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Cross-Reacting Typhus Antibodies in Rocky Mountain Spotted Fever.

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It is generally accepted that the rickettsial diseases of man may be separated into 4 distinct groups according to their immunological relationship. (Trench fever is not included since its etiology has not been definitely established.) While the members of the typhus group, the spotted fever group, the scrub typhus group, and the "Q" fever group confer an immunity to the diseases of their respective group, most observers consider that there is no cross immunity between the groups. However, an antigenic relationship between members of the typhus and spotted fever groups of diseases is suggested since both groups are characterized by the presence of an OX-19 agglutination. This hypothesis was investigated by Castaneda and Silva[1] who observed that when typhus-immune guinea pigs were challenged with a virulent strain of Rocky Mountain spotted fever, a febrile reaction of variable duration occurred with only 4 of 17 animals dying while all of the 19 normal control guinea pigs developed fever and died. While no complete cross immunity was demonstrated, these experiments did indicate that typhus-immune guinea pigs developed a partial immunity to Rocky Mountain spotted fever. The same authors performed rickettsial agglutination tests, using a suspension of murine rickettsiæ obtained from infected rat lungs and convalescent human serum from cases of Rocky Mountain spotted fever. Two macroscopic agglutination tests showed complete agglutination at titers of 1/160 and 1/20 respectively while the third specimen showed partial agglutination at 1/20. Microscopic agglutination tests performed on 6 specimens of human convalescent Rocky Mountain spotted fever serum with murine rickettsiæ were recorded as showing some agglutination. The sera of infected Rocky Mountain spotted fever guinea pigs likewise were tested with murine rickettsial suspensions and it was observed that no agglutination developed from the 1st to the 6th day of disease while specimens obtained from the 6th to the 31st day showed more or less agglutination, but none was observed after 32 days. From the experimental data presented the authors concluded that there was a definite immunological relationship between Rocky Mountain spotted fever and typhus.

Parker[2] observed in cross immunity tests in guinea pigs that while animals that had recovered from murine typhus were fully susceptible when inoculated with a strain of Rocky Mountain spotted fever isolated from *Amblyoma americanum,* the converse was not true. Of 4 guinea pigs recovered from Rocky Mountain spotted fever and challenged with a strain of murine typhus, 2 guinea pigs remained afebrile and 2 had 3 and 6 days of fever respectively, without the scrotal swelling noted in the controls. In similar studies with epidemic typhus versus Rocky Mountain spotted fever, of 15 guinea pigs recovered from epidemic typhus and challenged with Rocky Mountain spotted fever 6 guinea pigs remained afebrile and 9 developed fever of from one to 6 days' duration. In the reverse test, when 12 Rocky Mountain spotted fever immune guinea pigs were challenged with an epidemic strain, 4 remained afebrile and 8 developed fever of from 2 to 5 days' duration. It is apparent from these observations that a partial immunity between typhus and Rocky Mountain spotted fever was present, at least with the strains investigated.

The following study was undertaken to obtain further serological data on the possible immunological relationship between these two diseases.

A series of 32 serum samples were examined

* Member of the U. S. A. Typhus Commission.

1 Castaneda, M. R., and Silva, R., *J. Immunol.*, 1941, **42**, 1.

2 Parker, R. R., *Public Health Rep.*, 1943, **58**, 721.

TABLE I.
Serological Tests on Specimens from Cases of Rocky Mountain Spotted Fever.

Case No.	Day of Disease	Weil-Felix*		Complement fixation†			Rickettsial Agg.*		Mouse neut. 50% endpoint*
		OX-19	OX-2	RMSF	Epidemic	Murine	Epidemic	Murine	Epidemic
1	7	1/160	0	0	0	0	1/40	1/160	‡
	14	1/640	0	1/160	0	0	1/640	1/640	6510
	15	1/640	0	1/160	0	0	1/80	1/2560	1444
	21	1/640	0	1/320	0	0	1/80	1/320	‡
2	12	0	0	0	0	0	0	0	0
	16	1/2560	1/160	1/40	0	0	‡		64
	106	1/80	1/40	1/80	0	0	0	1/80	23
3	16	0	0	1/20	0	0	0	0	2888
		Fatal-strain isolated							
4	6	0	0	0	0	0	0	1/80	—
	49	1/320	1/40	1/40	0	0	0	1/160	6
5	44	0	0	1/80	0	0	1/40	1/80	—
	217	1/40	0	1/80	0	0	0	0	8
6	30	1/320	1/320	1/80	0	0	‡		—
	33	1/320	1/160	1/160	0	0	0	1/80	—
	40	1/320	1/320	1/160	0	0	0	1/80	45
	75	1/160	1/160	1/160	0	0	0	1/80	13
	155	1/320	1/160	1/640	0	0	0	1/40	—
7	4	0	0	1/10	0	0	0	1/20	12
8	35	0	1/80	1/40	0	0	1/40	1/40	—
	146	0	1/20	1/80	0	0	0	1/20	45
9	57	1/160	0	1/640	0	0	0	1/20	204
	120	1/20	1/20	1/1280	0	0	0	1/80	132
10	130	1/40	1/80	1/80	0	0	0	1/80	128
11	5	0	0	0	0	0	0	0	—
	12	1/640	0	1/80	0	0	1/80	1/160	—
	64	1/160	1/20	1/320	0	0	0	1/40	—
	75	1/80	0	1/320	0	0	0	0	16
12	30	0	1/640	1/1280	0	0	0	1/40	—
	71	0	1/160	1/1280	0	0	0	0	—
13	44	0	1/160	1/640	0	0	1/80	0	—
	96	0	1/160	1/320	0	0	1/40	1/20	—
14	29	1/160	1/2560	1/80	0	0	0	1/40	—

* Final dilution.
† Initial dilution.
‡ Not sufficient serum.

from 14 cases of Rocky Mountain spotted fever. Complement fixation tests for Rocky Mountain spotted fever,[3] epidemic and murine typhus,[4] rickettsial agglutination for epidemic and murine typhus,[5] Weil-Felix tests and epidemic neutralization tests[6,7] were performed on each specimen of serum. The results in Table I were observed.

It is noted that a positive complement fixation test for Rocky Mountain spotted fever was obtained some time or other during the

[3] Plotz, H., and Wertman, K., *Science*, 1942, **95**, 445.

[4] Plotz, H., *Science*, 1943, **97**, 20.

[5] Plotz, H., and Snyder, M., Report to the Surgeon General (withheld from publication).

[6] Gildermeister, E., and Haagen, E., *Deut. Med. Wchnschr.*, 1940, **66**, 878.

[7] Henderson, R. G., and Topping, N. H., *Public Health Rep.*, 1944, 58 (withheld from publication).

course of the disease in the 14 cases studied. In 6 cases a rise in complement fixation titer was found when early and late specimens were compared. The complement fixation reactions with an epidemic and murine rickettsial antigen were all negative.

Macroscopic agglutination tests, performed with purified epidemic and murine rickettsial suspensions obtained from yolk sac cultures, were made on 30 specimens and agglutinins were demonstrable in 24. Five cases showed both epidemic and murine agglutinins, 8 showed murine agglutinins alone and one early case showed no agglutination whatsoever. In 5 cases there was a rise in titer when early and late specimens were compared. The titers varied from 1/20 to 1/2560. The reactions were clear cut and represent complete agglutination at the titer recorded.

Mouse neutralization tests were performed with a toxic substance derived from yolk sac cultures of epidemic typhus. Sixteen specimens were tested for the presence of neutralizing antibody and positive tests were demonstrated in 15. The 50% endpoint titers (Reed and Muench) varied from 6 to 6510. In 3 cases rather high titers were found, the 50% endpoint being 6510, 2888, and 1444.

The results of the Weil-Felix tests were in accord with those reported by Davis and Parker.[8] In the 14 cases of Rocky Mountain spotted fever studied, the sera of 5 agglutinated Proteus OX-19 at a higher dilution, 3 agglutinated Proteus OX-2 at a higher dilution, and one agglutinated equally well with both Proteus OX-19 and OX-2. Significant titers were not obtained in the remaining five cases. One was an early fatality, 3 were obtained late in convalescence, and one was a single specimen obtained early in the febrile period.

These results indicate that epidemic and murine rickettsial agglutinins and epidemic neutralizing antibodies may occur in some convalescent specimens of Rocky Mountain spotted fever.

The presence of typhus antibodies in cases of Rocky Mountain spotted fever raises the question as to whether these antibodies were due to a previous attack of typhus fever or immunization with typhus vaccine. Since the specimens examined came from military personnel where immunization records are kept, we have verified that none had received typhus vaccine nor was there a history of a previous infection of typhus fever.

[8] Davis, G. E., and Parker, R. R., *Public Health Rep.*, 1932, **47**, 2.

TABLE II.
Epidemic Typhus Neutralizing Antibody in Late Convalescent Specimens from Epidemic Typhus Fever.

Case No.	Day of disease	Neutralizing antibody titer 50% endpoint
1344	286	51
1345	117	102
1495	137	180
1894	318	81
1902	99	256
2724	286	323
3558	251	45
3732	245	722
4569	104	814
4956	235	814
5038	241	51
5043	276	2888
5585	229	323
5587	226	202
6243	218	40
7932	209	90

TABLE III.
Epidemic Typhus Neutralizing Antibody in Specimens from Rocky Mountain Spotted Fever.

Case No.	Day of disease	Neutralizing antibody titer 50% endpoint
1	14	6510
	15	1444
2	12	0
	16	64
	106	23
3	16	2888
4	49	6
5	217	8
6	40	45
	75	13
7	4	12
8	146	45
9	57	204
	120	132
10	130	128
11	75	16

In view of the fact that one could be tempted to employ the neutralization test to demonstrate the existence of a past infection, it was of interest to compare the 50% endpoint neutralizing antibody titers against epidemic typhus obtained in late convalescent specimens from proven cases of epidemic typhus fever with those found in specimens from cases of Rocky Mountain spotted fever. The results are given in Tables II and III.

If we accept the figure 40 as representing the lowest 50% endpoint neutralizing titer (case 6243—strain isolated) which occurred in late convalescence in our series of epidemic typhus fever cases, it is observed that 9 of the 16 specimens studied from cases of Rocky Mountain spotted fever gave higher titers. As already pointed out three of the Rocky Mountain spotted fever specimens gave titers of epidemic neutralizing antibody as high as or higher than were found in late convalescent specimens from cases of epidemic typhus fever. These results must be taken into consideration if the neutralization test is used for survey purposes.

Conclusion. Serological studies on convalescent specimens from cases of Rocky Mountain spotted fever show the presence of epidemic and murine agglutinins and epidemic neutralizing antibodies although there is no evidence of cross complement fixation. This evidence fits in with the observations of Castaneda and Silva and those of Parker, and indicates that there is a serologic relationship between epidemic typhus fever and Rocky Mountain spotted fever. The data presented in this paper must be considered in evaluating the results obtained with rickettsial agglutination or the neutralizing antibody when these tests are used as diagnostic procedures.

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pH of Nasal Mucosa of Some Laboratory Animals.

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The susceptibility of individuals to infections of the respiratory tract shows seasonal variations. This also appears to be true of some of the laboratory animals. A number of physiological factors are involved in the defense mechanism against bacterial and viral invasions, and in determining the severity of the infection. Of these factors, the pH of the nasal mucosa may play a role. There is disagreement as to what constitutes the normal pH of nasal mucosa. Using a glass electrode *in situ,* Fabricant[1] reported a surprisingly low average value of 6.2 for man with large fluctuations. Nungester and Atkinson[2] pointed out several errors in the method used by Fabricant. Their determinations yielded a value of pH = 7.0. Neither investigator recorded the temperature at which the readings were made. They also introduced a saline bridge without testing the effect of the additional boundary potentials. The effect of these boundary potentials has, however, been shown to be small.[3]

Hamsters were selected for the study of pH of nasal mucosa because the animals are susceptible to some viral diseases. Several readings were also made using rats and ferrets. This article deals with normal values. Special glass electrodes were constructed so that the entire glass membrane would be in contact with the nasal mucosa. The saturated calomel cell made direct contact with the animal through an asbestos-fiber wick.

Methods. The glass electrodes were made

[1] Fabricant, N. D., *Arch. Otolaryng.,* 1941, **34**, 150.

[2] Nungester, W. J., and Atkinson, A. K., *Arch. Otolaryng.,* 1944, **39**, 342.

[3] Voegtlin, C., Kahler, H., and Fitch, R. H., *Nat. Inst. Health Bull.,* 1935, **164**, 15.