first determined in a control group of guinea pigs. It was found that approximately 5 times this amount was required to kill all animals previously treated with 3 mg/kg of Benadryl, while 35 times the lethal dose of histamine was necessary to produce 100% mortality in animals receiving 3 mg/kg of Pyribenzamine. No apparent difference was discernible between the 2 drugs in preventing anaphylaxis in passively sensitized guinea pigs. One mg/kg of either compound gave significant protection against a shocking dose of antigen, while 3.0 mg/kg prevented fatal anaphylaxis in all animals tested.

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Bed-side Agglutination Test with Whole Blood for Rapid Diagnosis of Tularemia.*

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The agglutination test has been the most practical method for the laboratory diagnosis of tularemia. McCoy and Chapin[1] showed the presence of agglutinins in the serum of patients infected with *B. tularense* and Francis[2] applied the agglutination test to the serological diagnosis of the infection. Recently Damond and Johnson[3] described what they call the "shake method" of agglutination by which it is possible to accelerate the reaction and read the results in 3 minutes.

Considering the possibility of a further improvement by using the method recommended by Castaneda and collaborators for typhus fever[4] and brucellosis,[5] we prepared a concentrated antigen conveniently stained and used either with whole blood as a bed-side test or with serum as in the case of the so-called rapid antigens developed by Huddleson[6] and Welch.[7]

Preparation of the Antigen. The strain of *B. tularense* No. 408, obtained by courtesy of Dr. R. R. Parker from the Rocky Mountain Laboratory of Hamilton, Montana, was used for the preparation of the antigen. The culture medium, recently described,[8] consisted briefly in a concentrated liver infusion with cystine, glucose, sodium chloride, peptone and agar, without blood or hemoglobin and distributed in Roux's bottles. Each bottle was inoculated with a concentrated emulsion of *B. tularense* and after 72 hours of incubation at 37°C the organisms were emulsified with isotonic saline containing 10% formaline (40%), filtered through wet cotton and left at ordinary temperature for 72 hours. The emulsion was centrifuged and the supernatant fluid was discarded; the organisms were emulsified in a small amount of isotonic saline. The concentration of the emulsion was standardized in order that one-tenth of antigen diluted with 10 cc of saline gave a turbidity corresponding to No. 3 of McFarland's Nephelometer. When the concentration of the antigen was adequate, enough aqueous solution of methylene blue was added to stain the antigen to a deep blue color. After 24 hours the antigen was centrifuged at high speed and the supernatant fluid was discarded.

* This work was aided by grants from the University of Mexico and Eli Lilly Co. of Indianapolis, Indiana.

1 McCoy, G. W., and Chapin, C. W., *J. Infect. Dis.*, 1912, **10**, 61.

2 Francis, E., *Medicine*, 1928, **7**, 411.

3 Damond, S. R., and Johnson, M. B., *J. Lab. and Clin. Med.*, 1944, **29**, 976.

4 Castaneda, M. R., Silva, R. G., and Monnier, A., *Rev. Med. del Hosp. General*, 1940, **8**, 382.

5 Castaneda, M. R., "Brucelosis," First edition, *Medicina*, 1942.

6 Huddleson, I. F., Tech. Bull. No. 123, Mich. Agric. Exp. Station, 1932.

7 Welch, H., and Stuart, C. A., *J. Lab. and Clin. Med.*, 1936, **21**, 411.

8 Tovar, R. M., *Rev. del Inst. de Salubridad y Enf. Trop.*, 1945, **6**, 181.

The stained organisms were emulsified in a small amount of isotonic sodium citrate solution (1.1%), containing merthiolate to a concentration of 1:5000.

Samples of the concentrated material are diluted to various proportions with isotonic citrate and each dilution tested for specificity and sensitivity using samples of serums with titers previously determined by the tube agglutination method. The test is performed mixing a drop of antigen with a drop of serum in a slide, and the sample giving definite agglutination within one minute with a serum of 1:50 titer is selected as a basis for further titrations. Then the antigen is tested for sensitivity which is made comparing the titer of a serum determined by the standard tube agglutination method and the results of tests performed on a glass slide using the rapid antigen following the method of Huddleson for brucellosis.[6]

The specificity of the antigen has been determined with a few available sera from patients suffering from tularemia, 2 from Mexico and 4 obtained by courtesy of Dr. Parker (Hamilton, Montana); all of them were confirmed cases. We also used sera from guinea pigs and rabbits experimentally infected with a non-virulent *B. tularense* strain. The inactivity of the antigen when mixed with normal sera or with serum from persons suffering from various infections, not including tularemia or brucellosis, was demonstrated performing 1600 comparative tests using the rapid antigen and the tube agglutination method.

When the antigen is found satisfactory it is submitted to tests with whole blood of guinea pigs or rabbits infected with *B. tularense*.

Technic of the test. Bed-side test with whole blood. The agglutination test with whole blood is performed at the bed side, mixing on a clean slide the antigen with blood taken by puncture of the ear or from the finger. The amount of antigen is that carried by a wire loop of 4 mm diameter and that of the blood is what is taken with a 2 mm loop. The slide is moved to and fro and the effect observed in front of a window or a bright light. The positive test appears within one minute, consisting of a definite separation of colors (blue of the antigen and red of the blood) and the immediate formation of clumps of antigen, which because of the rotation of the mixture, have a tendency to be accumulated in the periphery forming a blue ring. In the positive reactions there are different intensities that we called 1_t, 2_t, 3_t and 4_t, according to the time of clumping and size of clumps of antigen; they are related to a low or high agglutinating titer of the blood serum. The rapid reaction with whole blood is positive only in cases in which the agglutinating titer of the serum is above 1:100. In the negative tests there is neither color separation nor clumping of the antigen, the mixture remains homogeneous until drying.

The spontaneous clumping of red blood cells may be confused with a positive result for which it is necessary to perform a new test using the blood serum.

Rapid test with blood serum. The antigen has been satisfactory, used as a screen test, mixing a droplet of antigen with an equal amount of serum. When the test is positive there is an immediate formation of blue clumps of antigen and if it is negative the mixture remains homogeneous until drying. The screen test is positive when the agglutinating titer of the serum is above 1:20. The screen test with serum is particularly useful to pick up suspicious cases when a considerable number of samples is submitted to the laboratory. The positive serums are further tested by the rapid method following the technic of Huddleson and finally by tube agglutination using suitable emulsions of *B. tularense* to determine the agglutinating titer.

Results. The bed-side test with whole blood was performed with known cases of tularemia, suspicious cases detected by previous agglutination tests and blood of animals experimentally infected. The negative controls were normal persons or patients suffering from various diseases. We included a group of 100 cases of brucellosis, which according to Francis and Evans[9] produces a high percentage of cross-agglutination with *B. tularense*. Table I

[9] Francis, E., and Evans, A. C., *Pub. Health Rep.*, 1926, **41**, 1273.

TABLE I.
Bed-side Agglutination Test with Rapid *B. tularense* Antigen and Whole Blood.

No. of cases	History	Positive tests	%
100	Normal adults	0	0
100	Adults suffering from various infectious diseases	0	0
100	Brucellosis patients	10	10
30	Serological reactors to *B. tularense**	30	100
2	Confirmed cases of tularemia	2	100
20	Guinea pigs and rabbits experimentally infected	20	100

* Reactors determined by tube agglutination tests.

summarizes the results of the tests.

It may be seen that in spite of the few positive cases, the results of the test were very significant. The test was repeated many times in each case. The positive tests were clear cut in the 30 persons in whom it was found that the serum had a significant titer of agglutinins for *B. tularense*. In regard to the 10 cases of cross-agglutination found in the group of 100 patients suffering from brucellosis this is not surprising because of the previous findings referred to above. These cases of cross-agglutination can be readily differentiated by means of selective agglutination reported elsewhere.[10]

In the guinea pigs experimentally infected with tularemia the test was positive after the 7th day of inoculation and remaining positive during 7 months until discarded.

All individuals showing a positive bed-side agglutination test with whole blood, including the 10 cases infected with *Br. melitensis*, gave allergic skin reaction when injected by intradermal route with "Tulargen,"[10] an extract obtained by grinding *B. tularense*. The opsonocytophagic test performed with killed *B. tularense* as antigen and blood of the same persons, was positive in all cases.

Summary. A bed-side test for the rapid serological diagnosis of tularemia is described. The test is performed mixing a droplet of a concentrated suspension of *B. tularense* with a droplet of whole blood taken from the ear or from the finger of the patient. The minimum agglutinative titer of the patient's serum must be 1:100 to be detected by the rapid test. The antigen may be used for screen tests with blood serum.

[10] Tovar, R. M., *Medicina*, 1945, **25**, 331.

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Some Pharmacological Properties of the Monoanilide of Aconitic Acid.

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The monoanilide of aconitic acid was prepared by one of us* from the *cis* anhydride according to the method of Nau, *et al.*[1] This compound was one of a series of derivatives of aconitic acid which were being tested as nonaqueous solvents for medicinal agents. The anilide group is attached to one of the carboxyl groups connected to the unsaturated carbon linkage. It was deemed of interest to compare the toxicity and the analgesic and antipyretic actions of this compound with those of acetanilide.

* Emil K. Ventre.

[1] Nau, C. A., Brown, E. B., and Bailey, J. R., *J. A. C. S.*, 1925, **47**, 2596.

Toxicity. Forty rats weighing approxi-