Complement-Fixation in Rickettsial Diseases*

IDA A. BENGTSON, PH.D., AND NORMAN H. TOPPING, M.D.

Senior Bacteriologist, and Passed Assistant Surgeon, U. S. Public Health Service, Washington, D. C.†

THE complement-fixation reaction has been found useful in the study of a number of bacterial diseases and, more recently, in certain virus diseases including influenza, psittacosis, equine encephalomyelitis, lymphocytic choriomeningitis, lymphogranuloma venereum, papilloma, vaccinia, and others.

Complement-fixation in rickettsial diseases has been investigated by comparatively few workers. Among the early publications was that of Davis and Petersen,[1] 1911, who studied complement-fixation in Rocky Mountain spotted fever, using as antigens the serum and the macerated organs of infected guinea pigs and also infected tick eggs. Alcoholic extracts of organs from fatal cases of European typhus were used as antigens by several workers including Cathoire,[2] Müller,[3] Markl,[4] Delba,[5] and Papamarku.[6] Papamarku[7] later used an extract of infected lice as did Jacobthal[8, 9] and Epstein.[10] None of these antigens yielded results which were very satisfactory. In all probability the number of rickettsiae in the infected organs was too small for the purpose of producing good antigen. Infected lice were unsuitable because similar results were obtained with both normal and with infected lice.

With the newer improved methods for the cultivation of rickettsiae it has been possible to obtain much more satisfactory antigens. This is particularly true of endemic typhus and "Q" fevers. Endemic typhus fever rickettsiae grow abundantly in the infected chick yolk sac (Cox[11]) in the lungs of mice and rats infected by the intranasal route (Castaneda[12]), and also by the agar-tissue culture method of Zinsser, Fitzpatrick, and Wei.[13] The rickettsiae of "Q" fever can be obtained in considerable concentration in the spleens of infected mice (Burnet and Freeman[14]), and in the infected yolk sac of chick embryos. It is a more difficult problem to obtain luxuriant growth of the rickettsiae of Rocky Mountain spotted fever and European typhus.

Castaneda,[15] 1936, obtained positive complement-fixation reactions in cases of active and past infection with Mexican typhus and Brill's disease, using rickettsiae from x-rayed typhus infected rats as antigen.

One of us has recently reported on complement-fixation in "Q" fever[16] and in endemic typhus.[17] Mouse spleens and infected yolk sacs were the source of the rickettsiae used for antigens in "Q" fever and infected rats' lungs and infected yolk sac were employed for the typhus antigens.

---

* Read before the Laboratory Section of the American Public Health Association at the Seventieth Annual Meeting in Atlantic City, N. J., October 16, 1941.

† From the Division of Infectious Diseases, National Institute of Health, U. S. Public Health Service, Bethesda, Md.

In the complement-fixation reaction as well as in other laboratory procedures used for determining active or past infection an important consideration is that of specificity, and the work here reported has been undertaken to obtain added information on this point, particularly in regard to endemic typhus fever and its differentiation from Rocky Mountain spotted fever. The Weil-Felix test has proved very useful in the differentiation of certain of the rickettsial diseases from other diseases, but it does not differentiate between typhus and spotted fever. The neutralization test in guinea pigs also fails at times to yield conclusive results, owing to secondary infections and nonspecific immunity (Badger [18]). The question of differentiation is of special importance in those sections of the country where both endemic typhus and Rocky Mountain spotted fever occur, as in the eastern and southeastern sections of the country (Dyer [19]).

Materials—The sera used in carrying on this study include: (1) sera from cases of past infection with either endemic typhus or Rocky Mountain spotted fever; (2) sera from active cases of endemic typhus or Rocky Mountain spotted fever; (3) sera from patients with other diseases.

The typhus antigens were prepared by grinding the yolk sacs of infected chick embryos in the 5th or 6th passage when they showed numerous rickettsiae, with sterile alundum, after draining to remove some of the yolk. A 10 per cent suspension in 0.85 per cent sterile saline with 1:10,000 merthiolate was prepared. This was centrifuged at low speed in the horizontal centrifuge in order to remove the larger particles. The supernatant fluid was then centrifuged for 1 hour at 4,000 r.p.m. The precipitate was suspended in 0.85 per cent saline containing merthiolate to the original volume. The precipitate which settled from this suspension after standing 1 to 2 days was discarded. Further tissue precipitate settles on standing, but it has been found that this is not anticomplementary and the suspension may be shaken at the time of titration or for later use. The antigens were titrated to determine the lowest concentration at which fixation was obtained with a pooled specimen of known sera.

Methods—All of the specimens, both from the typhus and spotted fever cases, were tested against the endemic typhus antigen. Only a few tests were made with spotted fever antigens. The test was carried out as has previously been described, using 0.2 ml. amounts of inactivated sera in dilutions ranging from 1:2 to 1:256 or higher, 0.2 ml. amounts of antigen, and 0.2 ml. amounts of complement. After 1 hour's incubation, 0.4 ml. of sensitized sheep cells was added and incubation continued for another hour. After storage in the refrigerator over night readings were made the following morning. Fixation at 3+ or 4+ was considered a positive test.

RESULTS

1. *Non-rickettsial diseases*—It might be expected that the complement-fixation test would be specific as far as non-rickettsial diseases are concerned. This was found to be true among those investigated. Included were 14 cases of tuberculosis, 10 cases of leprosy, 6 cases of malaria, 10 cases of syphilis, 10 cases of rheumatic fever, 13 cases of tularemia, 7 cases of undulant fever, 8 cases of typhoid fever, 8 cases of trachoma, 2 cases of lymphopathia venereum, 1 case of psittacosis, and 2 cases of amebiasis (Table 1). These sera were freshly drawn, with the exception of some of those from undulant fever, tularemia, and typhoid, which had been stored for periods of several weeks at icebox temperature. Slight fixation occurred in the lower dilutions in certain of the leprosy, undulant fever, and tula-

TABLE 1

Complement-fixation in Non-rickettsial Diseases

No. of specimens	*Disease*	*Complement-fixation*	*Remarks*
14	Tuberculosis	0	
10	Leprosy	0 to very slight	7 fixed complement in dilution 1:2 (1+ or 2+)
6	Malaria	0	2 cases active 2 cases cured 2 cases with tabes dorsalis
10	Syphilis	0	3 cases primary 3 cases secondary 4 cases tertiary
10	Rheumatic fever	0	
7	Undulant fever	0 to very slight	6 fixed complement in dilutions 1:2 to 1:4 (1+ or 2+). Titers against abortus antigen were 1:160 to 1:5120.
13	Tularemia	0 to very slight	7 fixed complement in dilutions 1:2 to 1:8 (1+ or 2+). Titers against tularense antigen were 1:8 to 1:1280.
8	Typhoid fever	0	
8	Trachoma	0	2 cases papillary 1 case granular 1 case cicatricial
2	Lymphopathia venereum	0	
1	Psittacosis	0	
2	Amebiasis	0	

remia cases, but these were usually incomplete and not higher than 1:2 or 1:4 dilutions. All of the undulant fever cases were positive by agglutination in dilutions 1:160 to 1:5,120 against Brucella antigen, and the tularemia cases were positive against tularense antigen in dilutions 1:8 to 1:1,280.

2. *Active and past endemic typhus infections*—The study of known typhus cases has been extended beyond that previously reported. The complement-fixation titers and the Weil-Felix titers of a series of specimens from a case of endemic typhus resulting from a laboratory infection have been determined (Figure 1). The complement-fixation titer increased from 1:8 on the 10th day to 1:4,096 on the 15th day, then fell to 1:2,048 on the 16th day. On the 85th day the titer was 1:1,024 and on the 180th day 1:512, thus showing a gradual decrease. The Weil-Felix titer ran approximately ten times as high as the complement-fixation titer on the 15th or 16th day and fell off more rapidly, reaching 1:320 on the 85th day and 1:160 on the 180th day. A positive test by the Weil-Felix reaction was evident earlier than by complement-fixation, a titer of 1:80 being reached on the 6th day, 1:320 on the 9th day, and 1:1,280 on the 10th day.

In another case of typhus, originating as the result of a laboratory infection, the development of complement-fixing antibodies was much slower (Figure 2), a titer of 1:2 being reached on the 6th day, 1:4 on the 7th day, and 1:8 on the 14th day. The corresponding Weil-Felix titers were 1:320 to 1:5,120. This appears to have been an exceptional case. By 3 months the complement-fixation titer was 1:512 and the Weil-Felix titer 1:640.

Sera from 53 cases of past infection with endemic typhus were tested by the complement-fixation and Weil-Felix

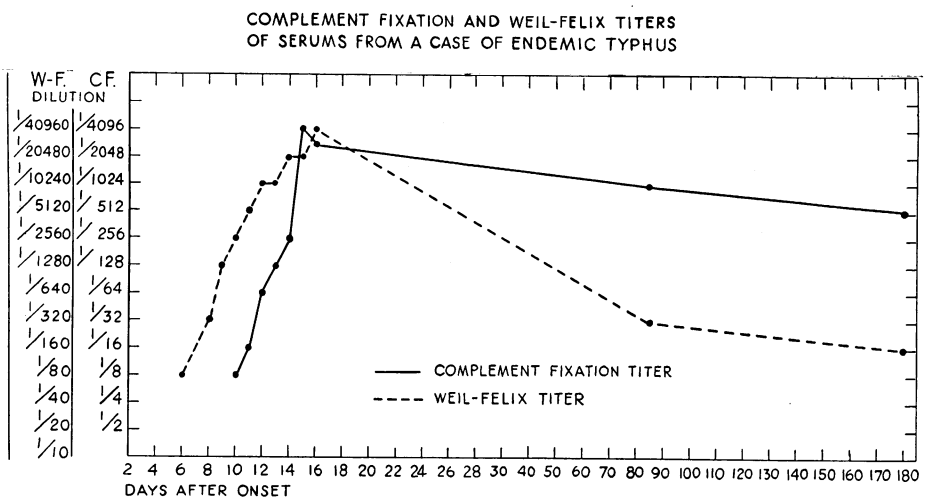

FIGURE 1

methods. All of these cases had occurred in Georgia and Alabama. The dates of occurrence varied from 2 months to 67 months prior to the time the sera were drawn. Fifteen cases were proved cases of endemic typhus, the virus having been isolated in guinea pigs. The diagnoses of the remaining 38 cases were based on clinical symptoms. It is possible that some of the cases which showed low titers may have been incorrectly diagnosed. It is significant, however, that the results of the tests agree so well with the diagnoses of the physicians, though it is to be considered that these cases occurred in a section of the country where the disease is endemic and therefore perhaps more likely to be correctly diagnosed.

The results obtained with these 53 cases are shown in Figure 3. The number of months elapsing between the date of onset and the date when the serum was obtained are represented by the abscissae and the complement-fixation and Weil-Felix titers by the ordinates. The graphs represent the average titers of all of the sera which were drawn at approximately the same length of time after onset of illness. It is very probable that the severity of the infection, as well as the length of time elapsing since onset, influences the titer of the serum, hence the irregularities in the titers. The general trend of the graph representing the complement-fixation titers indicates a rather gradual decrease in this titer. If we assume that, at certain stages at least, the Weil-Felix titer is approximately ten times that of the complement-fixation titer, it is obvious that in general the Weil-Felix titers in these samples are much lower than the corresponding complement-fixation titers, though it is to be noted that all of these cases had occurred 2 months or more prior to the time the serum was obtained. Only one serum

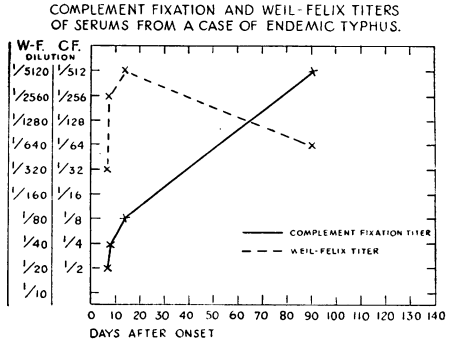

FIGURE 2

TABLE 2

Complement-fixation in Endemic Typhus

Patient	*Sex*	*Age*	*Race*	*Locality*	*Tick, flea or rat exposure*	*Symptoms*	*First appearance of rash*	*Physician's diagnosis*	*Date of onset*	*Serum collected*	*Weil-Felix titer*	*Complement-fixation-titer (Typhus)*
1. WB	M	24	W	Ohio	Rats	Chill, fever, cough	Face and neck Dec. 10	Typhus	Dec. 6, 1940	8 months	1:80	1:256
2. PC	M	45	W	Md. D. C.	Rats	Chill, fever, aching, sweats	Body, Dec. 24	Typhus	Dec. 20, 1940	85 days	1:1280	1:128
3. EP	M	*	W	Ga.	Laboratory infection	Typical typhus symptoms. Severe case.	6th day	Typhus	Feb. 1, 1941	74 days	1:1280	1:1024
4. WG	M	*	W	Ga.	Laboratory infection	Typical typhus symptoms. Mild case.	6th day	Typhus	Feb. 1, 1941	74 days	1:40	1:512
5. AF	M	9	W	N. J.		Chill, fever	Feb. 25	Brill's disease	Feb. 20, 1941	31 days	1:2560	1:256
6. SW	F	55	W	Mass.		Fever	Body		Mar. 12, 1941	13 days	1:1280	1:128
6a. "				Fla.				Typhus or RMSF		16 "	1:5120	1:128
7. AR	M	*	W	Mass. Fla.		Chill, fever, headache, sweats	Body, Mar. 24	Typhus or RMSF	Mar. 17, 1941	22 days	1:20480	1:4096
8. RF	M	*	W	N. Y.	Tick bite	Severe headache, semi-consciousness	Body, Mar. 26	Typhus or Brill's dis.	Mar. 20, 1941	16 days	1:2560	1:8192
9. EW	M	64	W	Cuba		Malaise, headache, fever	No skin manifestation	Typhus?	Aug. 8, 1941	9 days	1:2560	1:256
10. MC	F		W	Ga.		Chill, fever, headache	Body, June 1	Typhus	May 27, 1941	10 days	1:1280	1:256
11. LM	M	31	W						June 13,	11 days	1:20480	1:4096
11a. "				N. C.		Fever, sweats, headache	Rash	Typhus	1941 †	35 days	1:5120	1:8192
12. SC	M	20	W	Conn. Fla.	Rats	Fever, malaise, headache, sweats	Trunk and extremities, June 9–16	Brill's disease	June 5, 1941	June 18 approx.	1:20480	1:3192
13. JR	M	*	W	Ala.	Rats	Chill, fever, sweats headache	Body, June 29	Typhus	June 23, 1941	30 days (?)	1:2560	1:128
14. MS	F	35	W	Okla. Texas		Chill, fever, headache	Upper portions of extremities	Typhus or RMSF	July 9, 1941	15 days	1:1280	1:1024
15. WG	M	36	W	Va. D. C.	Rats	Fever, headache	None	Typhus	Aug. 26, 1941	20 days	1:10240	1:8192

* Adults

† Estimated

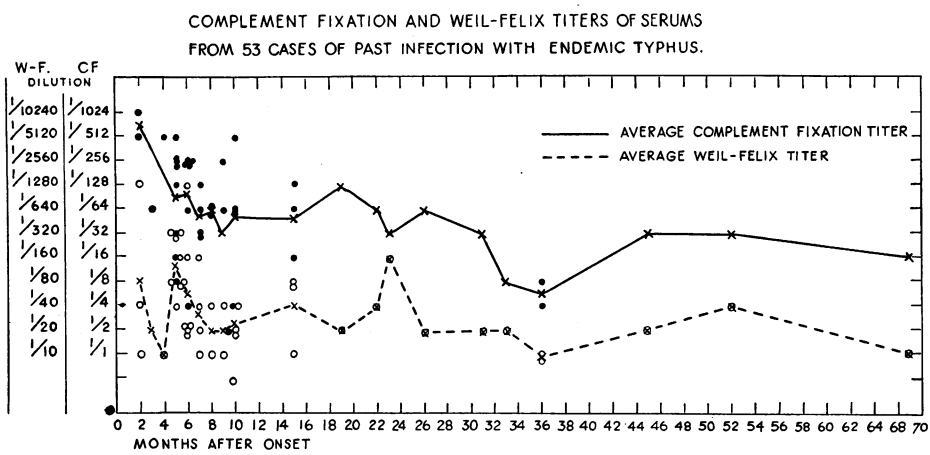

FIGURE 3

had a Weil-Felix titer higher than 1:320 in 6 months, and after this none was higher than 1:160, and the majority had titers of 1:80, 1:40, or lower. The complement-fixation titers with a few exceptions were 1:16 or higher even in one case which had occurred 5½ years previously. It is, therefore, evident that the complement-fixation titer of the serum is a much better criterion of past infection than is the Weil-Felix titer.

3. *Endemic typhus and Rocky Mountain spotted fevers*—The specificity of the complement-fixation test for endemic typhus has been further investigated by a comparative study of the results obtained with serum from active cases of endemic typhus and Rocky Mountain spotted fevers against a typhus antigen. A few cases of past infection are also included in this group. In the typhus group are 11 active 1941 cases and 4 cases of past infection. The Rocky Mountain spotted fever cases include 20 active 1941 cases and 10 cases of past infection, 2 of which occurred in 1939 and 8 in 1940. The sera from most of the active cases are those received at the National Institute of Health with requests for the Weil-Felix test, or for the complement-fixation test, or for tests to differentiate between endemic typhus and Rocky Mountain spotted fever. A number of sera from cases of both endemic typhus and Rocky Mountain spotted fevers have been received from the branch Typhus Research Laboratory of the National Institute of Health at Savannah, Ga., and others from the Georgia State Health Department.

In order to evaluate the results of the tests, an effort has been made to obtain all the information necessary from the clinical standpoint for a diagnosis, using records from the hospitals or attending physicians. This information includes age and sex of patient, locality where the case occurred, date of onset of illness, history of tick or flea exposure, clinical symptoms, date of appearance, location and description of rash, the date when the specimen was obtained, and the physician's diagnosis.

Weil-Felix tests and complement-fixation tests were done on all specimens, using an endemic typhus antigen as previously stated in all the complement-fixation tests whether the sera were from cases diagnosed as endemic typhus or Rocky Mountain spotted fever. It was anticipated that positive results would be obtained in all the typhus cases and that probably negative

TABLE 3

Complement-fixation in Rocky Mountain Spotted Fever (Typhus Antigen)

Patient	Sex	Age	Race	Locality	Tick, flea, or rat exposure	Symptoms	First appearance of rash	Physician's diagnosis	Date of onset	Serum collected	Weil-Felix titer	Complement-fixation titer	Remarks
1. GH	F	6	W	Md.		Fever, headache		RMSF	1939	2 years	1:10	0	
2. VH	F	13	W	Md.		Fever, headache		RMSF	1939	2 years	1:20	0	
3. GB	F	35	W	Ga.	Ticks	Chill, malaise, fever	Thighs, wrists June 5	RMSF	May 29, 1940	11 months	1:80	0	Confirmed by cross-immunity test *
4. HE	M	11	W	Md.	Ticks?	Fever, headache, nausea	Extremities May 14	RMSF	May 12, 1940	9 months	1:160	0	
5. LS	M	..	W	Ga.	Ticks	Chill, fever, headache, delirium	Legs, forearms July 6	RMSF	June 28, 1940	10 months	1:20	0	Confirmed by cross-immunity test *
6. MS	F	5	W	Ga.	Ticks	Fever, headache, delirium	Legs, forearms July 5	RMSF	June 28, 1940	10 months	1:20	0	Confirmed by cross-immunity test *
7. MB	F	9	W	Ga.	Ticks	Fever, headache	Extremities July 22	RMSF	July 14, 1940	10 months	1:10	0	Confirmed by cross-immunity test *
8. RW	M	..	W	Ga.	Ticks	Fever, headache, moderately severe	Chest and abdomen July 17	RMSF	July 15, 1940	9 months	1:80	0	Confirmed by cross-immunity test *
9. MJB	F	10	W	Ga.	Ticks?	Fever, headache	Extremities July 30	RMSF	July 24, 1940	9 months	1:40	0	Confirmed by cross-immunity test *
10. EJ	M	5	W	Ga.		Fever, headache	Legs and forearms—Aug. 10	RMSF	Aug. 7, 1940	8 months	1:10	0	Confirmed by cross-immunity test *
11. LD	F	4	W	Md.	Ticks	Fever, labored respiration	Arms and legs	RMSF	Apr. 25, 1941	9 days	1:640	0	Fatal case
12. JR	M	10	W	Md.		Typical spotted fever	Extensive Apr. 28	RMSF	Apr. 23, 1941	13 days		1:16(2+)	Fatal case
13. MH							Extremities			5 days	0	0	
13a. "	F	..	W	Ga.	Ticks	Fever	May 5	RMSF	May 6, 1941	7 days	0	0	
14. FR										6 days	1:1280	0	
14a. "										12 "	1:5120	0	
14b. "	M	..	W	Md.	Ticks	Fever, cough	Hands and feet	RMSF	May 15, 1941	58 "	1:80	0	
15. KT						Fever, headache, aching in body and legs	Arms and thighs May 30	RMSF or Typhus	May 29, 1941	14 days	1:20480	1:256	
15a. "	F	3	C	Miss.	Ticks					54 "	1:1280	0	

TABLE 3 (Cont.)

Complement-fixation in Rocky Mountain Spotted Fever (Typhus Antigen)

Patient	Sex	Age	Race	Locality	Tick, flea, or rat exposure	Symptoms	First appearance of rash	Physician's diagnosis	Date of onset	Serum collected	Weil-Felix titer	Complement-fixation titer	Remarks
16. MM	F	5	W	Va.	Ticks	Chill, fever, aching	Extremities	RMSF	June 17, 1941	10 days	1:160	1:8	
17. Mrs. AV	F	..	W	Ky.			Extremities	Typhus or RMSF	June 1941	6–9–41	1:2560	0	
18. Mrs. JL	F	..	W	Conn.	Ticks?	Malaise, headache, fever	Body June 22	Typhus or RMSF	June 20, 1941 July 7,	1 day 18 days	1:10 1:20	0 0	
19. RH	F	36	W	Md.	Ticks?	Chill, fever, severe aching	Body July 1	RMSF or typhus	June 28, 1941	24 days	1:1280	1:32(2+)	
20. Mrs. S	F	46	W	Ga.	Ticks	Chill, fever, nausea	Wrists and ankles	RMSF	June 30, 1941	8 days	1:320	1:8(2+)	
21. LS	F	42	W	Ga.	Ticks?	Chill, fever, headache	Over entire body—July 9	RMSF or typhus	July 7, 1941	16 days	1:2560	0	
22. DP	M	4	W	Ga.	Ticks	Typical symptoms of RMSF	Extremities	RMSF	July 11, 1941	10 days	1:80	0	
23. Mr. P	M	35	W	Ga.	Ticks	Headache, fever, body pains	Extremities July 13	RMSF	July 12, 1941	5 days	0	1:4	
24. MB	F	28	W	Md.				RMSF	July	7–22–41	1:10240	1:32(2+)	
25. LR	F	69	W	Ky.	Ticks	Fever, sweats, delirium	Ankles	RMSF	July 13, 1941	11 days	1:160	0	Fatal case
26. DM	F	10	W	Pa.	Ticks	Fever, pain	Wrists and ankles	RMSF	July 20, 1941	24 days	1:1280	0	
27. OM	M	34	W	N. Y. Mont.	Ticks	Chill, fever, aching	No rash	RMSF	July 21, 1941	14 days	1:20	0	Had had course of vaccine
28. JS	M	28	W	Va.	Ticks	Fever, prostration	Characteristic rash. 4th day	RMSF	July 24	21 days	1:20	0	
29. WW 29a. "	M	19	C	Ky.	Ticks	Fever, aching	Body Aug. 6	RMSF	Aug. 2, 1941	13 days 25 "	1:320 1:640	0 1:4	
30. NN	M	36	W	W. Va.		Chill, fever	Generalized rash. 1 week	RMSF or typhus	Aug. 6, 1941	7 days	1:40	0	

* Immunity tests by Dr. George D. Brigham, Typhus Research Laboraotry, Savannah, Ga.

results would be obtained in the spotted fever cases.

Considering the endemic typhus cases (Table 2), it was found that all of these gave positive results in comparatively high dilutions, none being lower than 1:128 and several as high as 1:4,096 and 1:8,192. Among the active cases, the shortest time recorded between the date of onset and the date of obtaining the serum was 9 days, and in this case the complement-fixation titer was 1:256. One of the highest titers recorded, 1:8,192, was on the 16th day. All of the results obtained in these tests were definite and clear-cut, and usually the titer dropped sharply from positive to negative. In those cases having a high complement-fixation titer the Weil-Felix titers were usually correspondingly high. Also in the 3 cases of past infection the results with the complement-fixation test were definite, the titers ranging from 1:256 to 1:1,024.

The majority of these cases were adults living in the eastern or southeastern sections of the country and several gave a history of contact with rats. The rash in most cases was typical of endemic typhus, occurring first on the body.

The 30 cases of Rocky Mountain spotted fever (Table 3) in contrast to the endemic typhus fever gave negative results for the most part in tests against endemic typhus antigen, though positive Weil-Felix agglutination titers were obtained in fairly high dilutions in a number of cases. All of the 10 cases of past infection which occurred from 8 months to 2 years prior to the test gave negative results in the complement-fixation test.

The active cases of Rocky Mountain spotted fever date from April 23, 1941. Eighteen specimens from 14 cases were all completely negative against endemic typhus antigen (Table 4).

The sera from 8 cases showed some cross-fixation with typhus antigen (Table 5). In probably none of the above cases was fixation present in a high enough titer to be considered significant, with the exception of the one case, K.T., in which complete fixation was obtained in the dilution of 1:256

TABLE 4

Complement-fixation in Rocky Mountain Spotted Fever (Typhus Antigen)
No Cross-Fixation

Case	*Days after onset*	*Weil-Felix titer*	*Complement-fixation titer*
11. LD	9	1:640	0
13. MH (1st specimen)	5	0	0
(2nd specimen)	7	0	0
14. FR (1st specimen)	6	1:1280	0
(2nd specimen)	12	1:5120	0
(3rd specimen)	58	1:80	0
15. KT (2nd specimen)	54	1:1280	0
17. Mrs. AV	..	1:2560	0
18. Mrs. JL (1st specimen)	1	1:10	0
(2nd specimen)	17	1:20	0
21. Mrs. LS	16	1:2560	0
22. DP	10	1:80	0
25. Mrs. LR	11	1:160	0
26. DM	24	1:1280	0
27. OM (2nd specimen)	14	1:20	0
28. JS	21	1:20	0
29. WW (1st specimen)	13	1:320	0
30. NN	7	1:40	0

TABLE 5

Complement-fixation in Rocky Mountain Spotted Fever (Typhus Antigen)

Cross-Fixation

Case	Days after onset	Weil-Felix titer	Complement-fixxation Titer 1:2	1:4	1:8	1:16	1:32	1:64	1:128	1:256	1:512	1:1024	1:2048	1:4096	1:8192	1:16384	1:32768
12. JR	13	....	2	2	2	2	0	0									
15. KT	14	1:20480	4	4	4	4	4	4	4	4	3	2	0				
16. MM	20	1:160	4	3	2	0	0	0									
19. RH	24	1:1280	3	3	2	2	2	0									
20. Mrs. S	8	1:320	2	2	1	1	0	0									
23. MB	..	1:10240	3	2	2	2	2	1									
24. Mr. P	5	0	3	3	1	0	0	0									
29. WW	25	1:640	4	3	0	0	0	0									
Typhus Controls																	
15. WG	20	1:10240	4	4	4	4	4	4	4	4	4	4	4	4	4		
16. WD	..	1:320	4	4	4	4	4	4	4	4	4	4	4	4	4	4	3

and partial in the 1:512 dilution. This case was a 3 year old child from whom ticks had been removed and who had had a rash typical of Rocky Mountain spotted fever. This specimen was taken on the 14th day, at which time the Weil-Felix titer was 1:20,480. A second specimen taken on the 54th day of illness was completely negative for typhus by the complement-fixation test, while it had a positive Weil-Felix in the 1:1,280 dilution. The high Weil-Felix titer may bear some relationship to the high cross-fixation titer or the possibility may be considered that this case may have suffered a previous typhus infection, since the locality was one in which endemic typhus prevails.

In repeated tests on the above specimens in which some cross-fixation occurred, it was found that fixation could be reduced by using a more dilute antigen, titration of the antigen being made against known typhus sera in order to insure that dilution was not carried past the point where positive results would be obtained. It was thus found possible to dilute some of the antigens as much as 1:32.

COMMENT

The question of the best method for the early diagnosis and the differentiation between typhus and Rocky Mountain spotted fever is one which cannot be answered completely at the present time. The Weil-Felix test has proved very useful as a diagnostic test for certain rickettsial diseases without differentiating between them. The complement-fixation test for endemic typhus is positive in sufficiently high dilutions in general to exclude Rocky Mountain spotted fever. In some cases positive results were obtained in dilutions of 1:256 on the 9th or 10th day after onset. There may occasionally be some confusion between early cases of typhus or those of typhus in which complement-fixing antibodies develop slowly (see Figure 2), and cases of Rocky Mountain spotted fever which are more advanced or in which there is a rapid development of antibodies. However, this subject can be more adequately studied when studies similar to these reported are made with spotted fever antigens.

SUMMARY

The complement-fixation test for endemic typhus is of value in the detection of active or past infection. Titers of 1:128 and 1:256 may be reached on the 9th or 10th day of illness and 1:4,096 and 1:8,192 on the 14th or 15th day.

The complement-fixation reaction is a better criterion of past infection with endemic typhus than is the Weil-Felix

test as complement-fixing antibodies may be present in significant dilutions up to 5 or more years after the illness.

The complement-fixation test may probably be used to differentiate between endemic typhus and Rocky Mountain spotted fevers. Spotted fever sera tested against a typhus antigen as a rule give a negative reaction, while at the same time a positive Weil-Felix reaction may be obtained in quite high dilutions of serum. Occasionally there may be some cross-fixation of typhus antigen by spotted fever sera, but usually in low dilutions. Tests similar to those reported in which a spotted fever antigen is used may elucidate this phase of the problem.

REFERENCES

1. Davis, Benjamin F., and Petersen, William F. Complement Deviation in Rocky Mountain Spotted Fever. *J. Infec. Dis.* 8:330–338, 1911.

2. Cathoire, E. Recherche de la déviation du complément dans la typhus exanthématique. *Compt. rend. Soc. de biol.*, 69:117–118, 1910.

3. Müller, Paul Th. Bakteriologische Untersuchungen bei Flecktyphus. *Arch. f. Hyg.*, Bd. 81:307–332, 1913.

4. Markl. Beitrag zur serologischen Diagnose des Flecktyphus. *Wein. klin. Wchnschr.* 26:1234, 1913.

5. Deltá, Constantin. Sur la réaction de Wassermann dans le Typhus exanthematique. *Centralbl. f. Bakt.*, Abt. I, Orig., 76:50–54, 1915.

6. Papamarku, P. Beiträge zur Serodiagnostik des Fleckfiebers. *Centralbl. f. Bakt.*, I. Abt. Orig., 77: 186–197, 1915–16.

7. Papamarku, P. Weitere Beiträge zur Komplement-bindungsreaktion beim Fleckfieber. *Berl. klin. Wchnsch.* 54:649–651, 1917.

8. Jacobsthal, E. Eine Anregung zur Anstellung von Kutisreaktionen bei Fleckfieber. *Deutsche. med. Wchnschr.*, 42:1094, 1916.

9. Jacobsthal, E. Zur Komplement-bindungsreaktion zwischen Fleckfieber Läuseextrakt und Fleckfieberserum. *.Berl. klin. Wchnschr.*, 54:1028, 1917.

10. Epstein, H. Beiträge zur Kenntnis der Rickettsia prowazeki. *Centralbl. f. Bakt.* I. Abt. Orig., 87:553–556, 1922.

11. Cox, H. E. Use of Yolk Sac of Developing Chick Embryo as Medium for Growing Rickettsiae of Rocky Mountain Spotted Fever and Typhus Groups. *Pub. Health Rep.*, 53:2241–2247, 1938.

12. Castaneda, M. R. Experimental Pneumonia Produced by Typhus Rickettsiae. *Am. J. Path.*, 15: 467–475, 1939.

13. Zinsser, Hans, Fitzpatrick, Florence, and Wei, Hsi. A Study of Rickettsiae Grown on Agar Tissue Cultures. *J. Exper. Med.*, 69:179–190, 1939.

14. Burnet, F. M., and Freeman, Mavis. Experimental Studies on the Virus of "Q" Fever. *M. J. Australia*, 24(2):299–315, 1937.

15. Castaneda, M. R. Studies on the Mechanism of Immunity in Typhus Fever. *J. Immunol.*, 31: 285–291, 1936.

16. Bengston, Ida A. Complement Fixation in "Q" Fever. *Proc. Soc. Exper. Biol. & Med.*, 46:665–668, 1941.

17. Bengtson, Ida A. Complement-fixation in Endemic Typhus Fever. *Pub. Health Rep.*, 56:649–653, 1941.

18. Badger, L. F. The Laboratory Diagnosis of Endemic Typhus and Rocky Mountain Spotted Fever with Special Reference to Cross-Immunity Tests. *Am. J. Trop. Med.*, 13:179–190, 1933.

19. Dyer, R. E. The Rickettsioses of North America. *Tr. & Studies College Phys. of Phil.*, 4 ser. 7:232–259, 1939.