THE ANTIGENIC RELATIONSHIP BETWEEN PROTEUS X-19 AND TYPHUS RICKETTSIAE

A Study of the Weil-Felix Reaction

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In a preceding paper from this laboratory, Zinsser and Castaneda (1) reported upon the development of a method by which reliable agglutination reactions could be obtained, with *Rickettsia* suspensions, in sera from convalescent typhus patients and in those of convalescent or vaccinated animals. The experiments carried out by this method showed a definite antigenic relationship between our own Mexican *Rickettsia* vaccines obtained from rats and the Weigl vaccines produced with European *Rickettsiae* obtained from infected lice. It was also found that there was parallelism between the agglutinating powers of the investigated sera for *Rickettsia* and for *Bacillus proteus* X-19. These results were in keeping with the immunologic studies on typhus fever published from this laboratory during recent years and with the well known specificity of the Weil-Felix reaction in the several types of *Rickettsia* infection. The method furnished an opportunity for a more precise investigation of the antigenic mechanism responsible for this specific relationship.

The experiments described below represent agglutinin absorption studies applied to this problem.

Technique

The materials used for agglutination were the following: (1) Weigl's phenolized vaccines, which consist of triturated suspensions of *Rickettsia prowazeki* obtained from the intestines of lice infected with European typhus. (2) Suspensions of Mexican *Rickettsiae* obtained from rats by the method described. (3) A non-motile "O" culture of *Bacillus proteus* X-19. The suspensions of this organism used were made from fresh agar slants in every case.

The agglutinating sera used were as follows: (1) European and Mexican convalescent typhus sera. (These were unfortunately quite old.) (2) A serum from an endemic case of typhus occurring in Boston. (3) The serum of a horse immunized with formalinized Mexican *Rickettsiae*. (4) The sera of rabbits immunized with the stock strain of *Bacillus proteus* X-19.

Absorptions were carried out by the addition of thick suspensions of either *Bacillus proteus* X-19 or of the Mexican *Rickettsiae* to sera diluted two to four times with salt solution. In the experiments presented in Table III, the sera were diluted to from 1:30 to 1:75 before absorption. The mixtures were kept in a water bath at 37°C. for from 3 to 4 hours and were then allowed to stand at room temperature overnight. Controls of similarly diluted sera—unabsorbed—were,

TABLE I

Sera Absorbed with Proteus X-19

	Unabsorbed								Absorbed								Antigens
Serum dilutions.....	1:20	1:40	1:80	1:160	1:320	1:640	1:1,280	1:2,560	1:20	1:40	1:80	1:160	1:360	1:640	1:1,280	1:2,560	
Human typhus serum (Boston case)	4	4	4	4	3	1	—	—	2	1	—	—	—	—	—	—	*Proteus* X-19
	2	3	3	4	4	2			±	2	4	4	4	2			Weigl vaccine
		4	4	4	1	4	3			4	4	4	4	4	4	—	Mexican vaccine
Horse antityphus serum	4	4	4	1	—	—			—	—	—	—	—	—			*Proteus* X-19
				3	3	2			4	4	4	3	3	2			Weigl vaccine
Normal horse serum		2	—	—													*Proteus* X-19
		—	—														Weigl vaccine

4 = ++++, or complete. The other numerals are roughly in proportion.

in every case, identically treated. Before testing, the suspensions were centrifugalized at high speed in order to remove organisms.

In observing the reactions, the ordinary macroscopic test was used for the *Proteus* agglutinations, the tubes being placed in a water bath at 37° to 40°C. for 2 hours, and then left at room temperature overnight before final readings were made.

The *Rickettsia* agglutinations were carried out by methods described in the paper referred to (1).

Experiment I.—In this experiment, we used the serum of a woman who was admitted to the Beth Israel Hospital in Boston early in the winter of 1932–33, suffering from endemic typhus fever. The history and the clinical course were typical and the Weil-Felix reaction titrated up to 1:640. Agglutination of both

the European and the Mexican *Rickettsiae* in this case ran almost parallel with the Weil-Felix reaction, as will be noted from Table I. The fact that this serum was not from an epidemic Mexican or European case somewhat weakens the value of our experiment, since we were not able to obtain a virus from the patient, probably because our attempt to do this was made too late in the disease.

In the same experiment, we similarly absorbed our antityphus horse serum, controlling this part of the experiment with normal horse serum.

Table I illustrates the results.

Proteus X-19 removed, from the human serum as well as from the antityphus horse serum, practically all agglutinins for *Proteus* X-19, but did not affect the agglutination of the *Rickettsiae*.

In an experiment similar to our own, Krukowski (2) found that when European convalescent typhus serum was absorbed with

TABLE II

Sera Absorbed with Mexican Rickettsiae (Formalinized)

	Unabsorbed						Absorbed						Antigen
Serum dilutions.....	1:320	1:640	1:1,280	1:2,560	1:5,120	1:10,240	1:320	1:640	1:1,280	1:2,560	1:5,120	1:10,240	
Anti-*Proteus* Rabbit 1.....	4	4	4	4	4	—	4	4	4	4	4	—	*Proteus* X-19
Anti-*Proteus* Rabbit 2.....			4	2	—	—			4	2	—		*Proteus* X-19

Proteus X-19, the homologous agglutinins were removed, whereas those for the *Rickettsiae* were left unabsorbed. Our experiment fully confirms and extends this observation.

Experiment II.—In this experiment, the sera of two rabbits which had been immunized with *Proteus* X-19 were absorbed with Mexican *Rickettsiae*. Table II illustrates the results of this experiment.

It is plain from Table II that the absorption of an anti-*Proteus* rabbit serum with Mexican *Rickettsiae* has practically no effect upon the anti-*Proteus* agglutinins.

Experiment III.—In this experiment, four human typhus sera, one from the Boston case, two from convalescent Mexican typhus patients and one from a

Polish case, were absorbed by the method described with formalinized Mexican *Rickettsiae*. These sera agglutinated *Proteus* X-19, as well as the Mexican *Rickettsiae*, in all instances except in that of the Polish case. This serum agglutinated Mexican *Rickettsiae* up to 1:160, as reported in the paper of Zinsser and Castaneda alluded to above (1). We did not have enough of the serum to include the desirable low dilution controls in the present instance.

Table III shows the results of this experiment.

It is apparent from Table III that absorption with Mexican *Rickettsiae* removed, either completely or almost completely, agglutinins both for *Proteus* X-19 and for our Mexican *Rickettsia* suspensions.

TABLE III

Sera Absorbed with Mexican Rickettsiae (Formalinized)

	Unabsorbed									Absorbed								Antigens
Serum dilutions....	1:60	1:100	1:200	1:300	1:400	1:500	1:600	1:800	1:1,600	1:60	1:100	1:200	1:300	1:400	1:500	1:600	1:800	1:1,600	
Human typhus	4	4	1	1	—	—	—	—	—	—	—	—	—	—	—	—	—		*Proteus* X-19
Serum 1	4	4	4	1	4	4	4	4	—	2	±	—	—	—	—	—	—		Mexican vaccine
Human typhus			4	4			2					—	—			—			*Proteus* X-19
Serum 2			4	2			1		1			—	—						Mexican vaccine
Human typhus			4	4			4		2			2	2			1		—	*Proteus* X-19
Serum 3			2	3		4			3			2	—		—			—	Mexican vaccine
Human typhus			4	3			2		1			—	—			—		—	*Proteus* X -19
Serum 4			±	—								±	—						Mexican vaccine

Serum 1, from Boston case on the 12th day of disease. Serum 2, Mexican typhus. Serum 3, Mexican typhus. Serum 4, European typhus from Poland.

In past experiments, it has invariably appeared that animals immunized with *Proteus* X-19 possessed no agglutinating power for *Rickettsia* suspensions. This has been the experience of others and repeatedly noted in this laboratory. We have confirmed this observation with the Weigl vaccine, as well as with our own *Rickettsia* suspensions—fresh, formalinized and carbolized. Nevertheless, from the experiments described above, it seems reasonable to suppose that Mexican *Rickettsiae* contain an antigenic factor common to both

Rickettsia and to *Proteus* X-19. But the failure of detection of *Rickettsia* agglutinins in anti-*Proteus* serum would seem to be opposed to such a conception. In discussing this matter with Dr. Grinnell, who has studied the differences in agglutinability between heated and unheated *Bacillus typhosus*, we were led to investigate similar phenomena with *Rickettsiae*. The unexpected results are presented in the following experiment.

Rickettsia suspensions were prepared by washing the peritoneal cavity of X-rayed rats in the same way in which this is done in the preparation of typhus vaccine. Emulsions were made in saline and in formalinized saline. Both suspensions were heated at 75°C. for 30 minutes. Unheated material was reserved for controls.

TABLE IV

Agglutination of Mexican Rickettsiae by Anti-Proteus Serum

	Non-formalinized *Rickettsiae*										Formalinized *Rickettsiae*											
	Unheated					Heated					Unheated							Heated						
Serum dilutions.....	1:20	1:40	1:80	1:160	1:320	1:20	1:40	1:80	1:160	1:320	1:20	1:40	1:80	1:160	1:320	1:640	1:1,280	1:20	1:40	1:80	1:160	1:320	1:640	1:1,280
Anti-*Proteus* Rabbit 1.....	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	1	2	3	3	3	3	1
Anti-*Proteus* Rabbit 2.....	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	4	4	4	3	3	3	—
Rabbit 1 before immunization.....	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—
Rabbit 2 before immunization.....	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—

It is clear from the experiment shown in Table IV that while heating does not produce any change in the non-formalinized suspensions, formalinized *Rickettsiae* become agglutinable, which means that heating removes a factor that under ordinary conditions prevents agglutination in anti-*Proteus* serum.

The complementary experiment shown in Table V indicates that the formalin factor is not active in the same way when added to the *Rickettsiae* 24 hours after such suspensions have been made in saline. A test with human typhus serum was set up in order to control agglutinability by the homologous antibody.

We cannot at present explain the mechanism of the formalin action except that, as found for pneumococcus by Zinsser and Tamiya (3), formalin preserves from deterioration or disintegration some delicate antigenic element present in the *Rickettsia* body upon which agglutination in anti-*Proteus* serum depends. Heating, it seems reasonable to assume, removes a factor which masks the action of the serum in a manner analogous to that by which the type-specific

TABLE V

Serum dilutions	1:20	1:40	1:80	1:160	1:320	1:640	Mexican *Rickettsiae* formalinized 24 hrs. after washings in saline
Anti-*Proteus* serum	—	—	—	—	—	—	Unheated
Anti-*Proteus* serum	—	—	—	—	—	—	Heated
Human typhus serum		1		3		1	Unheated
Human typhus serum		1		2		1	Heated

TABLE VI

Absorption of Anti-Proteus Serum by Mexican Rickettsiae (Formalinized, Unheated)

	Proteus X-19					Heated formalinized *Rickettsiae*			
Serum dilutions	1:200	1:400	1:800	1:1,600	1:2,500	1:40	1:80	1:160	1:320
Anti-*Proteus* serum unabsorbed	4	4	3	2	±	1	2	1	—
Anti-*Proteus* serum absorbed	4	4	3	±	—	—	—	—	—

carbohydrate factor masks species-specific agglutination in the pneumococcus group.

Since we had thus succeeded in obtaining agglutination of formalinized and heated *Rickettsia* suspensions in anti-*Proteus* serum, we proceeded next to study the absorption of such serum with *Rickettsiae*. We found that, although only the treated *Rickettsiae* were agglutinated, the results of absorption were the same whether or not this procedure was carried out with normal or with formalinized heated organisms. In the experiment shown in Table VI, the absorption was carried out with normal *Rickettsiae*.

From experiments like the one given in Table VI, it is clear that

there is an antigenic relationship between *Rickettsiae* and *Proteus* X-19. Cross-agglutinations and absorptions follow with considerable accuracy the conditions as we know them concerning the so called major (*Haupt*) and minor (*Mit*) agglutinins. In a serum which is taken from a typhus patient, that is, a *Rickettsia*-infected body, major agglutinins for *Rickettsiae* and minor agglutinins for *Proteus* X-19 appear. These are both removed by absorption with *Rickettsiae*. Absorption with the minor antigen, *i.e. Proteus* X-19, removes only the minor agglutinins. A serum which is prepared by immunization with *Proteus* X-19 yields agglutinins of both types; *i.e.*, major agglutinins for *Proteus* X-19 and minor agglutinins, in this case for *Rickettsiae*. Absorption of such a serum with *Rickettsiae* removes only the corresponding minor agglutinins. When anti-*Proteus* serum is absorbed with *Proteus* X-19, all agglutinins for *Proteus* and *Rickettsiae* disappear.

The experiments as a whole demonstrate that the Weil-Felix reaction depends upon the presence of a common antigenic factor in *Proteus* X-19 and *Rickettsia prowazeki*. A more accurate study of this common element is being undertaken.

SUMMARY

1. The absorption of typhus sera (human or antityphus horse serum) with *Proteus* X-19 removes only the *Proteus* agglutinins, leaving the *Rickettsia* agglutinins intact.

2. The absorption of typhus sera with Mexican *Rickettsiae* removes the agglutinins for both the *Rickettsia* and *Proteus* X-19.

3. While normal or formalinized *Rickettsiae* are not agglutinated by anti-*Proteus* serum, these organisms—when formalinized and heated at 75°C.—become agglutinable by such serum.

4. The absorption of anti-*Proteus* serum with Mexican *Rickettsiae* removes agglutinins for formalinized and heated *Rickettsiae* but does not affect those for *Proteus* X-19.

CONCLUSIONS

There is a common antigenic factor in *Rickettsiae* and *Proteus* X-19 which explains the Weil-Felix reaction.

Incidentally, these experiments confirm the basic facts of *Rickettsia*

agglutination, both in European and Mexican, established by Zinsser and Castaneda in a preceding communication.

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