THE ETIOLOGY OF TYPHUS EXANTHEMATICUS IN MEXICO (TABARDILLO) *

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In a preliminary report¹ we presented an outline of the results of our investigations on typhus exanthematicus in Mexico. It is our purpose, in this communication, to state the facts which led us to our conclusions as well as to bring forward other phases of the work not mentioned in the first paper.

The studies here reported were made in Matehuala, in Central Mexico, during the epidemic of typhus fever which prevailed throughout the winter, or dry months, of 1915-1916. An expedition consisting of the authors was made possible through the generosity of the board of directors of Mount Sinai Hospital. A complete laboratory equipment including animals for experimental purposes was taken with us in a special car. We wish to thank Dr. F. S. Mandlebaum, pathologist to the hospital, for his great aid in this direction. These investigations could not have been made without the assistance of the American Smelting and Refining company, who magnanimously offered to take the expedition into Mexico privately and allowed the use of one of their plants as a base for operations.

Matehuala, where we did our work, is a town of 10,000 inhabitants. The extent of the epidemic both in this locality and in the remainder of Mexico was extremely difficult to determine on account of the absence of vital statistics, and the lack of adequate governmental or municipal control of health and sanitation in the district in which we worked. All the available data, however, pointed to a widespread epidemic. From reports of individuals and official newspapers it was estimated that there were 30,000 cases, or 10% of the population, in Mexico City alone. We estimated that there were at least 500 typhus-fever patients in Matehuala during the dry season.

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¹ Jour. Am. Med. Assn., 1916, 66, p. 1692.

It is important to note, when comparing our observations in Mexico with those on endemic typhus fever in New York and on the severe type in Southeastern Europe in 1913 and 1915, that this epidemic, while wide in extent, was but moderate in severity; the mortality in our series of cases was 20%. Arranged in the order of severity, indicated by the rate of mortality, we find:

1. Mildest, the endemic typhus cases in New York (Brill's disease).
2. Next severe, the epidemic typhus cases in Bulgaria and Russia in 1915-1916.
3. Still more severe, the epidemic typhus cases in Mexico.
4. Severest, the epidemic typhus occurring in immigrants from the Balkans in 1914.

The details of the cases included in (1) and (4) were given in the original publication by Plotz, Olitsky, and Baehr;² those in (2) will form the subject of a paper, shortly to appear, by Plotz and Baehr; while those mentioned in (3) will be discussed in this communication.

Our work, which was begun Feb. 1, 1916, was interrupted by the unsettled political conditions in Mexico on March 8, 1916. About the same time Dr. Husk became infected with typhus fever and died.

VIRUS STUDIES

In its larger features the disease as the authors found it in Matenhuala corresponded to the classical type. Yet it became essential, since influenza, relapsing fever, typhoid fever, etc., are confused with typhus fever, to identify absolutely the nature of the virus. To this end, the virus from patients was transmitted to guinea-pigs. Previous observers have demonstrated that such virus circulating in the blood during the febrile period, when inoculated into guinea-pigs (or monkeys), produces a febrile reaction of from 4 to 11 days' duration after an incubation period of usually from 7 to 14 days (Ricketts and Wilder*). The guinea-pig's blood during the febrile period is infective for other guinea-pigs.

In the following series of experiments (see Table 1) we have demonstrated that the virus with which we were dealing was typical of typhus fever. The usual procedure was to inject into guinea-pigs

* Anderson (Jour. Med. Research, 1914, 30, p. 467) in an exhaustive study finds the incubation period among guinea-pigs to be from 4 to 29 days; in most cases between 7 and 10 days, and in 91% of cases between 7 and 14 days. The duration of illness is from 4 to 18 days; in 80% of cases it is between 7 and 11 days.

² Jour. Infect. Dis., 1915, 17, p. 1.

intraperitoneally 3 c.c. of the patient's blood (defibrinated) plus 3 c.c. of normal salt solution. After an average incubation period of 12 days the guinea-pigs would develop fever. Such animals were bled and the virus transmitted to others. The virus was maintained for 2 generations, the work being then interrupted. The period of incubation, the type of the febrile reaction, and the findings at autopsy — enlarged spleen with prominent Malpighian bodies (first noted by Baehr) and absence of other gross lesion — corresponded in every way with the results obtained in previous work with the virus of endemic and epidemic typhus fever.

TABLE 1
VIRUS TRANSMISSION EXPERIMENTS

Patient	Incubation Period in Guinea-pig, Days	Febrile Period	Autopsy	Transmission
1	14	Died after 5 days	Spleen slightly enlarged; prominent Malpighian bodies. No other lesion	None
6	11	Bled after 2 days	Same.....	After 10 days guinea-pig developed fever. Re-transmitted
14	12	Died after 1 day	Same.....	None
16	12	Bled after 3 days	Same.....	Transmitted, but guinea-pig died in 2 days, no cause being found

Several guinea-pigs injected with the blood of patients not suffering from typhus fever failed to show the reactions described.

RESULTS OF BLOOD CULTURES

Blood cultures were taken on all the available cases of typhus fever and on numerous controls. It is important to note that the absence of hospital facilities and nursing, and the necessity of working in the filthy houses of ignorant natives, forced an adaptation of the technic to the surroundings and may have interfered somewhat with our results.

Blood cultures were taken on 31 cases of typhus fever as well as on several patients not having this disease. With the exception to be mentioned, the technic and the culture media employed were those used in the previous studies by Plotz.²

Briefly, the technic was as follows: After preliminary sterilization, 12 to 20 c.c. of blood were aspirated from a vein in the antecubital space. Two cubic centimeters were inoculated into each of 6 to 9 tubes. These tubes, measuring 15 cm. in length and 2 cm. in diameter, contained 20 c.c. of 2% glucose agar (+0.9 reaction), to which 4 c.c. of ascitic fluid were added. We wish to emphasize at this point that the ascitic fluid used was tested previously both aerobically and anaerobically, that it was bile-free, and had a specific gravity of at least 1.015. The blood and nutrient media were carefully poured from one sterile tube into another, so that thorough mixing took place. The contents were then allowed to solidify in an upright position, the surface was layered over with melted agar, and the whole then incubated at 37 C.

Of the 31 blood cultures made on typhus-fever patients, 3 were taken with 0.5% glucose serum agar. Of these three 1 was positive (see Table 2). Of the 28 cultures made with 2% glucose serum agar, only 8 could be followed a sufficiently long time, unsettled political conditions forcing our departure before the remaining cultures could be satisfactorily studied. All of these eight showed an organism the identity of which with Plotz's bacillus—*B. typhi-exanthematici*—will be discussed later.

TABLE 2
RESULTS OF BLOOD CULTURES

Case	Time of the Taking of Blood Cultures		Amount of Blood Cultured, c.c.	Number of Colonies	Day of Appearance of Colonies in Culture	Media
	Days Before Crisis	Day of Disease				
1	5	6th	10	1	7th	0.5% glucose serum agar
3	At crisis	At crisis	12	1	12th	
9	At crisis	At crisis	15	8	14th	
				Negative		
12	5	9th	8	2	9th	2% glucose serum agar
13	8	6th	8	3	7th	
				1	9th	
14	6	9th	16	3	5th	
				2	7th	
15	3	8th	16	2	6th	
16	3	6th	2	1	9th	
21	5	5th	12	1	9th	
22	Died	4th	9	3	6th	
40	Unknown	3rd	12	6	4th	

As Table 2 shows, the two cases in which there were no organisms were cultured at the crisis. This fact, as well as the greater number of colonies appearing in those cultured up to the 6th day of the disease, conforms with findings made in the original studies by Plotz, Olitsky, and Baehr. They found that blood cultures on patients suffering from the disease showed a larger number of colonies on the 4th and 5th days before the crisis than on the few days immediately preceding the

crisis, and that those on guinea-pigs inoculated with typhus virus showed the largest number on the 2nd and 3rd days of illness. In either case blood cultures were rarely positive at the time of the crisis.

The total number of colonies, 34, as compared with the total number of cubic centimeters of blood cultured, 121, shows the interesting relation of the degree of bacteriemia to the severity of the cases. Comparing the results obtained in Mexico with those obtained in New York (in studies made on endemic typhus fever as well as on the epidemic type in Serbian immigrants) we note the following:

TABLE 3
COMPARISON OF DATA SHOWING RELATIONSHIP BETWEEN BACTERIEMIA AND SEVERITY OF DISEASE IN TYPHUS FEVER

Type of Disease	Mortality	Cubic Centimeters of Blood Cultured	Number of Colonies	Average Number of Colonies per c.c.
Endemic typhus (New York).....	About 0.3%	436	33	0.07
Mexican typhus.....	20%	120	34	0.28
Epidemic (Balkan, 1914).....	18 - 60%*	51	74	1.45

* Eighteen percent represents the mortality in our small series of cases (11) studied in New York. Reports from the Balkans place the average percentage at much higher figures. In a report based on studies of typhus fever in Bulgaria and Russia in 1915-1916 by Plotz and Baehr, which will appear shortly, the results of blood cultures correspond with those given here; the epidemic was midway, as regards severity, between the endemic and the Mexican types, the blood cultures showing more positives than in the endemic, and fewer positives than in the Mexican cases.

Thus we see that the more severe the disease, the greater is the number of colonies. This is exemplified also in our series in Case 1. This patient was most desperately ill and was in extremis at the time of the crisis, but recovered after a very stormy convalescence. Our fatal case likewise had more than the average number of colonies.

The day of appearance of these colonies in the original blood cultures fell between the 4th and the 14th days, usually about the 9th day. Since these colonies are first seen only after they have attained considerable size (the opacity of the culture media interfering with their detection), it is logical to assume that the colony grows very slowly at first, requiring a considerable period of time before reaching a visible size.

Blood cultures were also taken at the same time in a number of patients suffering from fevers other than typhus fever, such as influenza, bronchitis, bronchopneumonia, pneumonia, cystitis, etc. All were taken during the febrile period of the disease, the same serum and media and technic being used as for the typhus cases. The total

number of cubic centimeters of such blood cultured was 76, as against 120 of typhus-fever blood. The blood cultures in control cases were observed a sufficiently long time, usually for about 21 days. All these cultures were either negative or contained the exciting cause of the disease, as influenza bacilli, pneumococci, etc.

RESULTS OF AGGLUTINATION TESTS

In studying the serologic reactions found in cases of Mexican typhus fever, we were forced, because of a lack of facilities and animals, to place our main reliance upon agglutination tests. The serum obtained from patients was usually small in amount, hence the microscopic method was used. Wherever the quantity of serum was sufficient, however, macroscopic tests were employed as well, the result usually confirming the microscopic.

The method of setting up these tests was as follows:

The blood was collected in capillary tubes and the serum allowed to separate by standing or by centrifugation; it was then heated to 56 C. for one-half hour. Dilutions of the inactivated serum of 1:25, 1:50, 1:100, and 1:200 were made in small sterile tubes, so that by the addition of equal quantities of agglutinin these dilutions were doubled; that is, they were from 1:50 to 1:400. The agglutinin was prepared from growths of the organism on agar slants, a sufficient amount being washed off into 0.9% salt solution to make a suspension of proper opacity. This was shaken and then centrifuged gently so as to throw down any clumped masses of bacteria. The tests were set up in hollow ground slides, kept at room temperature for exactly 1 hour, and then read. In reading, the usual caution was taken to avoid the natural clumping incident to drying or that which occurs about the very edge of the material. Proper controls were always employed. When macroscopic tests were made, 1 c.c. of each of the dilutions of the inactivated sera was added to 1 c.c. of the bacterial suspension. Such tests, put up in sterile tubes, were incubated 1 hour, then kept at room temperature 24 hours, and afterward read.

In regard to the strains used in the agglutinin, we found, on several occasions, a freshly recovered organism to be inagglutinable, even in the serum of the patient from which the organism was obtained. However, such serum would agglutinate actively older strains—for instance, Strain 10, isolated about 2 years previously from an epidemic case. This experience corresponds with that encountered at times with the typhoid bacillus.

Again, as a consequence of the lack of hospital facilities, most of our patients could be bled only once, either before or after the crisis, and we were able, therefore, to test for the presence of agglutinins at but one period of the disease. In a few, however, we could trace the

TABLE 4
RESULTS OF AGGLUTINATION TESTS AT DIFFERENT STAGES OF THE DISEASE

Case	Days before Crisis	Result	Critical	Days after Crisis	Result
1	...			5	1:50 +
3	...			9	1:100 +
				13	1:200 +
6	...			3	1:600 +
7	...			16	1:200 ++
9	...			19	1:100 ++
11	4	1:200 +	1:200 +	16	1:400 +
12	1	1:50 —		4	1:400 +
13	...			4	1:800 +
14	...			6	1:400 +
15	3	1:100 +		..	
18	...			8	1:50 +
19	...			7	1:800 +
20	...			2	1:400 +
21	...			9	1:50 —
23	...		1:50 +	8	1:200 +
25	5	1:50 —		..	
27	8	1:50 —		..	
28	6	1:50 —		..	
29	2	1:50 —		6	1:100 +
30	1	1:50 —		8	1:800 +
31	...		1:200 +	7	1:200 ++
32	6	1:50 —		..	
34	6	1:50 —		..	
36	...		1:50 +	..	
37	8	1:50 —		..	
38	6	1:50 +		..	
39	10	1:50 —		..	
40	10	1:50 —		..	
41	8	1:50 —		..	
42	2	1:50 —		..	
43	9	1:50 —		..	
44	11	1:50 —		..	
46	...			?	1:800 +

development of these antibodies during the various stages of the disease.

The total number of tests made was 41; 18 being made at the height of the disease, 4 at the crisis, and 19 after the crisis. There were 16% positive results in the antecritical period. In all these the agglutination showed a low titer. Of 4 tests made on bloods taken at the crisis, all were positive, but here as well the agglutination limits were low (from 1:50 to 1:200 positive). Of the tests made after the crisis, all but 1 were positive, giving a percentage of positives of 95. Also, the agglutinin titer of the serum showed much stronger reactions, several agglutinations occurring in dilutions of 1:800.

These results correspond for the most part with those obtained in the study of endemic and epidemic typhus fever in New York. That is, the agglutinins in typhus fever are usually negative at the height of the disease but increase in quantity as the crisis is reached and become almost uniformly positive in the postcritical period.

At the same time control agglutination tests made with the same technic were set up with serum obtained from patients suffering from diseases other than typhus. In this group were included febrile cases in which the tests were made not only at the height of the fever, but also in the postcritical stage. In most of these cases, the tests were negative; in one there was agglutination in a dilution of serum of 1:50. However, it has been emphasized previously that reactions in dilutions of serum up to 1:50 may not be specific. This occurs as well with other organisms.

We shall state later the results obtained in agglutination tests with typhus immune serum against agglutinogens of other than typhus organisms.

IDENTIFICATION OF THE ORGANISM

The identification of the organism recovered from the blood of Mexican typhus-fever patients was based upon its definite characteristics. These refer to the appearance of the colony, the morphology of the bacillus, the cultural characteristics including fermentation reactions, and finally its behavior towards typhus immune serum, especially in regard to agglutination and complement-fixation. In all these respects the organisms we have cultured from typhus in Mexico, including the organisms obtained from cultures from lice and from experimental typhus fever in guinea-pigs, correspond with *B. typhi-exanthematici*.

The characteristics of the organisms isolated are as follows:

Colonies.—The colony as it appears in blood cultures usually attains its maximal size by the 9th day, in one case in our series (see Table 2) as early as the 4th day. At this time, the colony appears as a small (3 to 4 mm.) opaque spot surrounded by a well-defined brownish area of precipitation. On cross section it is brownish in color, and if allowed to develop in the culture tube for a time, it becomes Y-shaped, the arms of the Y being whitish and fusiform.

Morphology.—The organism is a small slender bacillus averaging about a micron in length. When very young, it appears to have a morphology similar to the small-type influenza bacillus. It is usually gram-positive, altho we have met with many strains, especially from louse cultures, which were decolorized by Gram's method. This is true in the early subcultures; in the later subcultures the predominating organisms are gram-positive, while numerous gram-negative bacilli may be seen scattered throughout the field. We have not been able to determine polar bodies in young cultures, but in older cultures they are apt to appear occasionally, along with degeneration and involution forms.

Cultural Characteristics and Fermentation Reactions.—Culturally the Mexican strains are identical with the organism isolated in New York. It is a strict anaerobe. It grows rather sparsely on 0.5-2% glucose serum agar slants, so

that it is necessary to transplant a considerable amount in order to obtain sufficient growth. This medium shows a growth in 3 days after inoculation and is precipitated more or less, depending on the age of the culture. In these and other respects it corresponds with the description detailed in the work previously cited.²

It is important to emphasize in regard to fermentation reactions that the purity of the carbohydrate used in the media for the tests is of greatest significance in obtaining uniform results. In the reactions to be described, for example, the observations were made on media prepared with crystallized lactose; media prepared with powdered lactose gave marked variations, possibly due to impurities in the latter.

In order to ascertain the reliability of the carbohydrates used, it is best to note previously the reactions of a standard strain of a known organism, for example, the typhoid bacillus, upon the same sugars. Again, as suggested by Dr. Libman, one may find it necessary to purify a sugar, as in the case of lactose containing traces of dextrose and galactose, by subjecting the carbohydrate to the growth of an organism which will use up the undesirable elements, this measure being based on the method of Theobald Smith for eliminating dextrose from meat infusions by inoculation with *B. coli*. In the case mentioned, lactose, the paratyphoid strains will ferment out the impurities and leave the lactose untouched. Of course, it becomes essential to test the resultant sugar for its efficacy, and this may be done by growing in it a known organism, as *B. coli* in the case of lactose. The *modus operandi* should also allow no extraneous factor to complicate the results. The pyrogalllic acid used in the production of anaerobiosis, if impure, may liberate volatile acids which cloud the readings.

Our methods of procedure were as follows: The medium was made of 3% agar, containing 1% of the carbohydrate to be studied, to which were added one-third as much of rich ascitic fluid, and a sufficient amount of litmus (Kahlbaum) to color the entire material a deep blue. Just before use reactions of such media were usually 0.8% acid. Slants made this way were inoculated with a quantity of the culture and were allowed to incubate for 6 days. For suitable anaerobic conditions we found that the use of jars subjected to a negative pressure of 600 mm. Hg, which at the same time had a compartment for the pyrogalllic acid with the potassium hydrate, were more suitable than Buchner tubes. All the tubes were influenced, in the former case, by the very same conditions, and the extraneous acid was less likely to complicate the results.

TABLE 5
RESULTS OF FERMENTATION TESTS, "A" SIGNIFYING ACID-PRODUCTION, AND "P" PRECIPITATION

Carbohydrate	Epidemic N. Y.	Endemic N. Y.	Mexican
Lactose.....	Negative	Negative	Negative
Glucose.....	A, P	A, P	A, P
Raffinose.....	Negative	Negative	Negative
Maltose.....	A, P	A, P	A, P
Mannite.....	Negative	Negative	Negative
Galactose.....	A, P	A, P	A, P
Inulin.....	A, P	A, P	A, P
Saccharose.....	Negative	Negative	Negative
Dextrin.....	Negative	Negative	Negative

The Mexican strains, tested in this manner, gave the following reactions: acid-production (but no gas-formation) on glucose, maltose, galactose, and inulin; no acid (nor gas-production) on lactose, raffinose, mannite, saccharose and dextrin. A comparison of these with the results obtained in New York for epidemic and endemic strains of the typhus organism is shown in Table 5.

Incidentally, we may state that growth was luxuriant on the inulin, maltose, and glucose media, but sparse on the lactose, mannite, and saccharose media.

Thus we see the complete correspondence, as regards the reactions on the various carbohydrates, of the Mexican strains of the typhus bacillus with those of the epidemic and endemic organisms isolated in New York.

Serum Reactions.—The most important methods for the identification of the organisms obtained from Mexican typhus fever are based on the immune-serum reactions. For the purpose of such study we depended on the use of human serum obtained from typhus immune subjects. In order to make comparative studies on an organism grown on human serum media, as in this instance, it is of prime importance to use human immune serum, since the use of artificially prepared immune serum, as that produced in rabbits by the repeated injection of the organism, may give results which are nonspecific. One of us (Olitsky) in collaboration with Bernstein³ has found that artificially prepared immune serum, as in rabbits, against organisms grown on serum media gives results which are nonspecific. This is due to the fact that such immune serum contains, besides the specific antibodies, antibodies developed against the serum (or protein) employed in the media upon which the bacteria in the antigen were grown. The serum antibodies give rise to nonspecific agglutinins, precipitins, complement-fixing bodies, and cellular antibodies.

In the following tests, we used endemic-typhus immune serum, as this type was the only one available.

(a) Agglutination Tests. For these studies there were available 5 strains of the Mexican organisms—No. 204, from a louse culture; No. 207, from the spleen of a guinea-pig infected with typhus virus; No. 208, from a human-blood culture in a case of typhus fever; Nos. 209 and 212, from cultures of lice.

The methods employed were the microscopic and macroscopic. The agglutinogens were prepared from each of these strains in the manner stated. The immune serum was inactivated and diluted so that the final dilutions were 1:50, 1:100, 1:200, and 1:400.

³ Jour. Infect. Dis., 1916, 19, p. 253.

TABLE 6
AGGLUTINATION TESTS: ENDEMIC-TYPHUS IMMUNE SERUM AGAINST MEXICAN STRAINS

Strain	Control	1:50	1:100	1:200	1:400
204.....	0	++	++	++	+
207.....	0	0	0	0	0
208.....	0	++	++	+	0
209.....	0	++	++	++	+
212.....	0	++	++	+	0
Non-typhus organisms...	0	0	0	0	0

By reference to Table 6, we find that of the 5 strains studied, 4 gave positive agglutination reactions with the endemic-typhus immune serum. One may explain the nonagglutinability of the remaining strain—which, however, bound complement—by the fact that endemic-typhus serum was used. It has been shown previously that various strains differ in their serum reactions and that polyvalency in such tests is of considerable importance. The same serum was found to agglutinate New York strains of the organisms strongly in dilutions of 1:400. Hence, as far as these tests go, one may conclude that the Mexican organisms are strains of the typhus bacillus.

Incidentally, the typhus immune serum was tested against agglutinogens made of organisms resembling somewhat the typhus bacilli. These bacteria were all gram-positive, but differed in one or more of the characteristics mentioned, from the typhus bacillus. Some grew aerobically after a few subcultures; others, while strictly anaerobic, differed in morphology and growth. One of these organisms was a typical *B. acne*; two others were isolated from pustules in cases of acne vulgaris; one was obtained from a blood culture from a patient suffering from chronic polyarthritis, and one from a blood culture in a case of chorea. However, when these bacteria were tested against the typhus immune serum, the same technic being used as indicated heretofore, the results were uniformly negative, no agglutinations occurring even in very low dilutions of the serum.

(b) Complement-Fixation Tests. All the available strains isolated from cases of Mexican typhus fever were studied by means of complement-fixation tests to determine their relationship to those obtained in New York.

For this purpose individual antigens were prepared from each strain. The methods of preparation were two. In the first instance the growth of the organism was suspended in distilled water and heated at 60 C. for 1 hour, subsequently being autolyzed at 37 C. for 24 hours. In the second case the

procedure was continued to include filtration through a Berkefeld candle (Size N— pressure 100 mm. Hg), and heating at 56 C. for one-half hour on each of 3 consecutive days. The second method is preferable, producing a lasting, nonanticomplementary “antigenic,” antigen; the first commends itself only for the rapidity of its preparation. Never more than one-fourth the anticomplementary unit was employed.

The serum used was obtained from a patient who had recovered from endemic typhus fever. It was inactivated at 56 C. for one-half hour and was absolutely nonanticomplementary.

The hemolytic system consisted of antsheep rabbit amboceptor (titration varying from 1:1000 to 1:10,000), a 5% suspension of sheep cells, and guinea-pig serum (1:10) as complement. The quantities employed were one-half the amounts used in the original Wassermann reaction. The system worked perfectly for the performance of routine Wassermann tests. The possible presence of natural amboceptor was always considered.

The time allowed for fixation was one-half hour at 37 C. in a water bath, then 3½ hours in the icebox.

The individual antigens were made up of the strains mentioned (see “Agglutination Tests”).

Altho no attempt was made to study quantitatively the amount of complement-fixing body by using higher dilutions of the immune serum, yet the correspondence of the results presented in Table 7 shows conclusively that the Mexican organisms are similar to those isolated in New York in cases of endemic and epidemic typhus fever.

At the same time comparative studies were made with organisms having some of the characteristics of the typhus bacillus. The source of these organisms was mentioned previously under “Agglutination Tests,” where it was stated that as agglutinogens they failed to give agglutination with typhus immune serum. Table 8 shows that typhus immune serum in the presence of such antigens bound no complement and that this serum did bind complement in the presence of typhus-bacillus antigen. In these tests all antigens, as well as the immune serum, completely hemolyzed by themselves in the control tubes. Altho the nontyphus antigens were used, as indicated in Table 8, in amounts nearer the anticomplementary unit and in generally greater quantities, the results were uniformly negative.

These tests in conjunction with the agglutination reactions formed the most reliable methods of differentiation of the Mexican strains from organisms resembling them in one way or another. An important factor, however, should not be overlooked, namely, that comparative studies should always be made with an immune serum from the same species from which the antigen to be investigated is drawn. Otherwise nonspecific results may be expected.

TABLE 7
COMPLEMENT-FIXATION TESTS WITH MEXICAN STRAINS

Strain in Antigen	Endemic-Typhus Immune Serum		Normal Human Serum	
	0.05	0.10	0.05	0.10
204.....	++++	++++	—	—
207.....	++++	++++	—	—
208.....	++++	++++	—	—
209.....	++++	++++	—	—
212.....	++++	++++	—	—
Polyvalent N. Y. typhus antigen....	++++	++++	—	—
Nontyphus antigen.....	—	—	—	—

TABLE 8
COMPLEMENT-FIXATION TESTS WITH NONTYPHUS ANTIGENS

Source of Antigen	Dosage of Antigen, c.c.	Endemic-Typhus Immune Serum		Normal Human Serum	
		0.05 c.c.	0.10 c.c.	0.05 c.c.	0.10 c.c.
B. acne.....	0.3	—	—	—	—
Gram-positive bacillus from an acne case.....	0.2	—	—	—	—
Gram-positive bacillus from another acne case..	0.3	—	—	—	—
Gram-positive bacillus from blood culture in chorea (strict anaerobe).....	0.2	—	—	—	—
Gram-positive bacillus from blood culture in chronic infectious arthritis (strict anaerobe)..	0.3	—	—	—	—
Polyvalent typhus bacilli.....	0.15	++++	++++	—	—

PATHOGENICITY OF THE ORGANISM

Previous experiments by Plotz, Olitsky, and Baehr² demonstrated that organisms obtained from epidemic typhus fever are pathogenic for guinea-pigs only when inoculated from early subcultures. These studies showed also that the organisms isolated from endemic cases fail to give such reactions. The conclusion was reached that there is a marked loss of virulence of the bacilli after transplantation on slants of 2% glucose ascitic agar. With this in mind, we attempted to inoculate into animals the original colony of the organism (as it occurs in the blood culture) and we obtained thereby very striking results.

Guinea-pig B1 was injected on Feb. 12, 1916, with 1 loopful of a colony growth from a blood culture (Case 1—the colony having appeared 7 days after the taking of the blood culture). The growth was emulsified in 2.5 c.c. of salt solution and injected intraperitoneally. There was a rise in temperature

Chart 1. Temperature record of Guinea-pig B1, injected with organisms directly from colony in blood culture.

for 48 hours after the injection. The animal then became gradually feebler and died on February 23, 11 days after inoculation (see Chart 1). The injection of typhus virus into guinea-pigs is followed occasionally by the same reaction. Autopsy showed what we consider typical of typhus fever in the guinea-pig. There was no peritonitis, nor any other lesion except the slight enlargement of the spleen with hypertrophy of the Malpighian bodies. At the same time the spleen was removed in sterile manner, macerated in salt solution, and cultured according to the usual technic. Six days later 3 colonies appeared containing the typical typhus bacillus.

To summarize, the main points of this experiment are: first, the initial rise of temperature corresponding with similar rises obtained with epidemic strains and not with endemic, due very probably to the endotoxins of the organism; second, death after a period corresponding with the average incubation period of typhus fever in guinea-pigs; third, the typical lesions of typhus at postmortem examination; and last, the recovery of the organism from the spleen culture. In other words, the injection of the organism caused typhus fever in this guinea-pig.

Guinea-pig B2 was injected intraperitoneally with 1 loopful of the agar emulsified in 2.5 c.c. salt solution, of the same blood culture, as a control on Guinea-pig B1. This was done to eliminate from the experiment the factor that the infective agent may have been carried over from the blood in the medium. This animal showed no such reaction.

To study the influence on virulence of growth on the media ordinarily used for culture, the following experiments were performed:

Guinea-pig C1 was injected with a 3-day growth on a 0.5% glucose serum agar slant of the identical organisms found in the foregoing colony—that is, the first transplant from the blood culture of Case 1. The entire growth on one standard slant was emulsified in 3 c.c. of salt solution and injected intraperitoneally. There was no preliminary rise in temperature as was seen in Guinea-pig B1 (which we have ascribed to endotoxin action). However, after 12 days the animal suddenly died, before we could make our blood cultures.

Chart 2. Temperature record of Guinea-pig C1, injected with the first transplant.

This death was preceded, the day before, by a marked drop in temperature (see Chart 2). The autopsy showed no lesions except in the spleen, which was not enlarged, but was slightly congested, with Malpighian bodies prominent. To summarize, there was no toxic action (showing itself in an early rise of temperature), the incubation period was typical, and the postmortem observations corresponded with those of the lesions in typhus fever in guinea-pigs. In other words, there was presumptive evidence of typhus fever as having been produced by the injection of the first transplant of the organism.

The effect of a retransplantation is shown as follows: Guinea-pig C5 was injected intraperitoneally with the second transplant of the very organism occurring in the colony just mentioned (from Case 1). The entire growth for 4 days on a slant of 0.5% glucose serum agar was emulsified in 3 c.c. of salt solution and inoculated. There was no rise in temperature, either early or after the usual incubation period. Here, then, we have absolute loss of virulence (see Chart 3).

Chart 3. Temperature record of Guinea-pig C5, injected with second transplant. This animal was alive and well after 19 days' observation, work having been interrupted at the end of this time.

In agreement with previous observations we may conclude that this organism is pathogenic only in its very youngest generation and that it loses its virulence almost immediately on being subcultured on the glucose serum media.

ANIMAL EXPERIMENTS WITH INFECTED LICE

It was our purpose to produce typhus fever in guinea-pigs by the intraperitoneal injection of infected lice so that the nature of the reaction could be studied bacteriologically.

Previous workers, notably Anderson and Goldberger, Nicolle and Conseil, Ricketts and Wilder, and others, have been able to infect monkeys by the injection of such body lice subcutaneously. In these instances the number of lice varied from 37 to over 100. Usually no fever or other manifestations of the disease were produced, except the development of an immunity to a subsequent inoculation of typhus fever virus. Direct transmission of the disease to guinea-pigs, with the typical rise in temperature, by means of infected lice has not thus far been attempted.

With this end in view, we collected from the clothing or skin of the patients ill with typhus fever, the lice which were used in the following experiments. That these lice either had fed recently or were still feeding actively when removed from the patient, was indicated by their distention with blood. No attempt, however, was made to start with normal lice and allow them to feed on patients in a definite manner. Interruption of the work precluded this. The lice were picked off the clothes or patient with forceps, gathered in a Stender jar containing a piece of sterilized cheese-cloth, and transplanted to the laboratory where they were immediately injected.

They were prepared for injection as follows: Into a sterile mortar were placed 4 c.c. of salt solution, the desired number of lice were put into this salt solution, and with the aid of forceps the lice were crushed thoroughly with the pestle until a homogeneous emulsion resulted. This was then injected intraperitoneally into guinea-pigs. No effort was made to study the infectivity of the separate parts of the louse (salivary glands, intestinal contents, etc.).

Guinea-pig C6 was injected with the emulsion of 30 lice obtained from Case 18 when this patient was in the 13th day of illness, or 1 day before his crisis. Since it has been previously determined that lice become infectious after 5 days from the time of their feeding on infected blood, it was presumed that they carried the infectious agent at that time. On the 9th day after injection the animal experienced a sudden drop in temperature followed by a rise to 104.4 F. (see Chart 4). For further study the animal was exsanguinated and its defibrinated blood injected into 3 other guinea-pigs. The work was then interrupted and the transmission of the virus could not be followed. However, autopsy on the original guinea-pig showed no lesion except in the spleen, which was enlarged and congested with its Malpighian bodies prominent—typical of typhus fever in guinea-pigs.

The spleen of this animal was removed in a sterile manner. It was macerated in sterile salt solution and the emulsion thus obtained was cultured in deep tubes of 2% glucose serum agar. Five days later, 12 colonies of the typhus bacillus were obtained in one tube—the rest of the culture could not be observed longer.

A still more striking reaction was noted in Guinea-pig C8, which was injected in similar manner with 65 lice from Case 21. These lice were removed from the patient after the latter had passed the crisis (3 days postcritically). Unfortunately this animal could not be studied thoroughly, as the work was interrupted at about the time

of its reaction. But the definite incubation period of 10 days and the type of the rise in temperature lead us to believe that the guinea-pig had typhus fever (see Chart 5).

Thus we see that the transmission of typhus fever to guinea-pigs by the injection of infected lice has been accomplished and the same organism, the typhus bacillus, recovered from the organs of reacting animals.

Work was begun on the question of the bedbug as a carrier of this disease (along with the louse), but it was interrupted before satisfactory conclusions could be reached.

RESULTS OF CULTURES FROM INFECTED LICE

At the same time that the experiments just detailed were made, some of the lice were cultured.

Two methods were employed. In one instance the louse was seized between 2 sterile forceps, one grasping the thorax, the other the lower end of the abdomen, and the insect was pulled apart. The intestinal contents thus liberated were gathered in a platinum loop and inoculated into the media. In the second case the total number of lice were macerated in a mortar, with salt solution, and this suspension was cultured. The culture medium employed consisted of either a 2% or a 0.5% glucose serum agar. The tubes after inoculation were allowed to solidify in the upright position. We found these methods to be of value in separating the colonies of the organism from those of contaminating bacteria which are of course incidental to cultures of intestinal contents. Colonies, when present, usually appeared in the lower third of the tube.

Six series of experiments could be observed a sufficiently long time to note the final results. They are as follows:

Series 1.—Five lice, distended with the blood of Case 6, having been obtained from the patient 2 days before the crisis, or on the 12th day of the disease, comprised this series. They were cultured by one of the methods mentioned,

Chart 4. Temperature record of Guinea-pig C6, following injection of infected lice.

Chart 5. Temperature record of Guinea-pig C8, showing reaction after injection of 65 lice.

in 2% glucose serum agar in deep tubes. After 4 days of incubation very numerous minute colonies appeared. These colonies had the same characters as those of the typhus bacillus. They were apparently anaerobic, as they developed only in the deep portion of the tube, but it was impossible for us to subculture the organism. However, instead of being gram-positive, all these bacilli were uniformly gram-negative. We shall discuss later the significance of this variation in the staining properties.

Series 2.—Three lice taken from the same patient as Series 1, but in this instance 1 day before the crisis, or the 13th day of the disease, were cultured in deep tubes of 0.5% glucose serum agar. In one tube particular pains were taken to culture the excreta of a louse, passed on a piece of previously sterilized cheese-cloth. In this case, after 7 days' incubation, the most anaerobic portion of the culture medium was riddled with pinpoint colonies. These were made up of organisms having the typical morphology of the typhus bacillus, but, as noted, they were also gram-negative. Only one subculture (grown anaerobically) could be made and the organisms still retained their gram-negative character.

Series 4.—Two lice taken from the clothing of Case 12 at a period corresponding to 2 days before the crisis, or the 12th day of the disease, were cultured in 0.5% glucose serum agar in deep tubes. On the 11th day of incubation, 3 colonies appeared; on the 12th, 4. These colonies were of an organism which was subsequently studied in New York; it agreed absolutely in cultural and serum reactions with the typhus bacillus. In smears from the original colonies, most organisms were stained gram-negative, a few gram-positive; in later subcultures they were all gram-positive.

Series 6.—Six lice collected from the clothing of Case 13 by Dr. N. Frazin, were sent to us for culture. We are greatly indebted to Dr. Frazin, physician at Minas Dolores, not only for this courtesy but for numerous kindnesses shown us during the progress of the work. These lice were taken from the patient, who was suffering from a very severe form of the disease, at a period corresponding to the 12th day of the illness, or 2 days before the crisis. They were cultured in 2% glucose 3% agar serum media.

The culture of Louse 1 showed 1 colony after 3 days' incubation. The organisms were gram-positive. Cultures of Louse 2 showed 8 colonies after 3 days' incubation. All the organisms were gram-negative. Cultures from Louse 3 showed 4 colonies after 9 days' incubation. One colony was made up of gram-negative organisms, the others of gram-positive organisms. (A loopful of the growth in a colony made up of gram-positive organisms was emulsified in salt solution and injected into a guinea-pig. Our observations upon this animal, however, were unfortunately interrupted.) However, these organisms were subcultured and were brought back with us to New York, where they were compared with standard endemic and epidemic strains of the typhus bacillus and were found to be identical with them. Cultures from Louse 2 were gram-positive after first subculture; cultures from Louse 3 were gram-positive after second subculture.

Series 8.—Five lice taken from the clothing covering Case 18 at a period corresponding to 1 day before the crisis, comprised the series for culture, while 30 lice collected at the same time under the same circumstances were injected into a guinea-pig. This guinea-pig (C6) showed evidences of having been infected with typhus fever. The culture in 0.5% glucose serum agar was positive after 4 days of incubation. Here again the organisms were gram-negative in the original colony.

Series 9.—This series is of great importance inasmuch as the lice were collected from the body of Case 7, 4 days after the crisis. Four lice were cultured in deep tubes of 2% glucose serum agar. After 6 days' incubation the culture showed 2 typically growing colonies, one containing gram-negative organisms, the other partly gram-positive. After 2 subcultures these were uniformly gram-positive.

A loopful of organisms from an original colony were emulsified in salt solution and injected into Guinea-pig B9. Twenty-four hours later there was a drop in temperature of almost 4 degrees and on the 3rd day after injection the animal died. On autopsy the guinea-pig showed the typical lesions of typhus fever in this species: the spleen was slightly enlarged, and the Malpighian bodies were prominent. All the other viscera were apparently normal. The spleen was removed in sterile manner, macerated in salt solution and cultured. After 4 days' incubation 5 colonies appeared in one of the tubes, all organisms being gram-positive. The other culture tubes could not be observed long enough, the work being interrupted at about this time.

Several of the cultures of this series were studied in New York and were proved identical with the endemic and epidemic strains of the typhus bacillus.

To summarize the results of these louse cultures we may state that in practically all cultures that were made the organism was obtained. It was shown that lice contained the bacilli when they were taken from the patient at the height of the disease and 4 days after the crisis. This agrees with the findings of previous workers, who observed that a louse feeding on a typhus patient becomes infective for others after a period of 5 days. This period of time is necessary for the multiplication of the bacilli or their increase in virulence in the body of the louse until sufficient to make it infective. All the lice used in these experiments could be, then, infective.

The other striking observation was that these organisms are usually gram-negative in the original colonies. After one or two subcultures they become definitely gram-positive. The relationship between the virulence of the organisms and their staining reaction is very suggestive. Furthermore, it may be of interest to note that in previous work it was observed that in the original colonies in blood cultures from typhus cases the organisms were stained partly gram-negative and partly gram-positive in the same smear preparation. In recent studies by da Rocha-Lima⁴ further corroboration of this fact is made; he finds that infective lice harbor an organism of similar morphology in enormous numbers, especially in the stomach wall, and remarkable to state, these organisms are gram-negative. Independently and at the same time Plotz and Baehr⁵ in their studies on typhus fever in the Balkans and Russia, comparing normal with infected lice in smear preparations,

⁴ Arch. f. Schiffs- u. Tropen-Hyg., 1916, 20, p. 17.

⁵ Reports will appear in the Journal of Infectious Diseases.

found in lice from typhus patients numerous bacilli decolorized by Gram's method.

Again, we have demonstrated that in a guinea-pig injected with organisms from an original colony of a louse culture—that is, before they had lost their virulence by artificial cultivation—the bacilli caused the death of the animal in 3 days. The guinea-pig showed the typical findings, on autopsy, of typhus fever in this species, and from the spleen we have been able to cultivate the same organism.

Hence, in our studies on infected lice, we have been able to produce typhus fever by the injection of such lice, to recover the organism from the infected guinea-pig, to culture the organism from the typhus lice, and to demonstrate the pathogenicity of a culture of the typhus bacilli from the insect.

CONCLUSIONS

In the short period of our work (during which we received much assistance from Mr. Clarence A. Grabel, superintendent of the plant) we were able to confirm the results obtained previously by Plotz, Olitsky, and Baehr² and to add further evidence concerning the etiologic factor of typhus fever.

The data bearing on the etiologic relationship between this organism and Mexican typhus fever may be summarized as follows:

Of 11 cases cultured, 9 were positive. Of these eleven 3 were cultured in 0.5% glucose serum agar; 1 was positive. Eight of the eleven were cultured in 2% glucose serum agar; all these were positive. At the same time and by the same methods, blood cultures taken on control cases were negative.

The bacteria were more numerous in the early stages of the infection and in the more severe cases.

The organism obtained is identical in all its features, especially in carbohydrate-fermentation and in its serum reactions, with the typhus bacillus isolated in New York from cases of endemic and epidemic forms of the disease.

The blood so cultured was true typhus virus; we were able to infect guinea-pigs with typhus fever by the injection of such blood.

Further evidence to support our views was added by the nature of the formation of antibodies in the Mexican patients. We have been able to trace the development of agglutinins with these results: Before the crisis, 16% of the cases showed positive agglutinations; after the crisis, 95%. Agglutinins were absent in numerous control cases in

which a history of typhus fever could be excluded. Agglutinogens of various organisms, including those morphologically similar to the typhus bacillus, showed no agglutination with typhus-fever immune serum. Antigens made of these organisms likewise failed to bind complement in the presence of typhus immune serum.

Thus we see that specific agglutinins are present as a direct result of the typhus bacillus circulating at the height of this disease. These antibodies are usually absent when the fever is present, that is, when the antigen, the organism, may be recovered from the blood stream. After the crisis the agglutinins are evident in their greatest concentration.

We have been able to demonstrate the pathogenicity of the organism, and to show that the virulence deteriorates very rapidly in cultures. When an animal was infected in this way, the organism was recovered from its spleen.

In animal experiments with infected lice, we have been able to produce the disease in guinea-pigs when a sufficient number of lice were injected. From the spleen of one of these animals we obtained numerous colonies of the same bacillus.

Cultures from infected lice showed uniform results. The organism was recovered in all the cultures that could be observed the proper time, 21 days. The injection of an original colony into a guinea-pig caused the death of the animal in 3 days, and from its spleen the same organism could be isolated. The most striking features of these infected-lice cultures is the tendency of the organisms to be decolorized by Gram's method. This has occurred in every culture. The gram-negative bacilli, however, become gram-positive on subculture. It is logical to conclude from the evidence presented that the reaction of the organism to gram-stain has a distinct relationship to the maintenance and loss of its virulence.

Work on prophylactic vaccination will be reported in another communication.