ALEXIN FIXATION AND AGGLUTINATION TEST IN BRUCELLA INFECTIONS

By P. MORALES OTERO and G. MONGE
From the Department of Bacteriology of the School of Tropical Medicine of the University of Puerto Rico under the auspices of Columbia University

Alexin fixation has been employed in the diagnosis of undulant fever as alternative to, or confirmatory of, the agglutination reaction. It has also been used in the detection of *Brucella abortus* infection in cattle. The value of the alexin fixation test for the diagnosis of *Brucella abortus* infection in animals was indicated by Holth [2]. In human beings, Larson and Sedgwick [3] reported that 17 per cent of 435 children examined gave positive alexin fixation tests for *Brucella abortus,* and that this reaction ran parallel with agglutination. Moore [6] reports that the alexin fixation test is considered equally as good as the agglutination test, and more satisfactory by some. Zeissig and Mansfield [8] in their comparative studies of agglutination and alexin fixation for the detection of abortus infection in cattle, conclude that both tests agree very closely in regard to the status of the animal under test, and of the two, alexin fixation seems to be the more accurate in classifying the animals as reactors and nonreactors.

Carpenter and Boak [1] are of the opinion that the alexin fixation test has no distinct advantage over the agglutination test. They claim it to be more complicated, and often sera are found to be anticomplementary, though these same sera are still satisfactory for the agglutination reaction.

Sasano, Caldwell and Medlar [7] examined the serums of 1,000 persons; 96 showed positive complement fixation and 78 positive agglutination reaction. In only five cases was the diagnosis of undulant fever made and in each of these five cases both tests were positive at some time during the illness. They also found that alexin fixing substances and agglutinins developed at about the same time and that the alexin fixing substances persisted longer than the agglutinins.

Various antigens have been used for the alexin fixation

test; the most suitable reported are carbolized and centrifuged autolysates of the organisms (Mohler and Traum [5]); extracts obtained by shaking and heat killed cultures, and centrifuging or filtering (Mohler and Eichhorn [4]) solutions in isotonic saline of alcoholic precipitates of filtrates of autolized cultures (Holth [2]) or fresh heat, killed suspensions of the organisms.

Several methods were used by us in endeavoring to isolate a fraction of the organisms which would react as a good antigen. Those portions of the organisms soluble or insoluble in acetic acid, trichloracetic acid, hydrochloric acid, picric acid, magnesium sulphate, barium hydroxide, and sodium carbonate were tested for antigenic qualities, and that portion soluble in sodium carbonate was found most satisfactory.

PREPARATION OF THE ANTIGEN

The organism was grown for 48 hours on plain agar, in Blake bottles. At the end of this period the growth was washed off with 8 cc. of normal saline and the suspension centrifuged at high speed. The supernatant saline was discarded. To every cubic centimeter of the packed cells, 10 cc. of ether were added. The mixture was shaken vigorously and allowed to stand for 48 hours, with occasional shaking. The ether was then decanted and evaporated in an evaporating dish. A yellowish wax-like substance, soluble in ether and alcohol and slightly soluble in ammonium linolyate remained. This substance was found to be of very little antigenic value.

To the ether insoluble residue 30 cc. of a 2 per cent solution of sodium carbonate was added and well shaken. A gelatinous mass was formed which on standing for 2 hours, partly went into solution. The mixture was filtered. The precipitate left was a gelatinous substance soluble in water. The filtrate was an opalescent solution. The opalescent solution was treated with 2 volumes of 95 per cent ethyl alcohol and a flaky precipitate was formed which gradually sank to the bottom. The solution was centrifuged and the supernatant fluid discarded, the precipitate redissolved in 2 per cent sodium carbonate solution and reprecipitated with 95 per cent ethyl alcohol. After a third treatment the

precipitate was emulsified in Coca's solution using 10 cc. of this solution for every 1 cc. of the original organism.

This solution was found to have very good antigenic properties.

The fraction soluble in water was also tested for its antigenic properties; it fixed alexin in the presence of abortus antisera very slightly, and had a tendency to give anti-complementary reactions.

SPECIFICITY OF THE ANTIGEN

After the routine preliminary titration of the antigen for its anticomplementary, antigenic and hemolytic values, we proceeded to test for specificity.

The technic used was essentially the same as that used in the diagnosis of syphilis. Several tests were made using the following sera: normal horse serum; antipneumococcus type I; antipneumococcus type II; antipnumococcus type III; antidysenteric polyvalent; normal rabbit serum; normal cattle serum; antimeningococcus serum; antityphoid serum; antiparatyphoid A serum; antiparatyphoid B serum; anti-abortus serum. In all instances the reaction was negative except in the case of the antiabortus serum which gave strong fixation.

IMMUNE BODIES IN INFECTED RABBITS

A group of four rabbits was inoculated intravenously with living suspensions of *Brucella abortus*. A first dose of .5 cc. of a suspension matching No. 3 nephrometer reading was given. On the third day a second dose of 1 cc. of the same suspension, and on the fifth day, a third dose of 1 cc. The animals were bled every two days throughout the experiment, and their serums were studied for both agglutination and alexin fixation. We found that agglutinins and alexin fixing substances appeared in the blood very early. Agglutinins could be demonstrated the third day after the first injection and alexin fixing bodies on the sixth day. Agglutinins increased rapidly reaching a high point on the ninth day after the first inoculation and remaining more or less at the same titer for a period of eight to ten days when they began to decline slowly. Alexin fixing bodies increased less rapidly, reaching the highest concentration on the twenty-ninth day

and remaining very high for eight to ten days when they began to decline. Sixty days after the first inoculation, agglutinins and alexin fixing bodies were still demonstrable in the serums of the inoculated animals. Composite curves of the agglutinins and complement fixing substances in the group of rabbits studied are shown in Charts I and II.

CHART I

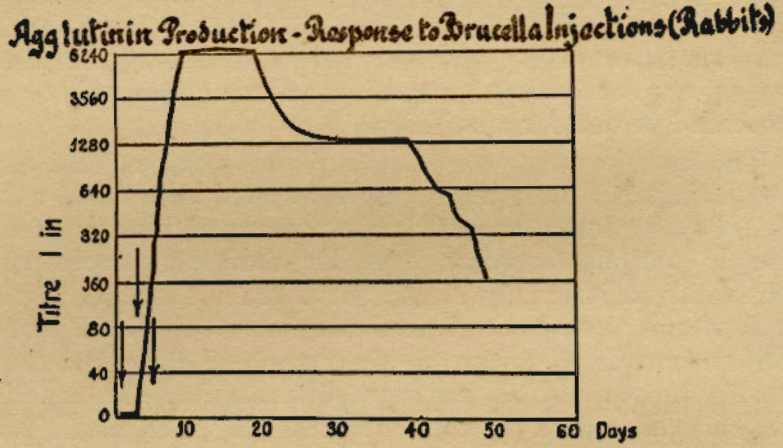
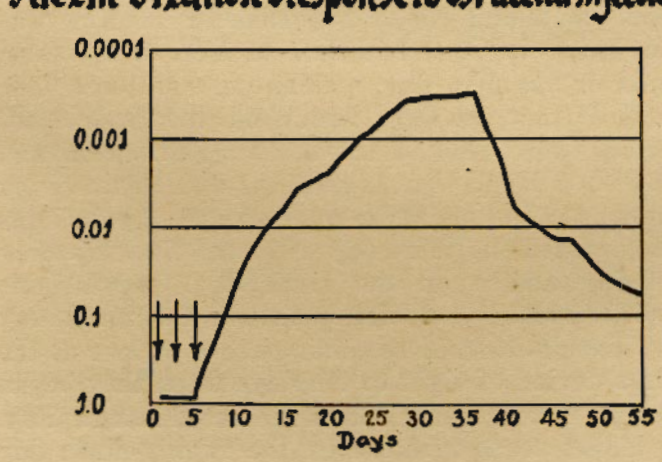


CHART II



COMPARISON OF ALEXIN FIXATION AND AGGLUTINATION TEST IN COW'S SERUM

The serums of two hundred and fifty cows were tested for both agglutination and alexin fixation. Of the two hundred and fifty serums, eight gave positive alexin fixation reaction (+ + + +), six gave positive agglutination reaction. Of the two cows whose serums fixed alexin but did not agglutinate, one had a definite history of abortus, and the other while having no history of abortion, had a history of retained placenta.

A summary of the experiment with cows' serums is given in Table I.

TABLE I

RESULTS OF TEST IN THE SERUM OF 250 COWS

Total number of serums examined	250
Positive complement fixation (+ + +) (+ + + +)	8
Positive agglutination 1/40 or higher	6
Negative to complement fixation	242
Negative agglutination	244
Agreement	248

Agglutinations lower than 1/40 and complement fixations lower than (+ +) have been considered as negative. Two serums gave strong complement fixation test without agglutination.

COMPARISON OF ALEXIN FIXATION AND AGGLUTINATION IN HUMAN SERUM

The serums of 225 patients from a general hospital were examined for both agglutination and alexin fixation. The results are summarized in Table II.

TABLE II

Diagnosis	No. of cases examined	Positive		Negative
		Agg.	Comp. fix.	
Malnutrition	6	0	0	6
Lues	53	1	0	52
Gonohrrea	15	0	0	15
Malignancy	10	0	0	10
Pregnancy	17	0	0	17
Abortion	10	0	0	10
Tonsilitis	13	1	0	12
Adenitis	7	0	0	7
Malaria	10	0	0	10
Endocarditis	5	0	0	5
Hookworm	17	0	0	17
Tuberculosis	8	0	0	8
Arthritis	22	1	0	21
Nephritis	14	0	0	14
Diabetes	5	0	0	5
Pneumonia	3	0	0	3
Tetanus	1	0	0	1
Hodgkin's disease	1	0	0	1
Acne	1	0	0	1
Dermatitis	5	0	0	5
Typhoid	2	0	0	2
TOTAL	225	3	0	222

Three cases in the whole group gave positive agglutination while none were positive to the alexin fixation reaction. The agglutination reactions were very weak, two having respectively a titer of 1 to 40 and a titer of 1 to 20. None of the cases giving positive reactions had a history of undulant fever.

SUMMARY

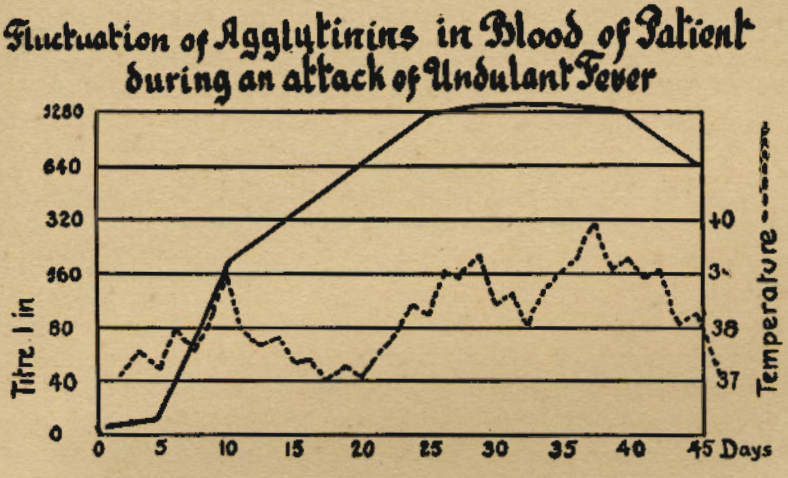
It was found that the portion of the Brucella organisms soluble in Na_2CO_3 precipitated by alcohol and resuspended in Coca's solution reacted as a suitable antigen. When this fraction was inoculated into rabbits, definite agglutinin response was produced in three days.

The development and persistence of immune substances in rabbits injected intravenously with living cultures of *Brucella abortus* were studied. Agglutinins and alexin fixing substances were produced when small doses of living organisms were injected. These immune substances were demonstrable about the third day after injection. With three injections on alternate days agglutinins increased rapidly, reaching their highest titer from the 10th to the 20th day,

and then slowly decreased as shown in Chart I. In the case of alexin fixing substances the increase was not as rapid and the height of the curve occurred around the 25th day, when the smallest unit of rabbit antisera that fixed the antigen was .0006 cc. The titer remained high for ten days, and then declined very slowly (see Chart II).

For comparison, the fluctuations of agglutinins in the blood of a patient during an attack of undulant fever are shown in Chart III. This case was due to a porcine strain and recovered after a long illness of forty-seven days. There is no relation between temperature and agglutinin curve.

CHART III



In testing different rabbit antisera with the abortus antigen described, no false reactions were recorded.

In testing cattle serum a disagreement of .8 per cent was found between agglutinin and alexin fixation test.

The examination of 225 serums from patients in a general hospital gave three positive agglutination reactions, while the alexin fixation was negative in all cases.

CONCLUSIONS

For general use the alexin fixation test has no marked advantage over the more widely used agglutination. It is,

however, reliable, and would be of great value as a confirmatory measure in cases in which the laboratory report conflicts with the clinical evidence.

REFERENCES

1. CARPENTER and BOAK (1930). J. Lab. & Clin. Med. **15**: 437.
2. HOLTH (1909). Bul. Tierartl. Wchnschr. **25**: 286.
3. LARSON and SEDGWICK (1913). Am. J. Dis. Child. **6**: 326.
4. MOHLER and EICHHORN (1911). Rep. U. S. Bur. Anim. Ind. No. 28 p. 119.
5. MOHLER and TRAUM (1911). Rep. U. S. Bur. Anim. Ind. No. 28 p. 147.
6. MOORE (1929). J. Am. Vet. M. A. **74**: 614.
7. SASANO, CALDWELL and MEDLAR (1931). J. of Infect. Dis. **48**: 577.
8. ZEISSIG and MANSFIELD (1930). J. Am. Vet. M. A. **29**: 211.