STUDIES ON TYPHUS FEVER*

IX. On the Serum Reactions of Mexican and European Typhus Rickettsia

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In a communication published in April, 1932 (1), the writers described a method of obtaining large numbers of *Rickettsiae* by the inoculation of rats previously exposed to severe short wave-length X-ray radiation. The primary purpose of these experiments was to supply us with *Rickettsia* material for further study of active immunization against typhus fever. Incidentally, the method furnished us with suspensions, relatively free from extraneous materials, which could be employed for the reliable performance of serum reactions. The importance of studying such reactions, both for the etiological questions raised by Nicolle and Laigret (2) and for the correct interpretation of our immunological observations, is obvious.

Agglutination with typhus *Rickettsia* suspensions has been carried out by Otto and Dietrich, (3), by Weigl (4), by Krukowski (5), and by da Rocha-Lima (6). All of these observers use suspensions of the carbolized intestinal contents of infected lice (Weigl vaccine), a material containing much extraneous debris but rich in *Rickettsia* and, with proper precautions, quite suitable for agglutination experiments. All of them found that the blood of typhus convalescents developed agglutinins for such suspensions, Krukowski stating that the *Rickettsia* agglutinins appeared earlier and attained higher potencies than did the agglutinins for Proteus X-19. This investigator also observed that guinea pigs—animals on which the Weil-Felix reaction rarely appears—developed *Rickettsia* agglutinins. Reviewing these

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experiments, Otto and Munter (7) express the opinion that *Rickettsia* agglutination is more specifically diagnostic of typhus fever than is the Weil-Felix reaction.

All such experiments up to the present time have been done with the European variety of typhus and with *Rickettsiae* obtained from lice. The etiological rôle of these organisms, however, is not in any way questioned today. There are still investigators, on the other hand, who are not yet convinced of the specific significance of the organisms observed in the tunica cells of Mexican typhus guinea pigs (see Nicolle and Laigret cited above). It was for the purpose of elucidating this point and to investigate the serological relationships between the Mexican (New World) and Old World varieties of the disease that the following experiments were undertaken.

Technique

The materials used for agglutination were the Weigl vaccine, of which we obtained several ampoules from Dr. Mooser, and our own suspensions of Mexican *Rickettsia* produced by the rat X-ray method referred to above.

In a large series of preliminary experiments we discovered that the formalin-treated *Rickettsiae*, as described in our published technique, were not as readily agglutinable as were similarly prepared carbolized suspensions. The reason for this is not clear, but in all the protocols cited the material used was carbolized (0.5 per cent phenol).

We devised a number of agglutination methods in which the organisms were observed microscopically and macroscopically, with and without staining; and while some of these were reasonably satisfactory and gave us much preliminary information, we report below only the results obtained by our final method, of the reliability of which we are satisfied. Microscopic methods were eventually given up entirely as possessing too many pitfalls of false clumping under conditions of prolonged observation. The method as finally adopted depends upon the use of small, flat tubes especially made for us, which can be used with minute quantities of material (this for reasons of economy) and which can be observed with convenience under a binocular of 30 diameters' magnification. Serum dilutions and *Rickettsia* suspensions are run into the tubes with capillary pipettes, and mixed by drawing them in and out several times. The tubes are then incubated in a water bath at 40°C. and observed at intervals of 2 to 3 hours. Agglutination was invariably slow, rarely clear—even with strong sera—in less than 2 hours. After 5 hours, agglutination was usually complete. At about that time the tubes were left at room temperature for subsequent reading and were again read after a night in the ice chest. Readings were independently made by two, and sometimes by three observers. All readings were made by comparing the tube under observation with a series of controls.

In the code used, + means slight agglutination, but definite; ++ means strong agglutination with some turbidity left between clumps; +++ means almost complete agglutination; ++++ means complete agglutination; − means negative agglutination; 0 means that this particular dilution was not set up.

EXPERIMENTS

Our first experiments concerned themselves with agglutinin development in guinea pigs convalescent from infection with a European (Breinl) strain of typhus, a Tunisian strain, and our own Mexican strain.

Experiment I.—The occasional omission of a dilution (0) in the comparative series was due to the necessity of economy with the Weigl vaccine, of which we had only a meagre supply.

The sera listed in the tables below are as follows:

Mexican Guinea Pig 1.—Bled 29 days after inoculation, 15 day after defervescence, 17 days after subsidence of swelling.

Mexican Guinea Pig 2.—Bled 21 days after inoculation, 2 days after defervescence, 9 days after subsidence of swelling.

European Guinea Pig 1.—Bled 17 days after inoculation, 4 days after defervescence.

European Guinea Pig 1a.—Same animal bled 6 days later.

European Guinea Pig 2.—Bled 23 days after inoculation, 10 days after defervescence.

European Guinea Pig 2a.—Same guinea pig bled 8 days later. None of the sera employed were heated.

In Table I, which shows the results, the sera are set up against the Weigl vaccine in 1–40 dilution only, a potency indicated by preliminary experience, and the controls are selected to give adequate safeguard without waste of material.

The foregoing experiment confirmed preliminary ones in showing that the sera of guinea pigs convalescent from either the Mexican or European typhus infection agglutinated both varieties of *Rickettsiae.* It was noticeable here, as in the earlier experiments, that the Mexican serum usually agglutinated the Weigl vaccine more powerfully than the European serum agglutinated our own *Rickettsia.*

Experiment II.—For the foregoing reason, it seemed desirable to us to make a comparison between two of the above sera in greater detail, setting up reactions at relatively short dilution intervals. A cross-agglutination of Mexican *Rickettsia* and of Weigl vaccine with convalescent guinea pig sera was therefore carried out. The results of this experiment are shown in Table II.

TABLE I

Serum	Dilution	Mexican *Rickettsia*	Weigl vaccine
Mexican Guinea Pig 1	1–40	++++	++ to +++
	1–80	++++	0
Mexican Guinea Pig 2	1–40	++++	++ to +++
	1–80	++++	0
European Guinea Pig 1	1–10	++	0
	1–20	++	0
	1–40	+	+++ to ++++
	1–80	–	0
European Guinea Pig 1*a*	1–10	+++	0
	1–40	0	+++
	1–80	–	0
European Guinea Pig 2	1–10	+++	0
	1–40	0	+++
	1–80	–	0
European Guinea Pig 2*a*	1–10	+++	+++ to ++++
	1–40	++	+++
	1–80	–	–
Normal Guinea Pig 1	1–10	–	0
	1–20	–	0
	1–40	–	–
Normal Guinea Pig 2	1–10	–	0
	1–20	–	0
	1–40	–	–
Normal Guinea Pig 3	1–20	–	–
	1–40	–	–
	1–80	–	0
Normal Guinea Pig 4	1–20	–	–
	1–40	–	0
	1–80	–	0

It was again noticeable that the Weigl vaccine was more quickly and completely agglutinated by the Mexican serum than was our own suspension by the European serum. While this may be due to im-

munological conditions, it is not impossible that it is partly a consequence of physical circumstances, since the Weigl material consists of a coarsely granular and heavy suspension, whereas our own *Rickettsia* vaccine is a much more finely divided suspension, particles under the binocular being exceedingly small.

TABLE II

Serum	Dilution	Mexican *Rickettsia*	Weigl vaccine
Mexican Guinea Pig 1	1–6	+++	+++
	1–10	++++	++++
	1–20	+++	++++
	1–30	+++	+++
	1–40	++++	++
	1–50	++++	++
	1–80	+++	+
	1–100	+++	+
European Guinea Pig 2	1–6	+	+++ to ++++
	1–10	++	++++
	1–20	+++	++++
	1–30	++	++++
	1–40	++	++++
	1–50	++	++++
	1–80	–	+++
	1–100	–	+++
Normal Guinea Pig 5	1–6	–	–
	1–20	–	–
	1–40	–	–
Normal Guinea Pig 6	1–6	–	–
	1–20	–	–
	1–40	–	–
Salt solution control		–	–

These and other reactions with guinea pig sera were sufficiently sharp to convince us that guinea pig convalescent serum from both varieties of typhus had acquired agglutinating properties for both types of *Rickettsiae*.

Under ordinary circumstances these experiments would convince us that our *Rickettsiae* are truly concerned with typhus and that vac-

cination against the disease with these suspensions is a rational procedure. The observations of Nicolle and Laigret, however, have suggested the possibility that the tunica organisms studied by Mooser and by ourselves might be adventitious saprophytes present in the stock guinea pigs and rats, stimulated into multiplication by the insult of intraperitoneal injections. In that case our agglutination experiments with guinea pig sera would carry little conviction.

We proceeded, therefore, to study human convalescent sera in the same manner in which we had studied the guinea pig sera. Our materials were as follows:

1. A Polish convalescent serum obtained through Dr. Mooser (Weil-Felix = 1–1280).

2. A Tunisian convalescent protective serum (Weil-Felix = 1–1280).

3. A Mexican convalescent serum, A, sent us by Dr. Mooser (Weil-Felix = ++ 1–3200).

4. A Mexican convalescent serum, B (Weil-Felix = 1–500, weakly 1–1000).

5. Two Mexican sera which were very old—over 2 years—and gave weak Weil-Felix reactions. Mexican Serum 7 (Weil-Felix = +++ 1–80). Mexican Serum 9 (Weil-Felix = +++ 1–160).

Experiment III.—Table III shows the results of agglutination experiments of human convalescent sera tested against our own carbolized *Rickettsia* suspension.

This experiment shows definite agglutination of our *Rickettsia* by all the convalescent sera, whatever their origins. But it is noticeable that the European sera agglutinate the Mexican organisms much more feebly than do the homologous sera, and there is apparent, in the European sera, a prozone of exceptional breadth. This may be due to the age of these sera. It will be remembered that Shibley (8) has shown that heating agglutinating sera is apt to extend the prozone considerably, and it is reasonable to assume that ageing may do the same thing. Nevertheless, we decided to run these sera out to their agglutinating limits and to so control them that we might make sure that the positive reactions in high serum dilutions were not non-specific phenomena.

Experiment IV.—Table IV represents consolidated titrations of two consecutive experiments done with identical materials.

Here again the zones are apparent in the Old World sera much more than in the Mexican, but there could be not the slightest question

TABLE III

Serum	Dilution	Result
Polish convalescent serum	1–20	±
	1–80	+ to ++
	1–160	++ to +++
Tunisian convalescent serum	1–20	+
	1–80	++
	1–160	+
Mexican convalescent Serum A	1–20	++++
	1–80	++
	1–160	++
Mexican convalescent Serum B	1–20	+
	1–80	++++
	1–160	++ to +++
Mexican convalescent Serum 7	1–20	+ to ++
	1–80	+
Mexican convalescent Serum 9	1–20	±
	1–80	+
Normal human Serum 1	1–20	−
Normal human Serum 2	1–20	−

TABLE IV

Dilution	Sera									
	Polish	Tunisian	Mexican A	Mexican B	Normal 3	Normal 4	Normal 5	Normal 6	Normal 7	Normal 8
1–20	−	−	++++	++	−	−	−	−	1–10−	1–10−
1–50	−	−	+++	+	−	−	−	−	−	−
1–100	±	−	+++	+	−	−	−	−	−	−
1–200	±	±	−	++	−	0	0	0	−	−
1–300	++	±	+	++++	0	0	0	0	0	0
1–400	+	+	±	++	0	0	0	0	0	0
1–500	++	++	+	+++	0	0	0	0	−	−
1–750	+++	+ to ++	0	0	0	0	0	0	0	0
1–1000	+	+	0	0	0	0	0	0	−	−

about the ++ and +++ agglutinations of the Mexican *Rickettsia* which occurred in the Old World serum dilutions above 1–200.

TABLE V

Human serum	Dilutions	Mexican *Rickettsia*	Weigl vaccine
Mexican Serum D	1–20	–	–
	1–50	–	–
	1–100	–	–
Mexican Serum E	1–20	–	–
	1–50	–	–
	1–100	–	±
Mexican Serum F	1–20	+++	+++
	1–50	+++	+++
	1–100	+++	+++
	1–200	+ to ++	0
	1–500	++	0
	1–1000	+	0
Mexican Serum G	1–10	++++	++++
	1–20	++++	++++
	1–50	+++	+++
	1–100	+++	++++
Normal Serum 5	1–10	–	–
	1–20	–	–
	1–50	–	–
	1–100	–	–
Normal Serum 7	1–10	–	0
	1–50	–	0
Normal Serum 8	1–100	±	0
	1–200	–	0
	1–500	–	0
	1–1000	–	0

Experiment V.—At this time, May, 1932, we made an observation which convinced us that agglutination of our *Rickettsia* in human serum indicated typhus. Dr. Varela of the Mexican Institute of Hygiene was good enough to send us four convalescent sera supposed to have been taken from patients at the period of defervescence.

Agglutinations done with these sera and with both varieties of *Rickettsia* vac-

cines are shown in Table V, which is a consolidated tabulation of two experiments carried out on consecutive days.

The results of this experiment were disquieting, since all the sera received were supposed to have come from hospital cases diagnosed as typhus fever, or tabardillo. Weil-Felix reactions were therefore promptly carried out with these specimens. The results are shown in Table VI.

The correspondence of the Weil-Felix reactions of these sera with their agglutination power for both the Mexican *Rickettsia* and the European louse *Rickettsia* is so striking that we have no hesitation in concluding that we are dealing with specific immunological phenomena.

Although the experiments described in the preceding paragraphs appeared to us to eliminate any possible uncertainty as to the etiological relationship of the Mexican tunica *Rickettsia* to the disease in human beings, there was obviously another line of simple experimentation by which this important fact could be further consolidated and by which the relationship between the two types of agent—the Old World *Rickettsia* of the louse (as found in the Weigl vaccine) and our own *Rickettsia*—could be investigated. This consisted in immunizing rabbits with each of the two vaccines respectively and carrying out cross-agglutinations, a procedure made possible by the fact that we were still in possession of a few phials of the carbolized louse vaccine prepared by Weigl. Accordingly, the following experiment was carried out.

Experiment VI.—Two rabbits were injected as follows:

Rabbit 1.—Weil-Felix negative. Was intravenously injected three times, at 5, 6, and 7 day intervals, with 3 cc. of our formalinized X-ray rat vaccine of Mexican *Rickettsia.* 11 days after the last injection the serum of this rabbit gave a Weil-Felix: 1–20 ++++; 1–40 +++; 1–80 +.[1]

Rabbit 2.—Weil-Felix negative. Was intravenously injected three times, at 5 day intervals, with 1 cc. of a Weigl European louse *Rickettsia* vaccine. A small specimen of blood was taken from the ear of this animal 5 days after the last injection. Although this was obviously too early for the most potent results, the

[1] Tested on guinea pigs this serum gave no protection against the Mexican disease, a fact in contrast with similarly prepared horse serum to be discussed in another paper but in harmony with similar rabbit serum observations made by Brienl.

serum gave a Weil-Felix: 1–20 +++; 1–40 ++; 1–80 +. For this reason we decided to use it for the experiment here recorded.

We set up a complete set of cross-agglutinations with the two sera and their respective vaccines. As will be seen, each serum highly agglutinated its homolo-

TABLE VI

Weil-Felix Reaction (Proteus X-19)

Dilution	Mexican Serum D	Mexican Serum E	Mexican Serum F	Mexican Serum G
1–20	–	–	++++	++++
1–40	–	–	++++	++++
1–80	–	–	++++	++++
1–160	–	–	++++	++++
1–320	–	–	++++	++++
1–640	–	–	++++	++++

TABLE VII

Agglutination of Mexican Rickettsia and Weigl Vaccine (European Louse Rickettsia) by Rabbit 1 Immunized with Mexican Vaccine from X-Rayed Rats

Serum Rabbit 1 before immunization (Weil-Felix negative)	Mexican *Rickettsia*	Weigl vaccine
1–10	–	–
1–50	–	–
1–100	–	–
1–200	–	–
1–500	–	–
1–1000	–	–
Serum Rabbit 1 after immunization (Weil-Felix 1-40)	**Mexican *Rickettsia***	**Weigl vaccine**
1–10	+++	++++
1–50	+++	++
1–100	+++	+++
1–200	+++	+
1–500	+++	–
1–1000	++	–

gous *Rickettsia*, but since, in each case, the rabbit had received, with the vaccines, a certain amount of either rat or louse protein, these results might justly be criticized. Agglutinations of either one of the vaccines by the heterologous serum cannot be subject to this objection. The reactions are shown in Tables VII and VIII.

Tables VII and VIII illustrate that the serum of a rabbit immunized with our formalinized Mexican *Rickettsia* vaccine will agglutinate the Weigl louse *Rickettsia* and that the serum of a rabbit immunized with the European louse vaccine develops agglutinating power for the Mexican *Rickettsia* as obtained by us from guinea pigs through X-rayed rats. Incidentally, the appearance of these agglutinins coincided in both animals with the appearance of Weil-Felix reactions.

TABLE VIII

Agglutination of Mexican Rickettsia and of Weigl Vaccine by Serum of Rabbit 2 Immunized with European Louse Vaccine (Weigl)

Serum Rabbit 2 before immunization (Weil-Felix negative)	Mexican *Rickettsia*	Weigl vaccine
1–10	–	+
1–50	–	–
1–100	–	–
1–200	–	–
1–500	–	–
1–1000	–	–
Serum Rabbit 2 after immunization (Weil-Felix 1–40)	**Mexican *Rickettsia***	**Weigl vaccine**
1–10	+++	++++
1–50	+++	++++
1–100	+++	++++
1–200	+++	++++
1–500	++	++++
1–1000	+	+++

DISCUSSION

The experiments recorded above seem to permit little further doubt that the *Rickettsia* suspensions obtained by us in prepared rats inoculated with tunica material from Mexican typhus guinea pigs represent organisms specifically related to the Old World typhus fever in man and closely related to, if not identical with the *Rickettsia prowazeki* seen in lice that have fed on cases of Old World typhus. Even though objections might be raised to this conclusion on the basis of our guinea pig serum agglutinations, it is not likely that an organism saprophytic

in guinea pigs and rats should also be present side by side with an undiscovered typhus virus in human beings infected with both the New World and the Old World diseases. Further than this, the agglutinations carried out with the sera of rabbits immunized with the two types of *Rickettsia* seem to us conclusive evidence that the European louse *Rickettsia* and the Mexican tunica organism are either identical or closely related. There is no other reasonable interpretation of these cross-agglutinations, and we can see no source of error in the experiments. If these results are considered together with the facts that, in both cases, the ultimate sources of the organisms are animals infected with material from active human cases, and that the rabbits developed the Weil-Felix reaction, which is acknowledgedly diagnostic of the human typhus group, the chain of evidence linking these organisms with the diseases seems complete.

Whether the comparative agglutinations indicate an antigenic overlapping of the *Rickettsiae* of the two sources we cannot decide with certainty on the basis of these tests, since the two antigens at our disposal—our rat vaccines and the Weigl louse vaccine—are quite incomparable in gross physical properties. However, from the differences in agglutinating titre manifested by individual European strain and Mexican strain guinea pig sera respectively upon one and the same vaccine, we are inclined to believe that there is a definite, though slight and overlapping, antigenic difference which must be taken into consideration in connection with the practical problems of specific prophylaxis and serum production.

CONCLUSIONS

1. The blood of guinea pigs convalescent from Old World and New World typhus infection develops agglutinating properties for the tunica and rat *Rickettsiae* of the New World diseases and for the louse *Rickettsia* of the Old World disease.

2. The two microorganisms are closely related, though probably not identical.

3. Human convalescents of both varieties of typhus develop agglutinins for both types of *Rickettsiae*. Such *Rickettsia*-agglutinating properties are parallel with the Weil-Felix reaction in the human sera.

4. Rabbits immunized with Weigl louse vaccines develop agglu-

tinins for our X-ray rat vaccines and *vice versa*. In both cases the rabbit sera develop agglutinins for Proteus X-19.

5. These experiments furnish a further and, we believe, conclusive proof of the etiological rôle, in New World typhus fever, of the *Rickettsia* bodies first seen in the tunica cells of inoculated guinea pigs by Mooser, and obtained in massive amounts by ourselves.

6. The serum reactions also provide a further logical basis for experiments in prophylactic vaccination with these *Rickettsiae*.

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