STUDIES ON BACILLUS MURISEPTICUS, OR THE ROTLAUF BACILLUS, ISOLATED FROM SWINE IN THE UNITED STATES.

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PLATE 24.

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No essential differences have been detected between the mouse septicemia bacillus described by Koch (1) and the rotlauf or swine erysipelas bacillus subsequently discovered by Loeffler (2). The studies to be reported here are on organisms obtained from swine which, if they had been isolated in Europe, would undoubtedly have been called rotlauf bacilli, but since swine erysipelas is a disease that has not thus far been recognized in the United States, and since Koch's organism was the first to be described, they are called mouse septicemia bacilli, or Bacillus murisepticus.

There are reports that organisms of this group have been isolated three times in this country. Smith (3) in 1885 obtained a culture from the spleen of a pig dead of hog-cholera, and Moore (4) in 1888 obtained a culture from the spleen of another pig affected with the same disease. Smith (5) in 1894 again obtained a culture from the cervical lymph node of a pig that presumably had hog-cholera. This last paper is of special interest, for in it we find the first description of scurvy in guinea pigs and a demonstration that such animals may be susceptible to organisms to which the normal guinea pig is immune. In addition, there is a discussion of the relation of feeding to disease, a subject about which even today we know very little, but the importance of which we are beginning to realize.

These organisms are not difficult to isolate, and if they were at all common in the bodies of pigs with hog-cholera they would have been reported more frequently. In Europe, and especially in Germany, there have appeared many reports concerning their presence in swine. Koch (1) obtained the mouse septicemia bacillus from mice that he had inoculated with putrid material. How many times he found it and how frequently it has subsequently been obtained in this way cannot be determined from the literature. In 1882 Loeffler (2) found similar organisms in the bodies of swine affected with rotlauf, rouget, or swine...
Bacillus Murisepticus

erysipelas. They were present in great numbers not only in lesions of the skin but generally distributed in the body, and he regarded them as the cause of the disease. Pasteur and Thuillier (6) had previously studied rouget and described as the cause an organism which from their description is difficult to identify. That they cultivated the organism described by Loeffler is shown by the observations of Schütt (7) and Smith (8), both of whom found the mouse septicemia bacillus in the vaccine prepared by Pasteur for protecting against rouget. The organisms found by Loeffler resembled Koch's bacillus in their morphology, growth in gelatin, and in being virulent for pigeons, white and gray mice, but not for field mice. Olt (9) quotes Loeffler as finding that animals which had recovered from an infection produced by the injection of roulet bacilli were immune to \textit{B. murisepticus} and \textit{vice versa}. This power of one organism to immunize against the other has repeatedly been confirmed, and Rosenbach (10) and others have shown that roulet serum will protect against the mouse septicemia bacilli as well as against the organisms obtained from swine.

Rosenbach (10) and Freisz (11) found the mouse septicemia bacillus to be slightly larger and to grow more rapidly in gelatin than the organisms obtained from swine. Rickmann (12), on the other hand, studied over 100 cultures obtained from swine with roulet and found considerable variation in their morphology, so that he could not differentiate some of them from the one culture of mouse septicemia he had, which was the one used by Rosenbach. Stickdorn (13) found that the frequent transfer of a virulent roulet culture or its passage through a series of mice or pigeons changed not only the virulence but the character of the growth in gelatin, and Smith (5) found that the same cultural character was markedly influenced by the reaction of the medium.

When injected immediately after its isolation the roulet bacillus may be virulent for swine, but after it has been on culture media for any length of time or after it has been passed through some of the experimental animals it loses this virulence and then resembles \textit{B. murisepticus} in that it fails to cause disease when injected into pigs.

Organisms that could not be differentiated from those found in swine with roulet have been isolated from chickens by Schipp (14) and from sheep with arthritis by Poels (15). In continental Europe bacilli of swine erysipelas have also been found in swine that were apparently normal. Bauermeister (16) inoculated fifteen mice with the secretion from the tonsils of fourteen pigs. From five of the mice he obtained cultures of what he called roulet bacilli. Olt (9) also found these organisms in normal swine, but he does not give the number of animals examined or the number of cases that were positive. Van Velzen (17) studied the tonsils of eleven normal pigs and from three obtained roulet bacilli. Pitt (18) inoculated mice with the secretion from the tonsils of 50 pigs and with the secretion from the glands at the ileocecal valve of 66 pigs. 56 per cent of these swine showed roulet bacilli in the tonsils and 40 per cent in the glands at the ileocecal valve.
That these organisms are the cause of rotlauf seems to be generally accepted. The fact that they are usually present in great numbers and the encouraging results obtained by vaccination are in favor of this view. It is, however, unusual to find such a large percentage of normal animals that are carriers of organisms that are the primary agent of a disease. Olt (9) has suggested that these bacteria may gain entrance to the body through lesions produced by intestinal parasites and then become virulent; Smith (5) emphasizes the relation of feeding to an invasion of the body. There is, furthermore, the possibility that the organisms found in normal swine differ from those found in rotlauf. Proof of any of these hypotheses is lacking and the facts as we now know them suggest that these organisms may be secondary invaders. There are diseases in which secondary invaders are almost invariably present and at times, due to their number or the case with which they are isolated, are regarded as the primary agents of a disease. In some cases it is only by the most careful work and the use of special methods that the primary agent of the disease is recognized. The object of this paper is to call attention to the fact that these organisms occur in the United States where the disease of swine erysipelas is unknown, to give the cultural reactions of the organisms isolated, to record some observations on the pathology of the disease produced in experimental animals, especially the mouse, and finally to raise the question as to whether these bacilli really are the primary agent of rotlauf in swine.

Isolation of Cultures from Swine.

In a study of the tonsils of pigs that had been inoculated with hog-cholera virus some of the exudate from an ulcer on the tonsil was injected into mice. These mice died in a few days and from them were isolated organisms that corresponded to the mouse septicemia bacillus. Following this first observation, material from the tonsils of all the swine that came to autopsy was injected into mice, and when these animals died, cultures were made from the spleen and heart’s blood and films from the lungs were stained by Gram’s method and examined for the intracellular Gram-positive rods, which are characteristic of Bacillus murisepticus.
BACILLUS MURISEPTICUS

Sixteen swine, all infected with hog-cholera virus, have been examined in this way and from the tonsils of five of these bacilli have been isolated which morphologically and culturally are mouse septicemia bacilli. The organisms were apparently localized in the tonsils, for they were not found in cultures made by transferring pea-sized bits of liver, spleen, and kidney to agar slants.

Four of the positive swine were from the same litter and the source of the fifth one is questionable, so that it appears that the infection is restricted. On the farm from which these animals came there is no record of any diseases among pigs, and before inoculation the animals were apparently well.

These organisms may have some relation to the ulcers that are so commonly present on the tonsils of pigs with hog-cholera, as four of the five positive cases showed ulcers, whereas only four of eleven negative cases showed them. It is just as possible, however, that a variety of organisms may be associated with these ulcers, as they are probably due to invasion by bacteria of a lesion produced by the hog-cholera virus. Bacilli of the swine-plague group were often present and killed the inoculated mice before the mouse septicemia bacilli had time to invade the bodies of these animals. It would be of interest to know how common and how widespread the mouse septicemia bacilli are in the United States, and if such a study is made it would be well to immunize part of the mice used for their isolation by the injection of anti-swine-plague serum as was done by Van Velzen (17).

Cultural Characters.

The ease with which mouse septicemia bacilli are identified probably accounts for the meager description of their cultural reactions. The morphology has been well described, as has the growth on agar and in gelatin, but this is as far as most descriptions go. It therefore seemed worth while to gather together the recorded reactions and to fill in the most obvious gaps. The five cultures isolated recently and the one culture isolated by Smith (5) in 1894 have been used, and all have been found to give the same results.

Morphology.—These organisms are non-motile, Gram-positive rods, varying considerably in length and diameter, the variation depending
upon the source from which preparations are made. In films from mice, dead after inoculation with a pure culture, they appear as very slender, straight or slightly curved rods from 1.5 to 2 μ in length. They may appear free or are grouped in a characteristic manner in large cells with an indefinite nucleus. From the surface of Loeffler serum slants and in gelatin incubated at 37°C, they appear as straight or slightly curved rods somewhat thicker than those found in the tissues of mice. In bouillon, and especially on agar slants, this increase in size is more marked. The organisms may measure up to 4 μ in length and show a decided tendency to curve and form clumps of interlacing bacilli. Branching forms have not been found in films made from cultures on a variety of media incubated up to 5 days. In sections of tissues stained by the Gram-Weigert method the organisms often have a beaded appearance, but in films stained by Gram's method, using Stirling's gentian violet and decolorizing with alcohol, the organisms stain uniformly.

**Agar.**—Growth appears in 24 hours in the form of fine, translucent, slightly gray colonies. It is very scanty, but becomes more abundant if serum or defibrinated blood is added to the medium.

**Gelatin.**—Stab cultures show the characteristic "test-tube brush" growth described by Loeffler (19) which has been serviceable in identifying these organisms. Petri and Maassen (20) obtained the same type of growth in a semisolid medium at incubator temperatures, but I have been unable to confirm this. Smith (5) noted that when the gelatin was acid this characteristic growth did not occur. After some weeks incubation gelatin stabs show a finger-like depression which is not found in uninoculated tubes that are incubated the same length of time. If this is due to a softening and evaporation of the gelatin, as has been stated, it is evident that it proceeds at a very slow rate and that it only occurs in the presence of oxygen. Gelatin cultures that have been incubated at 37°C. for 30 days will still harden when placed in the refrigerator.

**Bouillon.**—In 24 hours there is a uniform turbidity and when shaken a characteristic cloud-like effect is produced that has been considered of diagnostic value.

**Blood Serum.**—Several writers state that these organisms do not grow on this medium, while Poels (15) states that it grows but does
not liquefy the serum, this being one of the differential points between
the rotlauf bacillus and *Bacillus pyogenes*. All of my cultures have
formed a scanty growth on Loeffler serum slants and after 30 days
incubation there was no evidence of liquefaction.

*Milk.*—All writers agree that no visible change is produced in this
medium. Moore (4) found microscopic evidence of vigorous growth
of the one strain he studied, while Schipp (14) found no evidence of
growth of the three strains with which he worked. All of my cultures
have failed to produce acid in milk, yet all of them show microscopic
evidence of growth. As will be noted below, these organisms attack
lactose in bouillon, but not in milk.

*Ammonia.*—6 day cultures in fermented bouillon failed to show any
appreciable amount of ammonia.

*Hydrogen Sulfide.*—Petri and Maassen (20) found this gas in their
cultures, and in swine dying of rotlauf they found sulfmethemoglobin,
but they did not find sulfmethemoglobin in experimental animals in-
oculated with pure cultures. All six of my cultures blackened lead
acetate in peptone agar in 24 hours.

*Indole.*—Schipp (14) has the only reference that I have found to
indole production, his three cultures all giving a negative test. All
six of my strains in fermented bouillon cultures 5 days old gave a nega-
tive test with Ehrlich’s aldehyde as did a typhoid culture and the
un inoculated medium. A colon culture gave a positive reaction.

*Phenol.*—No reference has been found on phenol production.
Three of my cultures were grown for 10 days, each in 40 cc. of fer-
mented bouillon contained in 100 cc. Erlenmeyer flasks. They were
then distilled with steam and the distillate was tested with bromine
water and Millon’s reagent. Reactions for phenol were not obtained.

*Hemolysins.*—Van Nederveen (21) found that his culture did not
hemolyze swine blood agar in plates, or the blood of cattle, rabbits,
or pigeons in bouillon. The six cultures of the present study all pro-
duced a distinct zone of hemolysis around the deeper colonies in veal
infusion agar plates containing about 10 per cent of sterile defibri-
nated horse blood. The same blood in bouillon was not hemolyzed.

*Fermentation of Carbohydrates.*—The literature gives very little in-
formation about this important phase of the bacterial activity of these
organisms, and that found is not in agreement. Fermi (22) states that
the rotlauf bacillus forms acid from starch but that it does not possess a diastatic enzyme. Petri and Maassen (20) found that the growth of their rotlauf cultures was increased when dextrose, lactose, saccharose, or dextrin was added to the medium. Smith (5) studied one culture and found that acid but no gas was formed in bouillon containing dextrose or lactose, while no acid was formed in bouillon containing saccharose. Schipp (14) studied three cultures and found that acid was formed in a litmus peptone solution containing lactose or saccharose, while in the same medium containing dextrose the litmus was decolorized but was not reddened.

Preliminary tests with fermentation tubes containing fermented bouillon plus 1 per cent of either dextrose, lactose, or saccharose showed that these organisms did not form gas, but produced acid from the first two carbohydrates and that somewhat more acid was produced in the presence of oxygen than in its absence. In saccharose bouillon there was no acid or alkali formed. The great majority of the tests has been made in ordinary test-tubes containing 13.5 cc. of fermented bouillon to which 1.5 cc. of a previously autoclaved 10 per cent solution of the carbohydrate in distilled water was added. The tubes were steamed and incubated to insure sterility and were then inoculated from young bouillon cultures and titrated after from 5 to 7 days incubation. When acid was produced hydrogen ion determinations were also made, but when the carbohydrate was not acted upon this was not done. In Table I are given the extremes of reaction produced by the six cultures when acid was produced, the actual amount

<table>
<thead>
<tr>
<th>Carbohydrate</th>
<th>Initial reaction</th>
<th>Length of incubation</th>
<th>Final reaction</th>
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<tbody>
<tr>
<td></td>
<td>Acidity</td>
<td>pH</td>
<td>per cent</td>
</tr>
<tr>
<td>Dextrose</td>
<td>0.7</td>
<td>7.5</td>
<td>5</td>
</tr>
<tr>
<td>Lactose</td>
<td>0.9</td>
<td>7.5</td>
<td>7</td>
</tr>
<tr>
<td>Arabinose</td>
<td>1.3</td>
<td>7.3</td>
<td>10</td>
</tr>
<tr>
<td>Fermentation tubes</td>
<td>1.1</td>
<td>7</td>
<td></td>
</tr>
</tbody>
</table>
of acid formed being the difference between the initial and final reactions of the medium.

It is evident that dextrose and lactose are attacked. The small amount of acid formed in the arabinose medium might indicate that there were impurities present which were acted upon while the carbohydrate itself was not attacked. The lot of arabinose used was the best obtainable and had been used for some time with satisfactory results in differentiating the hog-cholera bacillus from the other paratyphoids. If the mouse septicemia bacilli attack it at all they do so very slowly.

Fermented bouillon cultures containing the following carbohydrates have also been titrated and no acid has been found.

<table>
<thead>
<tr>
<th>Xylose</th>
<th>Inulin</th>
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<tr>
<td>Dulcitol</td>
<td>Salicin</td>
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<tr>
<td>Maltose</td>
<td>Dextrin</td>
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<tr>
<td>Mannitol</td>
<td>Starch</td>
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<tr>
<td>Saccharose</td>
<td>Glycerol</td>
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Reduction.—The one attempt to study the reducing powers of these organisms was a failure as the presence of 1 per cent of either rosalic acid or methyl red apparently inhibited growth.

Pathogenicity.

Considerable work has been done on the virulence of the mouse septicemia bacilli for a variety of animals. Koch (1) found that they produced a fatal disease in white and gray mice, while the field mouse was immune. Loeffler (2) found that pigeons and sparrows were as susceptible as mice, and that frogs, salamanders, chickens, dogs, cats, and white rats were immune. In rabbits an erysipelas-like infection of the ears and a loss in weight followed an intravenous injection of the bacilli, but the animals usually recovered and later were immune. The virulence of the organisms isolated from pigs with rotlauf corresponds to that of the mouse septicemia bacillus except that very freshly isolated cultures may infect swine. This virulence is soon lost and the cultures are then the same as the organism isolated by Koch.

The strains which I have isolated are all virulent for white mice in as small amounts as 0.001 cc. of a 24 hour bouillon culture. The mice
are usually dead by the 3rd day. The culture isolated by Smith 26 years ago is also virulent for mice in the same amounts, but the animals die from 2 to 3 days later. These cultures when injected subcutaneously into pigeons cause death in about 4 days. Intravenous injection into rabbits causes a marked edema of the ear on the side of the injection and at times the opposite ear is also involved. The animals are very quiet, show a rise in temperature to around 41°C, and lose from 150 to 250 gm. in weight. Only one of the cultures will kill rabbits when injected either intravenously or subcutaneously. Whereas in mice and pigeons after death the organisms are abundant in all the organs and in the blood stream, in rabbits they are very scarce. They are rarely found in films, and in cultures made from bits of the various organs, or from several drops of blood, growth in agar slants occurs only in the condensation water.

Three pigs have been inoculated with the cultures isolated from the tonsils. One was given an intravenous injection of 1 cc. of a 24 hour bouillon culture. There was a slight rise in temperature without any signs of illness. The skin was normal and the appetite undiminished. The pig was then inoculated with hog-cholera virus and at autopsy cultures were made from the liver, spleen, and kidney, with negative results.

Two pigs were inoculated with another strain, one receiving 5 cc. of a 24 hour bouillon culture intramuscularly and the other 4 cc. intraperitoneally. Neither pig showed any effects from the inoculation. These results correspond to those obtained by Smith (5) and Moore (4) with the mouse septicemia bacilli which they isolated in this country.

One of the striking features of films made from mice and pigeons is the great number of organisms that is found in leucocytes. Koch (1) first called attention to this, and it has since been used as one of the means of identifying these organisms. Curiously enough, I have found no statement as to the type of cells in which these bacteria are found. From the drawings of Koch (1) and Moore (4) one might assume that they are polymorphonuclear leucocytes, since some of the cells show two nuclei. In the literature they are called leucocytes without further qualification.
Several years ago I made some experiments with an old stock culture of the mouse septicemia bacillus, one of the objects being to determine in which type of cell it occurred. After a subcutaneous injection of about 0.01 cc. of a 24 hour bouillon culture mice died on the 4th or 5th day. A number of inoculated mice were killed at intervals, and films, sections, and cultures made from the various organs. Cultures showed that the organisms were generally distributed at the end of 2½ days, but they were so scarce that they could not be found in films. On the 4th day, when the animals appeared to be sick, the bacilli could be found in films, especially in those from the lungs. Except when present in great numbers, they were always intracellular, and the only type of cell in which they could be found was the endothelial leucocyte (Figs. 1 and 2). Sections of the various organs showed that the bacilli at this stage were in the endothelial cells lining the veins and capillaries and also in endothelial cells free in the blood stream (Figs. 4 and 5). They were not found in endothelial cells lining the arteries or the heart. As the time of death approached the organisms were present in enormous numbers, filling the endothelial cells and crowding the nucleus to one side so that the cell resembled a sac containing a culture of the bacilli. When films were made these sacs were ruptured and the organisms were set free.

In pigeons the process was apparently the same except that the organisms were found generally distributed about 24 hours earlier than in the mouse. They were in the endothelial cells lining the veins and capillaries, and the type of cell in the blood containing them was apparently an endothelial leucocyte (Fig. 3). In both the mouse and the pigeon the organisms were at no time found in polymorphonuclear leucocytes.

Endothelial cells with only a few bacteria soon show evidences of injury. The cytoplasm contains small vacuoles and the nucleus when stained with a modified Romanowsky stain has a more reddish tinge than the nucleus of the same type of cell that is free from bacteria. As the bacilli become more numerous larger vacuoles appear in the cytoplasm and the nucleus stains distinctly red and shows evidences of disintegration.

The disease in the mouse appears, then, to be associated with an intracellular process. The organisms are taken up and instead of
being destroyed are able to multiply and finally to kill the cell. Some of these infected endothelial cells probably break away from the walls of the vessels and float free in the blood stream. Rous and Jones (23) have called attention to the fact that phagocytosed bacteria may be protected against immune substances in the body fluids. There are other instances of the multiplication of bacteria in cells. The leprosy bacillus may be found in the endothelial cells of blood vessels and the tubercle bacillus and *Treponema pallidum* are usually intracellular. Smith (24) has called attention to the localization of *Bacillus abortus* in the chorionic epithelial cells of cattle, and Tyzzer (25) to a disease of Japanese waltzing mice in which the organisms are found in the liver cells and cells of the intestinal mucosa. It is worthy of note that most of the diseases in which the organisms are found in cells are more or less slowly progressive, while the disease in the mouse produced by *Bacillus murisepticus* is acute.

**SUMMARY AND CONCLUSIONS.**

In the United States organisms, which culturally are mouse septicemia or swine erysipelas bacilli, have been isolated from the tonsils of five of sixteen pigs examined. These pigs all had hog-cholera, but it is probable that the bacilli were in the tonsils before they were infected with hog-cholera, and there is no evidence that they played any part in the disease. The distribution of the infection seemed to be restricted as most of the pigs from which the bacilli were obtained came from one litter. As we do not have clinical rotlauf, or swine erysipelas, in this country, as these organisms, in Europe, have been found in a large percentage of apparently normal swine, and as the disease is produced with difficulty by the injection of cultures, the question may be raised whether they are not secondary invaders rather than the primary cause of the disease with which they have been associated, or else whether the resistance of swine on the European continent does not differ from that of our breeds as a result of differences in foods.

It is possible that the mouse septicemia bacilli found in this country may differ culturally from those present in animals with swine erysipelas. With this in mind, the carbohydrate reactions, as well as
other cultural characters not necessary for the identification of the bacilli isolated, have been studied.

The disease produced by the injection of these bacilli into mice and pigeons has been studied and shown to be largely an intracellular process. The organisms are taken up by the endothelial cells lining the veins and capillaries; there they multiply and soon kill the cells. It has also been shown that the only type of cell in the blood stream which contains bacteria is the endothelial leucocyte, and the probabilities are that the free phagocytes have been detached from the lining of the vessels. The disease is acute, and the indications are that in the cells the bacilli find a favorable medium for their growth. While phagocytosis may in general be an immune reaction, in this case it appears to favor the parasite rather than the host.

The writer is indebted to Mr. Henry Hagens, of this laboratory, for valuable technical assistance.

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7. Schütz, Arb. k. Gesundhamsie, 1886, i, 56.


EXPLANATION OF PLATE 24.

Fig. 1. Film from the heart's blood of a mouse killed 3 days after the subcutaneous injection of 0.01 cc. of a bouillon culture of freshly isolated B. muri- septicus. Bacilli in an endothelial cell. × 1,000.

Fig. 2. Film from the lung of the same mouse. Bacilli in two endothelial cells. × 1,000.

Fig. 3. Film from the lung of a pigeon dead after a subcutaneous injection of 0.01 cc. of a bouillon culture of freshly isolated B. muri-septicus. Bacilli in a mononuclear cell which is probably an endothelial leucocyte. Free organisms from ruptured cells. × 1,000.

Figs. 4 and 5. Sections of the kidney of a mouse showing B. muri-septicus in the endothelial cells of a vein. × 1,000.