to a fatal issue. The bartonellas rapidly disappeared from the blood and the blood count returned to normal. There was a leucocytosis at the peak of the anemia.

Blood from these rabbits, containing *Bartonella muris*, again produced a characteristic bartonella anemia in young rats, showing that the virus can be passed through rabbits and not lose its potency for rats.
The following protocol shows the production of anemia in young rabbits.

Protocol Rabbit 17.

Young normal rabbit 3 weeks old. May 26, injected with 1 cc. blood intravenously from splenectomized Rat 17.

<table>
<thead>
<tr>
<th>Date</th>
<th>Red cells</th>
<th>White cells</th>
<th>Operations and observations</th>
</tr>
</thead>
<tbody>
<tr>
<td>May 26</td>
<td>5,672,000</td>
<td></td>
<td>Injected 1 cc. blood Rat 17</td>
</tr>
<tr>
<td>&quot;</td>
<td></td>
<td></td>
<td>No rods</td>
</tr>
<tr>
<td>&quot; 27</td>
<td></td>
<td></td>
<td>&quot; &quot;</td>
</tr>
<tr>
<td>&quot; 28</td>
<td></td>
<td></td>
<td>&quot; &quot;</td>
</tr>
<tr>
<td>&quot; 30</td>
<td></td>
<td></td>
<td>&quot; &quot;</td>
</tr>
<tr>
<td>&quot; 31</td>
<td></td>
<td></td>
<td>&quot; &quot;</td>
</tr>
<tr>
<td>June 1</td>
<td>6,128,000</td>
<td>9,200</td>
<td>Occasional rod</td>
</tr>
<tr>
<td>&quot; 2</td>
<td>4,616,000</td>
<td>9,600</td>
<td>Few rods, polychromatophilia</td>
</tr>
<tr>
<td>&quot; 3</td>
<td>3,936,000</td>
<td>9,000</td>
<td>Many rods</td>
</tr>
<tr>
<td>&quot; 4</td>
<td>3,448,000</td>
<td>7,600</td>
<td>&quot; &quot; anisocytosis</td>
</tr>
<tr>
<td>&quot; 7</td>
<td>3,040,000</td>
<td>6,800</td>
<td>Fewer &quot;</td>
</tr>
<tr>
<td>&quot; 8</td>
<td></td>
<td></td>
<td>Few &quot;</td>
</tr>
<tr>
<td>&quot; 9</td>
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<td>&quot; &quot;</td>
</tr>
<tr>
<td>&quot; 14</td>
<td></td>
<td></td>
<td>&quot; &quot;</td>
</tr>
<tr>
<td>&quot; 15</td>
<td></td>
<td></td>
<td>&quot; &quot;</td>
</tr>
</tbody>
</table>

"Littlestown" Rats.

Another strain of rats purchased from a dealer in Pennsylvania and designated by us as the "Littlestown" strain, did not develop anemia nor show Bartonella muris after splenectomy. There are some rats, therefore, which do not harbor the virus of rat anemia and do not come down with the disease until subsequently infected either by artificial injection of virus or by some natural mode of transfer. This is similar to the experience of Jaffé and Cannon who find some strains of rats always infected and some never, or only occasionally so. We have thus far splenectomized five animals in this group, which when kept in isolation did not show Bartonella muris nor develop anemia even after several months. In three others, which had been kept in the animal house in the same room with infected rats for several
months, splenectomy was followed by anemia with the appearance of bartonellas in the blood. Such adult Littlestown rats which do not harbor the virus are susceptible to the virus upon injection. After inoculation of 1 cc. of blood from an anemic animal, a few bartonellas appear on the blood cells and a light anemia develops. This is never as severe as in the young rats, and rapidly regresses, the bartonellas disappearing and the blood count returning to normal.

*Bartonella muris ratti in Wild Rats.*

All of the white and hooded rats used for splenectomy or transfer have been examined for bartonellas before operation or injection and in only one instance have we seen the rods. This was a young rat of the Littlestown strain which had been in the laboratory 2 weeks. In all we have examined about 300 rats. Wild rats on the other hand have shown a high incidence of bartonellas on the circulating blood cells. Out of thirty-nine examined, ten showed bartonellas. The rods were never numerous, usually only one or two in about four oil immersion fields, in two cases several on one cell in nearly every field. The rat in this series showing the heaviest infection with bartonella had many trypanosomes in the blood and a heavy infection with leptospirosis in the kidneys. This is in accord with Mayer’s observation that rats infected with trypanosomes may show bartonellas.

*C. Spontaneous Infection.*

As mentioned above, in the two strains of School of Hygiene rats, removal of the spleen is always followed by the appearance of *Bartonella muris* and a more or less severe anemia. Another strain of white rats (Littlestown strain) obtained from a dealer in Pennsylvania and reared in a room remote from infected rats showed no bartonellas and did not develop anemia following splenectomy. Others of these rats splenectomized about a month after removal to the common animal room however, came down with anemia in the usual way. If the uninfected splenectomized rats now are put in a cage with infected splenectomized rats they begin to show bartonellas in the blood in 7–14 days and usually come down with a fatal anemia.
a few days later. Similarly, convalescent splenectomized rats undergo relapses if placed with infected rats.

These observations indicate the highly contagious nature of the virus of rat anemia and point rather strongly to an insect vector. Cages and food cups are sterilized with steam twice weekly and care is taken not to mix food or water cups from cage to cage. The chances for transfer of the virus by direct contact with feces of infected animals or by biting are therefore remote. All of our rats are infested with Polyplax spinulosa. An occasional adult Cimex lectularius has been found around the cages and young forms have been picked from some of the rats. Experiments are under way to determine if these lice and bedbugs can transmit the virus to uninfected animals.

D. Attempts to Cultivate Bartonella muris ratti.

Repeated attempts have been made to cultivate Bartonella muris ratti on artificial media. For this purpose all the usual laboratory media have been utilized and in addition Noguchi's tissue media and leptospira media. Rat infusion agar from young normal animals was prepared and the reaction adjusted to pH 6.4, 7.0 and 7.4. Blood of rabbits, horses and rats has been used for enrichment. Thus far no successful cultivation has been effected and it seems very doubtful to us whether the organism has ever been grown artificially. Tissue cultures of blood or organs from infected animals have yielded no conclusive results.

E. Viability of the Virus.

Blood drawn from the heart of an infected rat into a glass syringe with heparin or sodium citrate remains infective for young rats for at least 2 hours. After 24 hours in the ice box the virus is definitely attenuated, ordinary doses giving only a light take with few bartonellas in the blood and little or no anemia. 24 hours at room temperature or 37°C. completely destroys the virus. The virus and the bartonellas are killed by heating the blood at 57°C. for $\frac{1}{2}$ hour. Injection of this heated blood causes neither anemia nor the appearance of bartonellas.
Contrasted with the short life of the virus in vitro, is its apparent indefinite survival in the animal body. A young rat injected with bartonella blood and kept in isolation for several months following recovery from anemia, will upon splenectomy again come down with typical bartonella anemia. A rat from the uninfected strain kept in similar isolation shows neither bartonellas nor anemia following splenectomy. Rats from the non-infected strain injected with bartonella blood or merely exposed for a period of 2 weeks or so to infection, develop bartonella anemia upon subsequent splenectomy. Rats therefore become bartonella carriers, either after exposure or as a result of injection and apparently remain carriers indefinitely. Any rat harboring bartonellas will develop anemia following splenectomy even though it may have had and recovered from a typical case by injection.

F. Immunity.

These cases of anemia in splenectomized rats which have once had the disease, and the spontaneous and induced relapses in recovered splenectomized rats point to the failure of the immune mechanism in the absence of the spleen. Moreover, serum from a normal adult animal will not protect a splenectomized animal from invasion by bartonella and the development of anemia, nor will it protect the young injected animal from the disease.

Natural infection among the rats presumably is effected by minute doses, either through the bite of an insect or the ingestion of infected material. Such doses are easily handled by the immune mechanism, Bartonella muris does not multiply on the blood cells and no anemia develops. This accounts for the failure to find demonstrable bartonellas in normal white rats, although there may be occasional organisms present in the circulating blood at times. The relatively enormous doses of virus given young rats in our injections so overwhelm the immune mechanism that infection readily occurs and anemia develops. The younger the rat the more severe the disease, and the more likely to be fatal. Normal adult rats cannot be infected even with large doses. Removal of the spleen however is all that is necessary to precipitate the latent infection. Why the young spleen is
unable to protect the animal against invasion with this virus and the adult spleen is usually so completely effective, is a matter for future investigation. As mentioned before, however, the adult "Littlestown" rat which is normally not infected, can be made to undergo a light attack after injection which indicates that previous infection probably has some influence on the protective power of the spleen. At all events, it appears evident that the immunity developed in growing rats is primarily dependent on the spleen.

With this in mind it was considered important to see if the adult splenic tissue acted as a reservoir of inhibitory substances for the bartonella anemia. The spleen from a normal rat was ground in a mortar with a little physiological saline solution and allowed to stand for 1 hour with half the quantity of blood from an anemic splenectomized animal. ½ cc. of the mixture was then injected into a young rat and a control rat was injected with an equal amount of blood without splenic tissue. The experiment was repeated four times, and in no case could we find any demonstrable difference between the control animals and the rats with splenic tissue. Both developed bartonella and a light or moderate anemia according to the potency of the virus used.

Attempts to produce an immune serum in rabbits have so far proven unsuccessful. A rabbit which has once had bartonella anemia cannot be made to undergo a relapse by further injections. Serum from such an "immune" rabbit which has recovered from the anemia and been given two subsequent injections of bartonella blood fails to protect splenectomized rats from infection and anemia. This is similar to Noguchi's experience with Bartonella bacilliformis.

G. Nature of Bartonella muris ratti.

In view of our inability to cultivate Bartonella muris, some question may arise as to its character, whether it is really a bacterium or represents a stage in the life cycle of some protozoan parasite.

According to our observations Bartonella muris ratti is a small bacillary body about 0.1 μ in width and 0.5-1.0 μ in length, which appears to lie on the surface of the red cells (Fig. 1). Stained by Wright's blood stain it takes a bluish hue with
the suggestion of redder granules at the ends. Stained by Giemsa the whole rod has a redder hue. It is decolorized by Gram's method and takes the fuchsin counterstain very faintly. When the rods first appear they are thick, dark and short, often diplococccoid, occur singly and only on an occasional cell. These forms are followed by dumb-bell-shaped or granular rods and long slender bacillary bodies. More cells are infected and there may be two or four rods on a cell. As the infection progresses the rods appear shorter, usually in parallel rows and chains and the majority of the red cells contain from a few to several dozen. Any time from the 5th–10th day after splenectomy, the majority of the infected cells disappear rather suddenly from the blood stream, and the remaining infected cells reveal larger deeply staining, highly pleomorphic bodies, with occasional peculiar prolongations of the cytoplasm. There may also be ring forms, thicker and more deeply stained on one side. These forms, which seem to be related to the earlier ones, suggest a stage in the life cycle of some protozoan.

Occasionally groups of bartonellas are found free in the plasma, but these are usually associated with fragments of laked corpuscles. The bartonellas do not stain well when they are free from the corpuscles, hence it is more difficult to identify them with certainty, particularly to distinguish them from granules of broken platelets. The platelet granules differ from the rods however, by their greater thickness and less delicate outline and the redder hue with Wright's stain. The rods are also readily distinguished from the basophilic granules of the red cells, from chromatin dust and the Howell-Jolly bodies, all of which tend to increase during the course of anemia. The reticulated red cells also increase during the infection. By staining with cresyl blue and then by Wright's the difference between the bartonellas and reticulum is well brought out. The reticulum appears as bluish rods or masses of rods principally in the center of the cell, while the bartonella rods are distinctly redder and appear toward the periphery of the cell. Only a few reticulated cells are infected with bartonellas and then usually very lightly. We have not been able to see *Bartonella muris* in unstained smears or on living cells in the hanging drop. Fresh dark field preparations, however, reveal the bartonellas on the cells as non-motile rods. They apparently do not stain with neutral red. Many cells in bartonella anemic blood may show neutral red-stained rods, but from their position and distribution we believe that they are reticulum rather than bartonellas.

**H. Relation of Bartonella muris ratti to Rat Anemia.**

Since *Bartonella muris* has never been grown artificially, and rat anemia produced by cultures, it cannot be regarded definitely as the cause of the disease. There is, however, some collateral evidence which makes it highly probable that this organism stands in etiological relation to it. *Bartonella muris* is always associated with the anemia.
and varies with the extent of blood destruction. If it appears on the
corpuscles in large numbers or if many corpuscles harbor it the anemia
is apt to be severe. When only a small number appear in the blood,
the anemia is mild. Moreover, the bartonellas seem to be destroying
the cells. In heavily infected blood one can find all stages of blood
destruction, frequently broken down corpuscles and shadows of cells
with bartonellas still clinging to them. Only blood containing *Bar-
tonella muris* can produce the anemia. Blood in which bartonellas
cannot be demonstrated has regularly failed to produce it. Red blood
corpuscles harboring bartonellas, freed from serum and washed re-
peatedly in physiological saline, produce the anemia. The bartonellas
can be demonstrated in stained films of these washed cells. The plasma
removed from these same cells does not produce immediate anemia
after injection. That a few bartonellas are actually present, however,
is demonstrated by their appearance in small numbers in the injected
rat after a prolonged incubation period and the subsequent produc-
tion of a moderate anemia. Blood from rabbits in which a mild
anemia has been caused by inoculation of bartonella rat blood and
which harbors *Bartonella muris*, will produce a characteristic anemia
in young rats which again show the bartonellas. As pointed out
above, the virus producing the anemia and the bartonellas are both
destroyed at 57°C. It can be said, therefore, that red blood corpuscles
plus *Bartonella muris* produce anemia and that *Bartonella muris*
either represents the virus or that the virus is attached to the cor-
puscles and *Bartonella muris* is an accompanying organism.

The following experiment illustrates the production of bartonella
anemia in young rats by the injection of whole blood or washed cells
and the failure of plasma to cause anemia except after a prolonged
incubation period.
TRANSFER OF RAT ANEMIA TO NORMAL ANIMALS

Experiment with Virus 30.

Rat 30 bled from heart 5 days after splenectomy. Rat N9 injected with 0.2 cc. and Rat N10 with 0.3 cc. whole blood. Blood centrifuged. Rat N12 injected with 0.5 cc. plasma. Cells washed in physiological salt solution, resuspended and 0.5 cc. injected into Rat N13.

<table>
<thead>
<tr>
<th>Weight</th>
<th>Injection</th>
<th>Bartonella</th>
<th>Hemoglobin</th>
</tr>
</thead>
<tbody>
<tr>
<td>30 gm.</td>
<td>0.2 cc. whole blood</td>
<td>Many</td>
<td>75</td>
</tr>
<tr>
<td>32 gm.</td>
<td>0.3 cc. whole blood</td>
<td>Many</td>
<td>77</td>
</tr>
<tr>
<td>24 gm.</td>
<td>0.5 cc. plasma</td>
<td>None</td>
<td>80</td>
</tr>
<tr>
<td>32 gm.</td>
<td>0.5 cc. washed cells</td>
<td>Many</td>
<td>72</td>
</tr>
</tbody>
</table>

SUMMARY AND CONCLUSIONS.

Fifty-eight white and hooded rats have been splenectomized and all of them have shown a more or less severe anemia and an infection of the red blood cells with Bartonella muris. Another strain of white rats obtained from Littlestown showed no anemia and no bartonellas in the blood after splenectomy, until exposed to infected rats. Others of these Littlestown rats, kept in the laboratory for some time before operation and exposed to infected rats, came down with bartonella anemia within 6 days after splenectomy.

Whole blood or the washed red blood corpuscles from splenectomized rats which show bartonellas and anemia will produce a similar condition in young rats when injected intraperitoneally. Adult rats of
strains which harbor the virus (as demonstrated by splenectomy) cannot be infected by injection.

Intravenous inoculation of young normal rabbits with blood from an infected rat will sometimes produce a similar infection and anemia in the rabbit, and the virus can then be transferred back to young rats.

The virus of rat anemia may be transferred from young normal rat to young normal rat with the appearance of *Bartonella muris* and the production of anemia. In the early transfers the disease may be fatal, but it usually becomes milder in successive passages.

Although we have not yet been able to cultivate *Bartonella muris* and prove its etiological relationship to rat anemia by inoculation of cultures, we have added to the evidence that *Bartonella muris* is the cause of the anemia. Washed red blood corpuscles, containing bartonellas, will produce the anemia in the usual way while plasma from the same cells will either fail to produce it altogether or only after a prolonged incubation period. Blood heated to 57°C. for ½ hour fails to produce anemia or the appearance of bartonellas in the blood of inoculated animals.

From these observations the following conclusions may be drawn:

1. All rats which harbor *Bartonella muris ratti* come down with a more or less severe anemia after splenectomy.

2. Young rats which have not yet developed an immunity undergo the typical anemia after intraperitoneal injection of blood from a splenectomized animal in the early stages of the anemia.

3. Young rabbits may show bartonellas and develop anemia following intravenous inoculation of infected blood.

4. The virus of rat anemia and *Bartonella muris ratti* may be transferred from normal animal to normal animal for successive generations. Such strains have now been transferred for five, nine and thirty generations.

5. The resistance of rats to bartonella anemia is almost wholly dependent on the spleen. Other organs do not take over this function of protection as shown by the relapse of splenectomized rats many months after recovery. Young rats which have recovered from an attack of anemia are not protected by this previous infection from the invasion of the virus following splenectomy. Adult splenic tissue
mixed with infected blood before injection does not inhibit or neutralize the virus.

6. The virus of rat anemia is highly contagious and rats exposed to infection acquire it in some unknown way.

7. *Bartonella muris ratti* represents the virus of rat anemia or at least cannot be separated from the virus because:
   
   (a) The anemia in splenectomized and injected animals is always preceded by the appearance of bartonellas and the grade of anemia is proportional to the degree of infection with bartonellas.

   (b) Washed corpuscles containing bartonellas always produce anemia. Plasma either fails to do so, or produces a mild anemia after a long incubation period with a few bartonellas in the blood.

   (c) The thermal death point of virus and bartonella is the same.

REFERENCES.


EXPLANATION OF PLATE 11.

Fig. 1. *Bartonella muris ratti* in the blood from a rat with severe anemia, 5 days after splenectomy. Stained by Wright's stain.
FIG. 1.

(Ford and Eliot: Transfer of rat anemia to normal animals.)