2-Mercaptoethanol Brucella Agglutination Test: Usefulness for Predicting Recovery from Brucellosis†

THOMAS M. BUCHANAN¹* AND LUKE C. FABER²‡

*Immunology Research Laboratory, U.S. Public Health Service Hospital, Seattle, Washington, 98114¹;
Departments of Medicine and Pathobiology, University of Washington, Seattle, Washington 98195²*

Ninety-two patients with brucellosis were studied for 18 months, and 15 to 29 sera from each patient were tested by the standard tube brucella agglutination test and by the 2-mercaptoethanol (2ME) brucella agglutination test. The standard tube test remained positive (≥ 160) for 1.5 years in 44 of the 92 patients (48%), despite adequate antibiotic treatment. The 2ME titers remained positive (≥ 160) in only 8 of 92 patients (9%) after 1 year, and in only 4 of 92 patients (4%) after 1.5 years. Positive 2ME titers 1 year after initiation of treatment were present in 3 of 10 patients (30%) with drug allergies that interfered with antibiotic treatment, in contrast to only 5 of 82 patients (6%) without any drug allergies ($P = 0.039$). None of 84 patients with negative 2ME titers after 12 months of treatment had significant signs or symptoms of brucellosis, and none developed chronic brucellosis. In contrast, four of eight patients with positive 2ME titers after 12 months of treatment still had signs and symptoms of brucellosis and required further treatment. The 2ME test is superior to the standard tube test in determining the adequacy of antibiotic therapy, and a negative 2ME test is strong evidence against a diagnosis of chronic brucellosis.

The 2-mercaptoethanol (2ME) brucella agglutination test is performed identically to the standard tube brucella agglutination test (STT test) except for the addition of 2ME to a final concentration of 0.05 M in each agglutination tube. The 2ME disrupts disulfide bonds, making immunoglobulin M antibodies inactive and permitting only brucella agglutination by immunoglobulin G agglutinating antibodies that are resistant to 2ME. Anderson et al. (1), Reddin et al. (5), and Buchanan et al. (3) have each observed that the 2ME test is a better indicator of recent infection than the STT, and Spink (6) has suggested that a positive 2ME test is evidence of an active infection and the need for antibiotic therapy. Each of the previous studies was conducted with few patients, and the 2ME serological responses were evaluated with few sera collected over relatively short time periods. This study is a more extensive evaluation of the 2ME test. From 15 to 25 sera were collected from each of 92 patients with brucellosis during 18 months after their diagnosis and initiation of therapy. Each serum specimen was tested by the STT and 2ME tests. The results indicate that the 2ME test is superior to the STT in evaluating the effectiveness of treatment and as a means to rule out a diagnosis of chronic brucellosis.

MATERIALS AND METHODS

Serum specimens. From 15 to 29 sera (median 20) were collected from each of 92 patients with brucellosis. These patients were all employees of a large slaughterhouse located in Iowa. Each patient had compatible signs and symptoms (2), plus seroconversion ($\geq$ fourfold change in brucella agglutination titer) or positive blood cultures (19 patients: 17 *Brucella abortus*, 2 *Brucella suis*) or both, as evidence of his brucellosis. Sera were generally collected biweekly from each patient during the first 4 to 6 months after diagnosis and initiation of treatment, monthly thereafter until 1 year after infection, and every 2 to 4 months from then on. Serum specimens were collected from each patient for at least 18 months, and from some patients for as long as 4 years.

Serological tests. The STT test was performed by the method of Hausler and Koontz (4) using a standardized suspension of brucella organisms prepared from *B. abortus* 1119 (7). The 2ME test was performed identically except that 2ME was added to each test tube to a final concentration of 0.05 M, and 0.85% saline was used to dilute the antigen rather than 0.85% saline containing 0.5% phenol. Briefly, the tests were conducted as follows.

(i) A 0.9-ml volume of 0.85% saline (containing 0.1 M 2ME for the 2ME test, or 0.5% phenol for the STT test) was added to a test tube (12 by 75 mm), and 0.5 ml of the same solution was added to eight more tubes of the same size. Also, 0.5 ml of the same solution was added to an antigen control tube, and 0.75 ml was added to a reading standard tube.

(ii) Twofold serum dilutions beginning with 1:10 were formed by adding 0.1 ml of serum to tube 1, followed by sequential mixing and removal of 0.5 ml

† Article no. 33 from the Immunology Research Laboratory, U.S. Public Health Service Hospital, Seattle, Wash.

‡ Present address: Medical Department, Dubuque Packing Co., Dubuque, IA 52001.

of the mixture to the subsequent tube. The final dilution of tube 9 was 1:2,560, and 0.5 ml of this tube was discarded. The antigen suspension was added in 0.5-ml amounts to tubes 1 through 9 and to the antigen standard tube, and 0.25 ml of this suspension was added to the reading standard tube. The reading standard tube was used to simulate 50% clearing of the antigen suspension after the agglutination reaction. The final serum dilutions in tubes 1 to 9 were 1:20 through 1:5,120.

(iii) The rack containing the test tubes was shaken 10 times to mix the antigen suspension and serum dilutions and was incubated in a water bath at 37°C for 44 to 48 h.

(iv) A fluorescent light and a black background were used to examine the tubes for agglutination. After 44 to 48 h, each tube was examined without mixing or centrifugation, and was read as 4+ if all organisms in the brucella antigen suspension were agglutinated and the supernatant was clear. A 3+ reading equaled 75% agglutination of the organisms, and a slightly cloudy supernatant. A 2+ reading indicated that 50% of the organisms were agglutinated and the supernatant was of equal density to the reading standard tube. A 1+ reading indicated agglutination of 25% of the organisms and a supernatant that was slightly less dense than the antigen control tube. A 0 reading indicated no organism agglutination and a supernatant density equal to the antigen standard.

(v) The endpoint was the highest dilution of serum producing a 2+ reading. The serum titer was the reciprocal of the serum dilution. The serum titer found in previous studies to be significant evidence of exposure to brucella antigens was ≥ 160 for both the STT and 2ME tests (3).

RESULTS AND DISCUSSION

The STT test became positive earlier than the 2ME test and was a more sensitive indicator of brucellosis in the first week of the illness. Serum specimens were obtained from 53 of the patients during their first week of illness. All of these specimens had elevated brucella-agglutinating antibodies as measured by the STT test, whereas 24 (45%) gave negative 2ME results. This is consistent with the slower development of immunoglobulin G brucella-agglutinating antibodies, as has been reported previously (3). Most of the patients initially negative for 2ME-resistant antibodies developed positive titers (≥ 160) within 1 to 3 weeks, and the failure to do so in some patients presumably reflected early initiation of effective antibiotic treatment. Treatment for all of the patients in this study, unless complicated by allergies, was streptomycin, given 1 g daily intramuscularly for 2 weeks, accompanied by tetracycline, 500 mg orally four times daily for a minimum of 3 months. This therapy, when initiated within 1 month of the onset of illness, has been shown to prevent complications or chronic brucellosis in this patient

population, and has been associated with a relapse rate of 3% or less (2).

The titers remained positive in the STT test much longer than in the 2ME test, and nearly half (44/92, 48%) had STT titers of ≥ 160 1.5 years after treatment was begun at the onset of their illness. In contrast, the 2ME agglutination test was much more discriminatory, and the number of patients with titers of ≥ 160 at 6, 9, 12, and 18 months, after onset of illness was 22 (24%), 12 (13%), 8 (9%), and 4 (4%), respectively (Table 1). A fall in the 2ME titer reflected a satisfactory response to antibiotic therapy. As shown in Table 1, more than 90% of the patients had 2ME titers less than 160 within 1 year, and these patients had no further sequelae and remained well during continued followup. Of the eight patients with titers of ≥ 160 after 1 year, four had continuing significant signs and symptoms of brucellosis, and these four patients still had positive (≥ 160) 2ME titers at 18 months after the onset of their illness. One of these patients developed complications and chronic brucellosis; this may have resulted in part from multiple drug allergies that prevented him from receiving adequate antibiotic therapy. The four less symptomatic patients, who had 2ME titers of ≥ 160 at 12 months but no signs of brucellosis, all had negative 2ME titers (≤ 80) by 18 months after the onset of their illnesses. One of these patients never received treatment, and a second patient had allergic complications that prevented him from receiving optimal antibiotic therapy. The alterations in treatment may have contributed to the slower decline in 2ME titers observed for these patients. Ten of the 92 patients developed allergic reactions that interfered with their antibiotic treatment, and 3 (30%) of these had positive 2ME titers (≥ 160) at 12 months. Two of these three still had significant signs and symptoms of brucellosis at 12 months. The 2ME titers of ≥ 160 at 12 months in 30% of patients with antibiotic allergies was significantly higher than the 6% (5/82) rate for the patients without antibiotic allergies ($P = 0.039$). Two patients experienced relapses, one 6 months

TABLE 1. Number of patients by titers and by time after onset of brucellosis

Titer	No. of patients							
	STT				2ME			
	6 mo ^a	9 mo	12 mo	18 mo	6 mo	9 mo	12 mo	18 mo
≥ 160	75	62	55	44	22	12	8	4
80	15	21	21	22	24	17	12	5
40	2	8	13	18	21	21	20	22
20	0	1	3	8	25	42	52	61

^a Time after onset of illness.

and the other 1 year after the initiation of therapy. Associated with the renewed signs and symptoms of brucellosis, the first patient showed a rise in 2ME titer from 160 to 320, and the 2ME titer of the second rose from 80 to 1,280. These data support the validity of the 2ME test as a monitor of adequate antibiotic therapy.

In summary, the STT test was more sensitive than the 2ME test and is indicated for initial screening of patients with signs and symptoms of acute brucellosis. However, in patients with a more insidious onset, or symptoms lasting 3 or more weeks, the 2ME test was more useful. A positive 2ME brucella agglutination titer was a better correlate of brucella infection requiring treatment than a positive STT titer, which persisted for 1.5 years in approximately one-half of our patients, despite adequate antibiotic therapy. The 2ME test also proved useful to monitor antibiotic therapy, and titers of ≥ 160 were present in only 9% of patients 1 year after initiation of treatment. The patients with negative (≤ 80) 2ME titers 12 months after the onset of illness were without signs or symptoms of brucellosis and continued healthy without any further antibiotic therapy. In contrast, half of the eight patients with positive 2ME titers 1 year after the initiation of treatment still had significant signs and symptoms of brucellosis and required further antibiotic treatment. Thus, a negative (≤ 80) 2ME titer is strong evidence against chronic brucellosis. It indicates a favorable response to antibiotic therapy and that no further antibiotic treatment is required.

ACKNOWLEDGMENTS

We thank Sally Palmer and Elizabeth Hesseling for their interactions with the brucellosis patients to obtain clinical histories and specimens, and while administering treatment; and Audra Bergman for performing the agglutination tests.

LITERATURE CITED

1. Anderson, R. K., R. Jenness, H. P. Brumfield, and P. Gough. 1964. Brucella agglutinating antibodies: relation of mercaptoethanol stability to complement fixation. *Science* 143:1334-1335.
2. Buchanan, T. M., L. C. Faber, and R. A. Feldman. 1974. Brucellosis in the United States, 1960-1972: an abattoir-associated disease. I. Clinical features and therapy. *Medicine* 53:403-414.
3. Buchanan, T. M., C. R. Sulzer, M. K. Frix, and R. A. Feldman. 1974. Brucellosis in the United States, 1960-1972: an abattoir-associated disease. II. Diagnostic aspects. *Medicine* 53:415-426.
4. Hausler, W. J., Jr., and F. P. Koontz. 1970. Brucellosis, p. 374-375. In H. L. Bodily, E. L. Updyke, and J. O. Mason (ed.), *Diagnostic procedures for bacterial, mycotic, and parasitic infections*, 5th ed. American Public Health Association, New York.
5. Reddin, L., R. K. Anderson, R. Jenness, and W. W. Spink. 1965. Significance of 7S and macroglobulin brucella agglutinins in human brucellosis. *N. Engl. J. Med.* 272:1263-1267.
6. Spink, W. W. 1977. Brucellosis, p. 856-858. In G. W. Thorn, R. D. Adams, E. Braunwald, K. J. Isselbacher, and R. G. Petersdorf (ed.), *Harrison's principles of internal medicine*, 8th ed. McGraw-Hill, New York.
7. Spink, W. W., N. B. McCullough, L. M. Hutchings, and C. K. Mingle. 1954. A standardized antigen and agglutination technique for human brucellosis. Report no. 3 of the National Research Council, Committee on Public Health Aspects of Brucellosis. *Am. J. Public Health* 24:496-498.