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Studies On Q Fever: Persistence of Complement-Fixing Antibodies After Naturally Acquired Infection.*

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Most of the information available with regard to the persistence of complement-fixing, agglutinating, or neutralizing antibodies in Q fever has come from the study of infections acquired accidentally by laboratory workers. Bengtson[1] demonstrated the persistence of complement-fixing antibodies in the sera of 2 laboratory workers 10 months after infection with Q fever. Huebner[2] reported that 14 individuals infected with Q fever in 1940 at the laboratory of the National Institute of Health escaped infection in the 1945-1946 outbreak. However, some of these individuals presumably were exposed to the agent in the laboratory at various times between the 1940 and 1946 outbreaks, and in addition, some of them may have been immunized with a vaccine which, it is believed, had some protective effect against *R. burneti.* In the Q fever outbreak in the Respiratory Diseases Commission laboratory,[3] rickettsial agglutinin titers tended to decline after 2 months but in some cases were detectable 3 to 4 months after infection.

Little information is available concerning persistence of antibodies following Q fever acquired under natural conditions. Australian workers[4] demonstrated rickettsial agglutinins in the sera of 2 individuals 17 months after naturally acquired Q fever; complement-fixation tests were not performed.

An outbreak of Q fever acquired under natural conditions at Amarillo, Texas, in March, 1946,[6] presented an opportunity to obtain data on the persistence of complement-fixing antibodies against *R. burneti.* It was felt that such data would be of value in the interpretation of the results of serological surveys for Q fever.[5]

The technique of the complement-fixation test, employing as antigen† yolk sacs of embryonated eggs infected with *R. burneti* (American Nine Mile), is described elsewhere.[5] The end point of the test was read as the highest serum dilution showing at least 3+ fixation.

Post-convalescent blood specimens were obtained from a group of 17 persons who were ill with Q fever in March, 1946. The diagnoses were established on the basis of clinical illness compatible with Q fever, isolation of *R. burneti* from the blood of several patients, and a rise in titer during convalescence of complement-fixing or neutralizing antibodies for *R. burneti.*[6] The blood samples obtained 5 to 7 weeks after onset of illness were tested in the laboratories of Dr. Herald R. Cox of Lederle Laboratories Divi-

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TABLE I.
Persistence of Complement-Fixing Antibodies Against *R. burneti* Antigen in Subjects Ill with Naturally-Acquired Q Fever.

Subject	Titer at indicated time after illness		
	5-7 wks	6 mo.*	17 mo.
1	1024	>32	64
2	256	>32	64
3	128	16	32
4	64	—	16
5	+	—	128
6	+	—	16
7	+	—	1024
8	64	32	—
9	256	32	—
10	256	>32	—
11	256	>32	—
12	128	>32	—
13	512	< 8†	—
14	128	>32	—
15	+	>32	—
16	+	32	—
17	+	8	—

Titer recorded as highest serum dilution yielding 3+ or greater fixation.
* Not tested in dilution greater than 1:32.
\+ Positive C.-F. test; titer unknown.
† 8 was lowest serum dilution measured.
— Not tested.

sion, American Cyanamid Company, and Dr. J. V. Irons, Texas State Health Department. Elevated titers of complement-fixing antibodies were present for 17 months after infection, as shown in the accompanying table. In all but 3 subjects tested 6 months after illness, titers of 32 or greater were obtained; in only one subject did the titer fall below 8. Likewise, 17 months after infection, titers of 32 or more were obtained in the serum of 5 of the 7 individuals tested.

All of the complement-fixation tests were performed with antigens of the same strain of *R. burneti* prepared from the infected yolk sacs of embryonated eggs. However, different lots of antigen were used for the tests on the early (5-7 weeks) convalescent specimens and the late or post-convalescent specimens (6 and 17 months). It is recognized that exact comparison cannot be made of titers obtained in different laboratories or even in the same laboratory at different times. Nevertheless, experience has indicated that the general trend of the titers has comparative significance.

Discussion. The demonstration that high titers of complement-fixing antibodies may persist for 1½ years after naturally acquired Q fever is important in both clinical and epidemiological studies of this disease. The clinical diagnosis of Q fever is difficult, particularly in sporadic cases. Diagnosis depends largely on the isolation of the rickettsia or on the results of serological studies. Because antibodies may persist for long periods the results of serological studies made during convalescence from a presumed attack of Q fever may be misinterpreted. As with other serological tests, the demonstration of a rise in antibody titer in convalescent phase as compared with acute phase blood specimens is of greater diagnostic value than a high titer present in a single specimen obtained during convalescence.

Little is known of the natural mode of infection with Q fever in this country. It is, therefore, conceivable that the persistence of elevated titers of antibodies in the subjects tested may be the result of continued contact with the rickettsial agent, subsequent to the initial illness. The unusual high titer in case No. 7, referred to in the table, suggests some such mechanism.

To determine whether or not Q fever may be endemic in the Amarillo area, 175 blood specimens from presumably normal individuals were tested for complement-fixing antibodies. These blood samples were collected for routine serological tests for syphilis and were obtained from the Amarillo City Health Department laboratory. Approximately 8% of these specimens showed complement-fixing antibodies against *R. burneti.* Only 2 individuals, however, had high titers (1:64 or more) and it was subsequently determined that both of these individuals had had Q fever during the epidemic. Clinical histories of previous illness were not available from other subjects who exhibited positive complement-fixation in low titers, and it is not possible, therefore, to assess the significance of these reactions.

Summary. Complement-fixation tests using an antigen of *R. burneti* (American Nine Mile) were performed on sera from a group of persons known to have had Q fever in 1946 in Amarillo, Texas. High titers of com-

plement-fixing antibodies were demonstrated 17 months after illness.

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Effect of Digitoxin On Creatinine and Histamine Output of the Isolated Heart.*

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In the course of some experiments on the isolated hearts of rabbits, cats and rats which were perfused with digitoxin to measure the amounts of fixation of the drug, some unexpected observations were made. In using the modified Baljet reaction[1,2,3] to determine the digitoxin colorimetrically, it was found that the intensity of the color in the perfusate was increased over that produced by the digitoxin in the perfusion fluid. This suggested the possibility that creatinine, which also gives the Baljet reaction, might be present in the perfusate. This was found to be the case. It was also observed that the perfusate contained a substance which caused a drop in the blood pressure of an anesthetized cat and a contraction of the isolated guinea pig intestine suspended in atropinized Tyrode solution. This led to the supposition that the perfusate contained histamine. Various investigators have found that the contracting heart muscle produces histamine;[4,5,6] however, this observation could not be confirmed by other workers.[7] Since the creatinine and histamine output of the heart muscle might be related to its physiological activity, it seemed worth while to perform experiments in which the release of these substances could be correlated with the action of cardiac glycosides.

Experimental. The effect of digitoxin in a final concentration of 1:40,000 was studied on isolated rat, cat, and rabbit heart preparations. The animals were sacrificed by a blow on the head, and the heart was immediately removed and washed in oxygenated Ringer-Locke solution. The hearts were perfused through the coronary arteries by means of a cannula inserted into the aorta. The temperature of the perfusion fluid was kept at 36-39°C. Samples of perfusate were collected at intervals before and after the introduction of digitoxin into the system. Digitoxin was dissolved in 95% alcohol. Five ml of this solution contained 25 mg which diluted to 1000 ml gives a final concentration of 1:40,000. Control measurements showed that this amount of alcohol did not affect the motility of the heart and did not influence the creatinine and histamine output. The volume of fluid perfused per unit of tissue was not affected by digitoxin. In the case

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