ON THE RELATION OF THE ORGANISMS IN THE TUNICA VAGINALIS OF ANIMALS INOCULATED WITH MEXICAN TYPHUS TO RICKETTSIA PROWAZEKI AND TO THE CAUSATIVE AGENT OF THAT DISEASE

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PLATE 5

(Received for publication, October 26, 1929)

INTRODUCTION

In earlier communications (1, 2) one of the present authors has advanced considerable evidence that the small intracellular organism discovered by him in the tunica vaginalis of guinea pigs reacting to the virus of tabardillo represents the specific organism of Mexican typhus. This organism can easily be demonstrated in all animals killed during the early scrotal reaction, whereas it never was met with in numerous normal control animals nor in animals which had recovered from the experimental disease. In its morphology, staining properties and its mode of intracellular multiplication, it exhibits a striking resemblance to Rickettsia prowazeki as found in the gut of lice fed upon typhus patients. The tunica vaginalis regularly proved to be the most infectious tissue, more so than brain, and a positive Weil-Felix reaction can be induced in rats and in rabbits more regularly with tunica than with blood or brain. The same small micro-organism was recently also demonstrated in both of these species. The rat proved to be an even more appropriate animal for the demonstration of this micro-organism than the guinea pig, as was first shown by Maxcy (3). It is frequently found in smears from the tunica of white rats in astonishing numbers within greatly swollen cells from the endothelial covering of the visceral and parietal surfaces. The relation of this organism to typhus fever has been so far cleared up that we were able

to state that it could not be separated etiologically from our strain of Mexican typhus. We still lacked, however, some very important evidence for the identity of the two, namely, the demonstration of the tunica organism in animals inoculated with lice infected with the virus of our strain. Although the findings of Maxcy in a strain of endemic typhus from North Carolina (4) and those of Pinkerton in a strain from Europe (5) have brought full confirmation of the findings of Mooser, the possibility that the tunica organism is a mere secondary invader had not yet been completely excluded. When one considers that the blood serum of a typhus patient regularly agglutinates in high dilutions B. proteus X19 and frequently the bacillus of Plotz, both of which have no etiological relation to that disease, even an organism found with such regularity as the tunica germ must be looked upon with suspicion. Progress can only be expected from experiments with infected lice. The present paper deals with this problem.

Material and Methods

1. Three monkeys have been used in these experiments, Monkeys 1, 2, and 3. As these three animals have not been inoculated at the same time and with the same material, the account of their reaction after inoculation and the result of lice feeding experiments will be presented separately.

2. The strain of body lice used in these experiments was carefully examined for Rickettsia-like organisms before we started. No suspicious organisms could be found in smears from very numerous specimens. The intestinal tract of the great majority proved to be free from demonstrable micro-organisms. The same was true for a number of lice fed for a week on a normal monkey. As the lice did not feed when applied to the monkeys within a Nuttal box, the felt with the lice on it was removed from the boxes each time for feeding and applied directly to the shaved abdomen. They were always closely watched for the whole time of feeding. As a rule they were applied twice a day to an infected monkey and, between feedings, they were kept in an incubator at 32°C.

Experiments with Monkeys

Experiment No. 1.—a. The Reaction of Monkey No. 1. Monkey No. 1 used in this experiment was a young female, a cross-breed between Macacus rhesus and the common macacus. She was inoculated intraperitoneally on May 12th with 5 cc. of blood and 5 cc. of testicular washings (4) from a guinea pig on the third day of fever and scrotal swelling. Examination of smears from the tunica had shown few extra and intracellular organisms. Chart No. 1 illustrates the reaction of this monkey. 120 young lice had been put on her on May 8th.

Twenty-four hours after inoculation the animal's temperature rose to 105 and it was listless and refused all food. Examination revealed that she was menstruating. The next day the fever had dropped and the animal was lively again. Five days after inoculation she was shivering in her cage, was listless again and did not touch any food for nearly two days, when she slowly began to recover, improving steadily despite a continued fever and appearing healthy on the day when the fever dropped to normal. On the second day after defervescence, the lice were transferred to a normal monkey, to avoid the action of the bactericidal power of the convalescent blood in making emulsions of these lice. The lice acted normally until May 26th when large numbers began to die. Before this date only

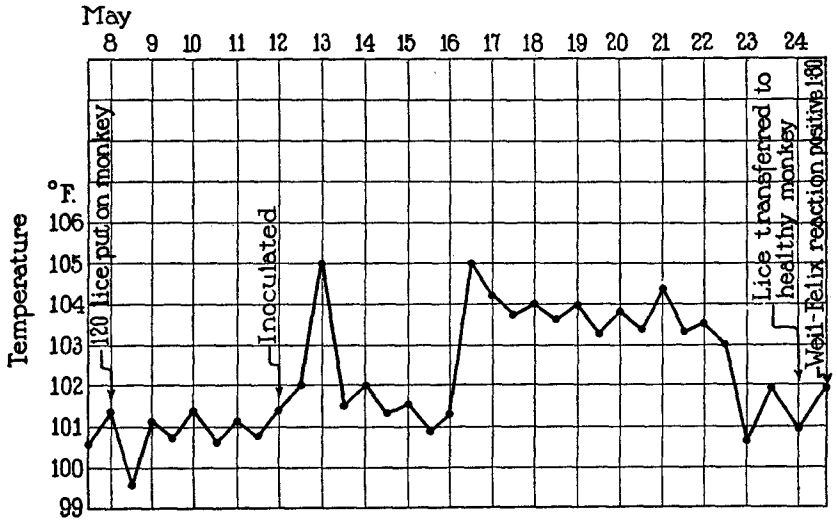

CHART 1. Monkey No. 1

four lice had died, but from then on the number of dead lice increased daily, and on May 30th, only 30 of them were still alive.

b. The Organisms Found in Lice Fed on Monkey No. 1. On May 24th scarce bipolar staining Rickettsiae were found in a dying louse. Every dead and dying louse was from then on examined carefully by making a smear from the dissected intestinal tract. On May 26th a second louse was found to be infected. It contained large numbers of the small red staining diplobacilliform organism described in typhus literature. On May 30th among a large number of dead lice, one was found to be very distended and discolored. It was the only one in which micro-organisms could be found. It contained very large numbers of minute red staining diplobacilli and coccoid forms and slightly larger bacillary forms exhibiting bipolar staining. Smears made from an emulsion of sixteen living lice did not reveal any organisms. Equal parts of this emulsion were inoculated into a

male guinea pig (G1) and a male white rat (Rat 1). The remaining fourteen living lice were then cut longitudinally into halves. From one side a smear was made and the other half was preserved for animal inoculation. In the smears of these fourteen lice Rickettsiae were found in two only. One contained large numbers of the small red staining diplobacillary and coccoid forms whereas the other showed numerous pale bipolar staining bacillary forms. The halves of these fourteen lice were emulsified in a mortar, taken up with 20 cc. of normal salt solution and inoculated intraperitoneally into a male rat (Rat 2) and three male guinea pigs (G2, G3, G4).

c. The Reaction of Guinea Pigs and White Rats Inoculated with Lice Fed upon Monkey No. 1. Nine days after inoculation G4 showed a moderate edema of the scrotum and a temperature of 104.6 F. G2 and G3 had had irregular fever since the time of the inoculation. On the day when G4 had fever and a suspicious reaction of the scrotum, Rat 2 was killed. The examination of its organs revealed a slightly moist peritoneum and there was a fine coat of fibrinous material covering the slightly hyperemic testicles and the parietal surfaces of the tunica. Stained smears made from the surfaces of both testicles and from the tunica parietalis showed numerous heavily infected endothelial cells crowded with reddish staining minute diplobacilli and other cells containing only few slightly larger, bluish staining diplobacilli exhibiting bipolar staining. Both these forms were morphologically and tinctorially indistinguishable from the organisms seen in the lice.

With a saline solution in which both testicles of this rat had been vigorously agitated two guinea pigs were inoculated intraperitoneally. Both developed high fever and a beginning scrotal swelling sixty hours after inoculation and numberous extra and intracellular organisms were found in smears from the tunica of both animals. A separate strain of typhus (louse strain No. 1) was started from one of these animals. This strain exhibited all the characteristics of the passage strain with the exception that in the first two transfers, it showed a remarkably short incubation period and there was an unusually severe general and scrotal reaction.

G4 was killed on the third day of fever. The slight scrotal swelling had completely subsided on the second day. The autopsy was negative except for the testicles which were hyperemic and covered by a fine film of fibrin. Smears made from both sides revealed the typical cytological picture that is found in animals reacting to tabardillo but it was only after a very prolonged search that a few intracellular organisms with characteristic morphology were found within an endothelial cell.

G1, which had been inoculated with an emulsion of sixteen lice, died of pneumonia twelve days after inoculation. No indication of a typhus infection could be found at autopsy.

The slight irregular fever of G2 and G3 lasted for about ten days but there was never any sign of scrotal swelling found in spite of daily examination.

d. Cross-Immunity Tests between the Strain Isolated from Rat No. 2 and the

Original Passage Strain. There was complete cross-immunity between the louse strain No. 1 and the original passage strain. Not only did animals previously inoculated with one of them not show any fever when subsequently inoculated with the other strain, but also the scrotal reaction failed to make its appearance and no tunica organisms could be found in these animals. Guinea pigs 2 and 3, however, reacted typically after inoculation with the passage virus, and the tunica organism could easily be demonstrated in smears from the processus vaginalis. Aside from the lesions mentioned, these two animals showed evidence of a healing diffuse peritonitis.

While there can not be any doubt that the virus of typhus was recovered from a rat inoculated with an emulsion of lice infected with Rickettsia prowazeki, the failure of two guinea pigs to react after inoculation with an equal part of the same emulsion was difficult to explain. The circumstance that the organisms in the tunica of another animal (G4) were found only after a very prolonged search, however, was not surprising as the animal was killed on the third day of fever when occasionally extremely few or no infected cells may be found in the tunica of animals reacting to tabardillo. Two previous observations may furnish an explanation for the failure of G2 and G3 to become infected. When the present strain of tabardillo was isolated nearly two years ago (6), only one of two guinea pigs inoculated with the same amount of blood of a case of Mexican typhus reacted, whereas the other not only did not show any evidence of disease, but reacted typically when inoculated later with the passage virus. More frequent was the observation that the virus does not take easily in an animal whose peritoneal cavity is not free from another kind of infection. The reaction of the tunica constitutes a primary lesion due to the intraperitoneal inoculation which brings the virus in contact with the endothelial lining of the tunica vaginalis. Acute inflammatory processes within the peritoneal cavity seem to be decidedly unfavorable for the primary localization of the virus within the endothelial cells of the peritoneum. The failure of G1 to react is not astonishing as we had not found any Rickettsia-like organisms in the emulsion from the sixteen lice with which it was inoculated. Rat 1, which was inoculated simultaneously, did not agglutinate proteus X19 a week and 12 days respectively after inoculation. It was concluded therefore that none of the sixteen lice inoculated into Rat 1 and G1 contained the virus of typhus.

Experiment No. 2.—Monkey No. 2, a Macacus rhesus, was a large adult female. She was inoculated intraperitoneally on June 25th with 10 cc. of blood and nearly the entire brain of a guinea pig, killed on the fourth day of fever. At the same time an emulsion made from the scrapings of the surface of both testicles and tunicae parietales was injected, 10 cc. subcutaneously over the abdomen and 10 cc. intraperitoneally. The scrotal swelling of this guinea pig had already begun to subside when it was killed. The careful examination of six smears from both sides revealed a single endothelial cell containing the organisms. It was for this reason that we have inoculated nearly the entire brain and 10 cc. of blood in addition to the tunica emulsion.

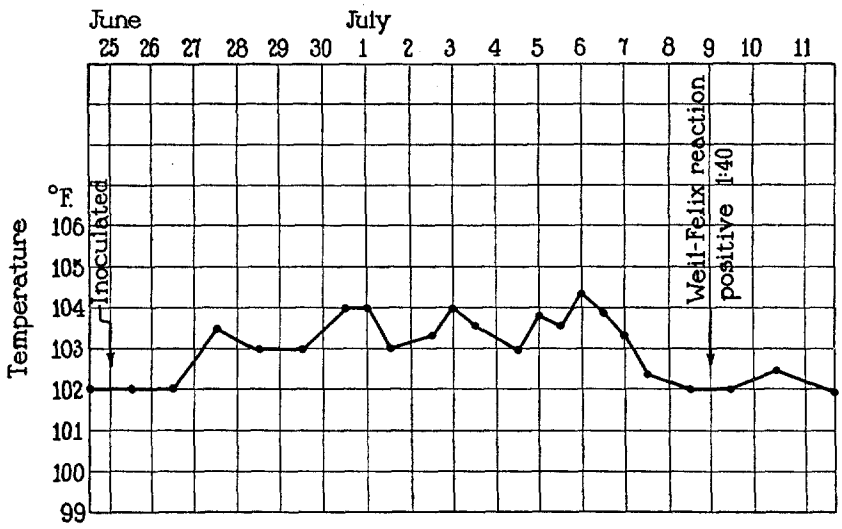

CHART 2. Monkey No. 2

Almost immediately after inoculation, this monkey showed signs of illness. She did not take any food for two days subsequently and remained crouched in a corner of her cage. On the third day, the place where the subcutaneous inoculation had been given became edematous and slightly inflamed. This reaction subsided completely within three days. The temperature of this monkey is illustrated in Chart 2.

350 lice, mostly nymphs, were put on this monkey on the day she was inoculated. They were allowed to feed twice daily as in Experiment No. 1. From the sixth day on, after they had begun to feed on this monkey, two or three of them were killed each day and examined for Rickettsiae. None were found. As the fever of Monkey No. 2 was very mild, we inoculated Monkey No. 3 on July 3rd and transferred the lice to the latter animal when it began to have fever. This was done on July 6th, eleven days after the lice had been put on Monkey No. 2.

This experiment was unsatisfactory and therefore not conclusive for the reason that we interrupted the louse feeding experiment too early. There is no doubt in our minds that the monkey had a mild attack of tabardillo on account of the typical local reaction (7) and a positive Weil-Felix reaction two weeks after inoculation. We transferred the lice to another monkey in order to take full advantage of the large crop of young lice carefully reared for this experiment. We were afraid that by feeding the lice unsuccessfully for a longer time on Monkey No. 2, they might have become too old to be used in another experiment, because adult lice live only about 25 to 30 days. That the lice had not become infected by feeding on this monkey and that our precaution was fully justified can be seen from the fact that Rickettsiae did not appear in them before the seventh day after feeding on Monkey No. 3, eighteen days after Monkey No. 2 had started to have fever.

Experiment No. 3.—a. The Reaction of Monkey No. 3. Monkey No. 3, a young male, was a cross-breed between Macacus rhesus and the common macacus. He was inoculated with an emulsion from both tunicas and scrapings from the surface of both testicles. This material was taken from a guinea pig on the third day of a very severe febrile reaction and on the second day of a very pronounced scrotal swelling. Part of the emulsion was inoculated intraperitoneally, the rest was introduced beneath the skin of the abdomen, infiltrating a large area of subcutaneous tissue. The examination of several smears from both tunicas before inoculation had revealed exceptionally numerous extra and intracellular organisms in every field of the oil immersion lens. Two guinea pigs inoculated with brain and one with a small amount of the tunica emulsion from the same animal were used as controls. Chart No. 3 gives the reaction of Monkey No. 3. All three guinea pigs developed high fever and a scrotal swelling 60 hours after inoculation. The reaction of the guinea pig inoculated with tunica was especially severe. Immediately after inoculation this monkey was an entirely changed animal. A lively and very friendly pet before, he acted very sick from the start, refusing all food for three entire days, sitting with a painful expression on his face in a corner of his cage, never budging an inch. On handling, he acted as if he had severe pain in his abdomen where the inoculation had been performed. 48 hours after inoculation an extensive inflammatory edema made its appearance at the place of injection. The central part of it became diffusely hemorrhagic the next day. This local reaction lasted for four days. Simultaneously with the swelling the fever started in this monkey. On the second day of fever, the lice were transferred from Monkey No. 2 to this monkey. They were fed upon him twice daily until the temperature had dropped to normal, when they were transferred to a

fresh monkey. Care was taken to feed the lice on the place of the local reaction as experiments with guinea pigs had shown that multiplication of the virus takes place in the skin after subcutaneous inoculation of material from the tunica. Monkey No. 3 had the severest reaction of all three animals. When the fever rose to 106 on July 9th a pronounced injection of the conjunctivae especially over the sclerae appeared. It exhibited a remarkable resemblance in respect to color and general appearance to the conjunctival reaction of human patients suffering from typhus. It persisted until the day on which the fever began to subside. At that time the monkey began to improve rapidly, returning to its normal condition within a few days.

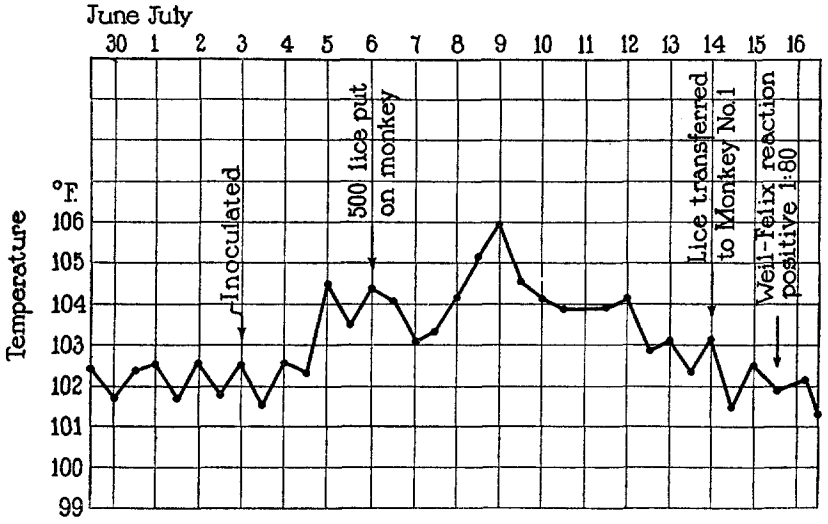

CHART 3. Monkey No. 3

b. The Organisms Appearing in Lice Fed on Monkey No. 3. The lice continued to feed eagerly on this monkey during the entire course of its fever with only an insignificant mortality of three or four lice daily. All lice which were found dead on opening the box were examined microscopically and also those which looked bad and refused to feed, one or two daily. No organisms were found until the seventh day after feeding on Monkey No. 3 when numerous small bipolar staining bacilli were found in teased preparations from the gut of a dying louse and from the guts of two dead lice. It was not until the tenth day that large numbers of the small red staining diplobacillary and minute coccoid forms made their appearance. The lice began to die now in great numbers and these small typical Rickettsiae were found in nearly all lice examined.

Three days after the lice had been transferred to a normal animal or twelve days after they had been put on Monkey No. 3, the box contained only 120 lice.

Sixteen were dead, 44 were dying and sixty fed normally, although the majority of them was distended and somewhat discolored. Smears made from ten lice taken at random on that day showed large numbers of the small red staining Rickettsiae in eight of them. A smear made from a greatly distended and discolored louse showed enormous numbers of these small micro-organisms.

c. The Reaction of Animals Inoculated with Lice Fed on Monkey No. 3. Fourteen rats and four guinea pigs were inoculated with emulsions of lice fed on Monkey No. 3. The lice were put into 95 per cent alcohol for five minutes. They were then transferred to a sterile Petri dish until thoroughly dry before making the emulsion.

The inoculations were made as follows:

July 13, 7th day after feeding: 7 lice into 2 rats (Rat 1 and Rat 2).
July 14, 16 lice into 3 rats (Rats 3, 4 and 5).
July 15, 25 " " 2 " (" 6 and 7).
July 16, 16 " " 2 " (" 8 and 9).
July 17, 14 " " 2 " (" 10 and 11) and 2 guinea pigs (G1 and G2).
July 18, 60 " " 2 " (" 12 and 13) and 2 " " (G3 and G4), and 30 lice separately into 1 rat (Rat 14).

The remaining twenty lice were fixed in Regaud's fluid for histological examination. Prior to every inoculation, a smear was made from each emulsion in order to look for Rickettsiae. This smear was made before the emulsion was taken up in saline solution. Rickettsiae were found in increasing numbers from July 13th to July 18th.

All four guinea pigs (G1, G2, G3, G4) showed fever 60 hours after inoculation and on the end of the third day a pronounced scrotal swelling developed in three of them (Figure 1) whereas the third had only a moderate swelling which increased and decreased slightly during the following three days. One animal (G3) was killed on the third day of fever. The autopsy was negative except for the pronounced and typical lesions of testicles and tunica vaginalis. The intracellular organisms were easily demonstrated in smears from both sides. From this animal a separate strain was started, louse strain No. 2. It induced the typical symptoms of tabardillo in guinea pigs and rats.

Seven rats (1, 3, 6, 8, 10, 12, 14) were killed between the 5th and 6th day after their respective inoculations. The organisms were easily demonstrated in the exudate around the testicles in all of them. In two rats (4, 11) killed after the 7th day, no organisms could be found. Although the lesions of the tunica in these three animals looked typical, the examination of smears indicated that the animals had been killed too late for the demonstration of Rickettsiae because the polynuclear leucocytes which accompany the Rickettsiae in the tunica exudate had already been replaced nearly completely by lymphocytes and large mononuclear leucocytes. From one rat which was positive a separate strain of tabardillo was started (louse strain No. 3). It produced the typical scrotal lesion in all guinea

pigs inoculated and smears from their tunicas revealed with regularity the small intracellular organisms.

The remaining five rats (2, 5, 7, 9, 13) were killed by bleeding twelve days after their respective inoculations. A Weil-Felix reaction performed with their blood gave the following results:

Rat 2 : Positive in a dilution of 1:20.
Rat 5 : " " " " " 1:20.
Rat 7 : " " " " " 1:10.
Rat 9 : " " " " " 1:80.
Rat 13: " " " " " 1:100.

d. Cross-Immunity Tests between the Two Lice Strains (1 and 2), the Original Passage Strain and a Strain of Endemic Typhus from North Carolina (Maxcy Strain). Several animals which had recovered from a typical reaction to these lice strains failed to react when subsequently inoculated with a heavy dose of passage virus and virus of the Maxcy strain respectively. Animals recovered after inoculation with the original passage strain and with Maxcy's strain proved to be immune to a subsequent inoculation with one of the louse strains. No organisms could be found in the tunica of these reinoculated animals.

The result of this experiment demonstrates more clearly than Experiment No. 1 that the virus of tabardillo which is characterized by the lesions of scrotum and tunica in guinea pigs is able to multiply in the body louse. Moreover, it demonstrates that the small intracellular organism constantly present in the tunica vaginalis of our passage strain can be recovered easily from rats and guinea pigs after the virus has passed through the louse. An observation made during these lice experiments has a special bearing on the significance of the tunica germ. Monkey No. 3 which was inoculated with material containing exceptionally numerous tunica germs had a very severe reaction and nearly all lice which were allowed to feed upon him became infected with Rickettsia prowazeki. Monkey No. 1 which had received blood and washings from a tunica which had shown relatively few organisms had a mild reaction and only a small percentage of lice became infected with Rickettsia prowazeki. Inoculation into guinea pigs and rats revealed that few of these lice contained the virus of typhus. In the tunica which was used to inoculate Monkey No. 2 only very scarce organisms could be demonstrated after a prolonged search. The reaction of this monkey was so mild that only the positive Weil-Felix reaction gave us the security that his mild fever

was really due to typhus. Not a single louse became infected with Rickettsia prowazeki by feeding on this monkey.

The Inoculation of Lice by the Method of Weigl (8)

5 cc. of human blood were drawn from a vein, defibrinated and some sodium citrate added. The testicles of a guinea pig killed within 24 hours after the appearance of the scrotal swelling were vigorously agitated in the blood. The sodium citrate was added in order to prevent coagulation of the fibrinogen which collects around the testicles during the early scrotal reaction. This mixture was inoculated rectally into 40 lice with a capillary glass pipette attached to a rubber bulb. After inoculation the lice were worn continuously by an immune person. Every day two lice were removed from the boxes for microscopical examination. The first Rickettsiae were found in a louse four days after inoculation. They were now found in increasing numbers in every louse examined. After the seventh day their number was enormous and the lice began to die. On the tenth day all lice which still remained in the box were found to be dying. Examination revealed that the epithelial lining of their guts was completely destroyed by Rickettsia prowazeki. This experiment was repeated twice with the same results. Several guinea pigs inoculated intraperitoneally with emulsions from these lice developed the typical symptoms of Mexican typhus and the tunica organism was easily demonstrated in all of them. The peritesticular exudate of three rats killed five days after the inoculation with such a louse emulsion contained enormous numbers of the small intracellular organisms.

If we compare the results of the preceding experiment with those of the feeding experiments we see that Rickettsia prowazeki appeared four days after a rectal inoculation and in 100 per cent of the lice, whereas in lice fed upon infected monkeys Rickettsia prowazeki did not appear until the seventh day and only a certain percentage of the lice became infected. This, however, is not astonishing as the organisms are always easily demonstrated during the early scrotal involvement whereas they never are found in the blood of animals reacting to typhus. More significant is the fact that the exudate around the testicles during the early scrotal reaction invariably contains several thousand infective doses of the virus of typhus. As a rule, the same amount of a tunica emulsion is at least five hundred to a thousand times more infectious than the same amount of blood. The experiments demonstrate therefore that lice inoculated rectally with concentrated virus of typhus became earlier and absolutely regularly infected with Rickettsia prowazeki as compared with lice which had fed on the much less infectious blood.

The following two experiments were carried out with the aim of separating the causative agent of typhus from Rickettsia prowazeki and from the organism in the tunica.

The Influence of Dilution of Tunica Emulsion on the Occurrence of Rickettsia and the Causative Agent of Typhus in Inoculated Lice

A guinea pig was killed at the beginning of the scrotal swelling and both testicles vigorously shaken in 10 cc. of citrate solution. This suspension was arbitrarily called dilution 1:10 although the amount of exudate in the scrotal sacs was far less than 1 cc. Dilutions were then made from this suspension and defibrinated human blood added to each dilution. Three separate groups containing 20 lice each were inoculated rectally, one group with a dilution of 1 to 100, one with a dilution of 1 to 1000, and one with a dilution of 1 to 10,000.

The following results were obtained:

In the first group of lice Rickettsia prowazeki appeared on the seventh day and the lice began to die on the twelfth day after inoculation. In the second and third groups Rickettsia prowazeki appeared on the ninth and twelfth day respectively. The lice of the second group began to die on the thirteenth day and several lice of the third group died on the fifteenth day. Examination of these dead and dying lice revealed large numbers of Rickettsia and the causative agent of typhus was demonstrated in several lice of each group separately by inoculation into guinea pigs. The tunica organism was easily found in these animals.

Sixteen days after inoculation all lice of Groups 1 and 2 had died. Of Group 3, however, eight lice continued to feed. They were killed on the twentieth day by emulsifying them in a few drops of normal saline solution. A loopful was used for making a smear. The rest was taken up with 10 cc. of normal saline solution and inoculated into two guinea pigs. No Rickettsiae could be found in the smear and the guinea pigs remained normal and did not show any immunity when later inoculated with an emulsion of tunica from the passage strain.

This experiment failed to separate Rickettsia prowazeki from the causative agent of typhus. Whereas the dilutions of 1:100 and 1:1000 of the tunica exudate infected all lice with Rickettsia prowazeki this organism appeared only in a small majority of the lice inoculated with a dilution of 1:10,000. Lice in which Rickettsia prowazeki was demonstrated harbored also the causative agent of typhus whereas eight lice of the third group which were free from Rickettsiae did not contain the causative agent of typhus. The amount inoculated into each louse was about 2 mgrs. Since the dilution inoculated into lice of group three was 1:10,000, the amount of tunica exudate received by each of these lice was one five millionth of tunica exudate. If Rickett-

sia prowazeki and the causative agent of typhus were two different organisms, we should expect that the inoculation of such a small quantity of tunica which is just beyond the limit where each dose contains the causative agent or Ricketstia prowazeki should separate them by the help of louse inoculation, given the extremely high susceptibility of the louse for both of them. One could of course, object that not all lice of Group 3 were equally susceptible to Rickettsia prowazeki and that our experiment does not prove that the quantity of tunica exudate inoculated into the eight lice which remained free from organisms did not contain the tunica organism. We are fully aware of this possibility. With such an assumption, however, we would presume that the degree of susceptibility of the lice for two different organisms is exactly alike, and that these two organisms always occur in the same number in an infectious material, and that the presence of one conditions the presence of the other and vice versa.

The Incubation Period of Mexican Typhus and the Time of the Appearance of the Organism in the Tunica Vaginalis

It is commonly thought that a febrile reaction in guinea pigs appearing earlier than six to seven days after inoculation is not due to the virus of typhus. The inoculation of concentrated tunica emulsion prepared from an animal during the early scrotal reaction is followed by fever and swelling of the scrotum within less than seventy-two hours. The Rickettsia-like organisms can regularly be demonstrated in smears from the tunica sixty hours after inoculation and repeatedly we were able to find them already at the end of the second day. We made, therefore, three successive transfers with tunica emulsions each sixty hours after the respective inoculation and injected twenty lice with an emulsion made from the tunica of an animal of the third transfer. This animal was killed fifty hours after it had been inoculated.

Result: All lice became heavily infected with Rickettsia prowazeki within eight days and the virus of typhus was demonstrated in six of them separately by inoculation into guinea pigs. In all six animals the tunica organism was demonstrated.

This experiment demonstrates that the virus of typhus and Rickettsia prowazeki make their appearance simultaneously in the tissue of guinea pigs. The experiment constitutes another fruitless attempt to separate Rickettsia prowazeki from the virus of typhus.

The Organisms in the Lice

The organisms found in our lice have already been referred to in our experiments as Rickettsiae for the sake of brevity and because they corresponded morphologically and tinctorially to the germs described in lice fed upon typhus patients by da Rocha Lima. We wish, however, to emphasize the morphological identity of the organism found in our lice with those found in the tunica of animals inoculated with the passage strain and with the three strains started from lice.

The organisms found in lice killed early after feeding on an infected monkey or after an infective enema were small pale bluish or purplish staining bacilli (with Giemsa solution) which exhibited more or less decided polar staining. As the infection of the lice progressed the minute red or purplish staining diplobacillary and coccoid forms began to show up in increasing numbers whereas the slightly larger bacilliform organisms became scarcer. Exactly the same phenomenon can always be seen in smears from the tunica of rats and guinea pigs provided that these animals are killed at the proper time. In endothelial cells which contain few organisms the larger pale bluish staining bacilli as a rule are present, whereas in heavily infected cells which are distended and about to rupture, the small red staining diplobacillary and coccoid forms are invariably met with. It seems, therefore, that the minute forms liberated from heavily infected cells by disintegration of the latter assume the form of somewhat larger bacilli after they invade a fresh cell of the louse or of an infected mammal. Wolbach, Todd and Palfrey (9) had already described this behavior in lice and our observation in guinea pigs and rats corroborates their findings in this respect. When the larger bacilliform organisms are distributed more or less evenly over the protoplasm of an infected tunica cell, or of an infected epithelial cell of the louse, they may be present in considerable numbers without the admixture of the minute forms. Very heavily infected cells, the protoplasm of which is completely filled with closely packed organisms, contain few or no large forms as a rule. Roundish or irregularly shaped colonies of closely packed organisms within large endothelial cells are also composed almost invariably of the minute red staining diplobacillary forms whereas loosely packed colonies are composed as a rule of the larger bipolar staining organism. Especially after careful differentiat on of the smears with 95 per cent alcohol the bipolar staining can be seen very clearly. At the first glance one would think that the small diplobacillary forms may not be properly recognized as such on account of their great number in which individual organisms can frequently be recognized only with difficulty. This, however, can easily be ruled out by the observation of large numbers of the small forms when found strewn about cells which had ruptured while making the smears or which had already disintegrated spontaneously in situ before the smear was made. It seems, therefore, that the larger bacillary forms develop only as long as there is enough room and perhaps

food within the cell, whereas the minute forms appear when these conditions become less favorable. We got the impression that the larger bipolar form represents the actively multiplying stage of the organism, whereas the small coccoid forms represent a resting stage. The size of a bipolar staining rod corresponds closely to the size of a diploform composed of the two minute coccoid organisms. A transitional form exhibiting hour-glass shape can frequently be observed. Arkwright and Bacot (10) and also Wolbach, Todd and Palfrey (9) described long bacillary and filamentous forms which showed indications of the formation of the small forms within their body. Similar pictures could occasionally be seen in the tunica cells of our guinea pigs and rats although much shorter ones than those mentioned by these investigators. The very large coiled forms described by other investigators in the gut of lice have never been seen by us in the tunica nor did we find them in sections or smears of lice.

GENERAL DISCUSSION

The result of these experiments is clear cut. Body lice fed upon monkeys inoculated with a strain of Mexican typhus carried along in guinea pigs for nearly two years became infected with the organism known as Rickettsia prowazeki. The inoculation of such lice into guinea pigs and rats induced in the latter animals the typical symptoms of tabardillo. The small intracellular organism found first by Mooser (1, 2) in guinea pigs inoculated with Mexican typhus and later by Pinkerton (5) in a strain of typhus from Europe and by Maxcy (3, 4) in guinea pigs and rats inoculated with endemic typhus from North Carolina could easily be demonstrated in animals inoculated with an emulsion of lice fed upon monkeys inoculated with Mexican typhus. The same results were regularly obtained with lice inoculated with an emulsion of tunica vaginalis by the method of Weigl (8).

Whereas up to the time of the conclusion of these experiments, we were only able to state that the organism found in the tunica vaginalis was constantly associated with our strain of Mexican typhus and that in its morphology and tinctorial behavior it is indistinguishable from Rickettsia prowazeki in lice, we know now that it accompanies the causative agent of typhus from the passage strain via monkey through the louse back into the tunica of rats and guinea pigs where it multiplies again exclusively within cells as it does in the louse, in other words, that the intracellular organism found in the tunica vaginalis of our animals is identical with Rickettsia prowazeki. The present experiments do not advance any startling new knowledge concerning

the etiology and pathology of typhus. They show again, however, and more impressively than had been demonstrated before that the causative agent of typhus cannot be separated from Rickettsia prowazeki. The causative agent of typhus seems to exist in the louse and in the mammal in one form only, namely, in the form of Rickettsia prowazeki. Even this is not an entirely new finding. Kuczynski (11) and especially Wolbach, Todd and Palfrey (9) had already advanced considerable evidence that the causative agent of typhus is present in man and in animals in the form of Rickettsia prowazeki. That their conclusions have not been universally accepted is due to the uncertainty of their method of demonstrating Rickettsia prowazeki in sections of mammalian tissue. This can clearly be seen from the circumstance that Wolbach, Todd and Palfrey (8) consider globular massing within endothelial cells as the most characteristic behavior of Rickettsia prowazeki in mammalian tissue and form their finding of Rickettsia prowazeki within the endothelial leucocytes which constitute the nodular lesion of typhus. Such globular massing can also be seen in smears from the tunica, but it represents only a transitional stage of the intracellular multiplication. The most characteristic finding in mammalian tissue is the occupation of the whole protoplasm of an infected cell by enormous numbers of closely packed organisms which cause great distention of the invaded cell and finally lead to its disintegration with liberation of the intracellular germs, exactly as is seen in the case of Rickettsia prowazeki in the louse. The same course of events can also be demonstrated within Regaud fixed tissue of guinea pigs after staining with Giemsa solution.

As long as the organisms are multiplying within an infected cell the surrounding tissue remains quiescent (12). As soon, however, as the organisms are liberated by disintegration of an infected cell, a sudden acute inflammatory reaction flares up around the disintegrating cell. The Rickettsiae which are not evacuated into the blood stream or which do not gain entrance into a new endothelial cell are taken up by polynucular leucocytes, where they are digested. The leucocytes in turn are taken up by large mononucular leucocytes which gather concentrically around them, giving rise to the typical nodule of the typhus literature. This nodule is not an early lesion but represents a healing stage of a typhus lesion and never contains any demonstrable Rickettsiae.

The demonstration that Rickettsia prowazeki multiplies within infected endothelial cells of the mammal exactly as it does in the cells of the stomach of the louse, causing great distension and finally disintegration of the invaded cells, constitutes alone strong evidence for the specificity of this organism in typhus.

All attempts to separate the causative agent of typhus from the tunica organism or Rickettsia prowazeki have failed completely. The following evidences have so far accumulated in respect to the specificity of Rickettsia prowazeki in typhus: Rickettsia prowazeki is only found in lice fed upon typhus patients (da Rocha Lima (13)). Its presence in lice is constantly associated with the virus of typhus (Wolbach, Todd and Palfrey (9)). Lice inoculated rectally with any material containing the virus of typhus invariably become infected with Rickettsia prowazeki and simultaneously there is a pronounced multiplication of the virus of typhus (Weigl (8)). Emulsions of Rickettsia prowazeki made from lice are agglutinated by serums of cases of typhus (Otto (14)) and also by serums of guinea pigs reacting to typhus which never give a positive Weil-Felix reaction (Weigl (8)). Organisms morphologically simulating Rickettsia prowazeki were demonstrated in sections of specific lesions of typhus (Kuczynski (11), Wolbach, Todd and Palfrey (9)). Recently the same organism was regularly demonstrated in smears from the tunica vaginalis of guinea pigs reacting to a strain of Mexican typhus (Mooser (2)). Pinkerton (5) found the same organism in a strain of European typhus, and Maxcy (4) in a strain of endemic typhus from this country. At this occasion it is well to remember that the virus of typhus is held back by bacterial filters (Anderson and Goldberger (15), Ricketts and Wilder (16), Olitsky (17)). The size of the specific organism must therefore lie within the range of microscopic visibility. Since in an infected louse only Rickettsia prowazeki can be found and since the same holds true for the tunica vaginalis, it is almost impossible to elude the conclusion that Rickettsia prowazeki and the causative agent of typhus are identical. All physical and chemical influences which kill the specific agent invariably kill also Rickettsia prowazeki. Furthermore, we have shown that the incubation period for both is the same in guinea pigs. Since the lesions in the tunica vaginalis show all the histologic characteristics of an uncomplicated typhus infection

(2) and since these lesions regularly contain no other organisms but Rickettsia prowazeki, we have to conclude that the latter organism is etiologically responsible for them. If we question therefore, the specificity of Rickettsia prowazeki in typhus, then we have also to question the specificity of the histopathology of that disease.

SUMMARY

Healthy lice became infected with Rickettsia prowazeki after feeding on monkeys inoculated with a strain of Mexican typhus. The same result was obtained in 100 per cent of lice by rectal inoculation of an emulsion of tunica vaginalis of guinea pigs reacting to the same strain. In the tunica vaginalis of guinea pigs and rats inoculated intraperitoneally with an emulsion of lice containing Rickettsia prowazeki the intracellular organism constantly associated with the passage strain appeared regularly. Rickettsia prowazeki found in lice and the organism constantly present in the tunica of guinea pigs and rats reacting to our strain of tabardillo are indistinguishable morphologically and tinctorially and their mode of intracellular multiplication is alike in every respect. It is concluded that they are identical. This organism is constantly associated with the causative agent of Mexican typhus, both in mammals and in lice, and all of our attempts to separate them have failed.

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EXPLANATION OF PLATE 5

FIG. 1. Scrotal swelling of a guinea pig (G4, Exp. 3) inoculated with an emulsion of lice fed on Monkey No. 3. The scrotal swelling in guinea pigs is characteristic of Mexican typhus.

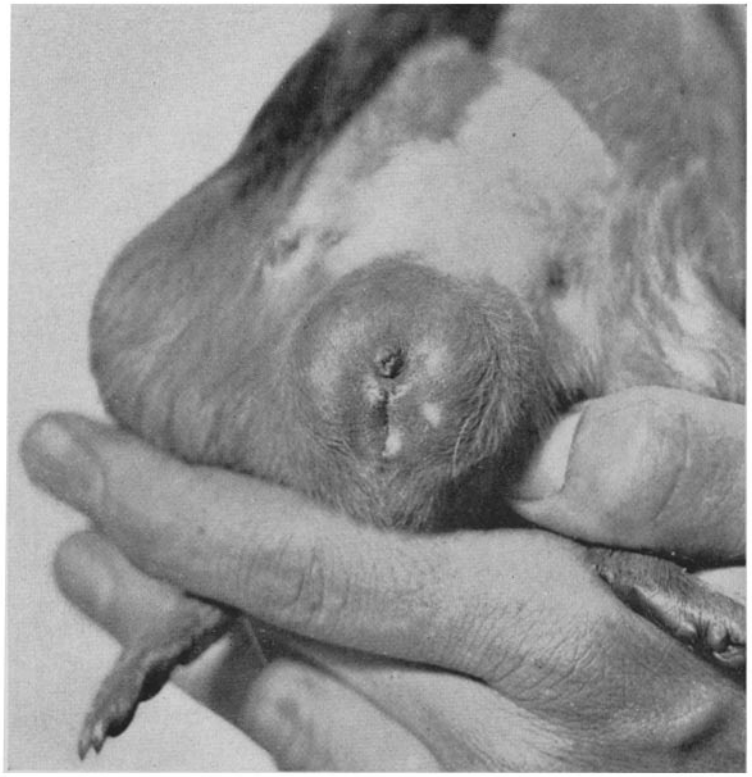

FIG. 1

(Mooser and Dummer: Mexican typhus and Rickettsia prowazeki)