atine, or formaldehyde when added to serum did not affect the sulfhydryl value previously determined.

The enzymes which are associated with anabolic processes, the cathepsins, are known to be activated by sulhydryl compounds. Altered protein synthesis and sulfhydryl content may be related to such intracellular enzyme systems. Huggins, *et al.*,(8) have studied changes in thermal coagulability, in the presence of iodoacetate, as a measure of serum protein alterations in disease. Iodoacetate is a well known reagent for sulhydryl groups. The iodoacetate indices determined by Huggins, *et al.*, for normal and abnormal sera are of the same order of magnitude as the sulfhydryl content determined with the amperometric method. Although we do not consider the determination of serum sulfhydryl to be a specific diagnostic test for cancer, it may serve as an additional objective indicator in following animals and patients receiving chemotherapy.

Summary. The sulfhydryl content of serum proteins has been quantitatively determined through an adaptation of an amperometric titration. Among normal adults, the values noted, expressed as milligrams of cysteine, were uniform, reproducible and averaged 6.5 ± 0.2 mg per 100 ml serum or 5.5±0.3 mg per gram serum nitrogen. The albumin component of serum contained 80% of the total sulfhydryl. The addition of desoxyribonucleic acid, creatine or formaldehyde did not change the sulfhydryl content of the serum. The addition of an SH reagent such as para-chloro-mercuri-benzoate abolished all reactant groups measured with this method.

Sera obtained from patients with various diseases showed a significant reduction in the sulfhydryl available for this titration. This quantitative reduction in sulfhydryl was still evident when corrected for differences in the albumin and globulin components and thus represented a qualitative change in the serum proteins. At the present time, the alteration in the sulfhydryl content of serum or its components is not characteristic enough to permit specific differentiation among the neoplastic, metabolic, or infectious diseases. The possible application of serial serum sulfhydryl determinations in animals and patients may be of value as an objective indicator of response and for study of the mechanism of action of chemotherapeutic and other agents.

8. Huggins, C., Miller, G. M., and Jensen, E. V., *Cancer Res.*, 1949, v9, 177.

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Surface Fixation. A New Method of Detecting Certain Immunological Reactions. (17570)

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The study of rapid agglutination tests for the diagnosis of a number of infections has shown that carefully prepared and standardized antigens are of considerable help to the practitioner as well as the epidemiologist. The use of a suspension of formalinized and blue-stained Proteus X-19 in slide agglutination tests with whole blood(1) has been a satisfactory preliminary method for the diagnosis of typhus fever. This encouraged us to apply the same method to brucellosis. We have had excellent results. Both tests are performed on a slide. A 3 mm wire loop of antigen is mixed with whole blood taken with a 2 mm loop. The mixture is rotated in order to favor the margination of the agglutinated antigen. In about 1 or 2 minutes a thick blue ring forms and surrounds the red cells which have separated from the antigen. In negative reactions the mixture remains a uniform greenish color often surrounded by a reddish ring formed by marginated red cells. The forma-

1. Castaneda, M. R., Silva, R., and Monnier, A., *Rev. Med. del Hospital General*, 1940, v8, 382.

tion of rings also occurs when the test is performed in a concave slide or in a white porcelain plate. This phenomenon can be of great usefulness when applied to the identification of closely related antigens such as *Br. melitensis* and *Br. abortus*. These organisms are stained in different colors; *i.e.*, blue and red. If the purple mixture is treated with a proper amount of anti-abortus or anti-melitensis serum and rotated a red or blue ring becomes apparent at the edge of the drop. The test which we described under the name of selective agglutination(2) seems to be the consequence of a very rapid union of homologous antigen and antibody leading to the clumping and margination of the particles which adhere to the porcelain wall. The formation of these rings either in blood tests or in selective agglutination is unexplained by the usual theories of orthodox agglutination tests. The work of Northrop(3), Boyd(4) and others, however, leads to a rather simple explanation. If a bacterial suspension is considered as a lyophobic colloid, which loses its zeta potential under the influence of an antibody coating, the agglutinated particles are readily pushed away from the menstruum to become attached to the edge of the drop. While studying this type of serological reaction we made an observation which, if previously known, has not been mentioned in the available medical literature. Since it may have theoretical and practical importance we wish to describe the finding. Its simplicity makes us rather dubious that others have not noticed it.

Preparation of Antigen and Test. The antigens used for this test are prepared as follows: *Brucella abortus* and *Br. melitensis* are cultivated during 48 hours in liver infusion agar. The growth is washed with saline and filtered through cotton. Formalin is added to a concentration of 10%. The suspension is left at room temperature for 24 hours, then centrifuged and the sediment resuspended in a small volume of distilled water. This suspension is treated with ferrous sulphate as recommended by Huddleson and Carrillo(5) for the preparation of milk antigens. The suspension is stained with a fresh aqueous solution of hematoxylin (Coleman and Bell) and becomes nearly black. The stained suspension is left over night, centrifuged and resuspended in 4 volumes of isotonic NaCl solution.

The filter paper used in this experiment was E. D. No. 609. The test may be performed by the two following methods:

(a) The serum and antigen are mixed on a slide by means of drops carried with a wire loop. One loopful of the mixture is transferred to the lower end of a strip of filter paper about 1 cm from the edge. As soon as possible, to avoid evaporation, stand the strip of paper in a layer of isotonic NaCl solution 0.5 cm deep in a manner similar to the procedures used in paper chromatography. The flow of fluid will go rapidly over the mixture which may or may not be washed upward with the ascending current of saline.

(b) The phenomenon may be observed placing a wire loopful of 4 mm of serum on a piece of filter paper. When the serum has reached its maximum spread, a drop of antigen taken with a 2 mm loop is deposited carefully in the center of the serum-spot. With the same loop drops of isotonic saline are placed over the antigen and may or may not push the antigen from the place where it was deposited.

Results. In procedure (a) if isotonic NaCl is used on the spot on the filter paper, the current of saline may wash upward part of the black antigen (Fig. 3), but most of it remains where it is on the strip just as occurs when diluted Parker No. 51 black ink is used instead of antigen (Fig. 1). If normal serum is used in the mixture most of the antigen is washed upwards with the ascending fluid leaving little or no trace of it at the bottom of the paper (Fig. 4). This occurs also when we use the ink instead of antigen (Fig. 2). When the mixture is prepared with antigen

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3. Northrop, J. H., *The Newer Knowledge of Bacteriology and Immunology*, pp. 782-801, Jordan and Folk, The Chicago University Press, 1929.

4. Boyd, W. C., *Fundamentals of Immunology*, Interscience Publishers, Inc., N.Y., 1943.

5. Huddleson, I. F., and Carrillo, C. D., *Veterinary Medicine*, Chicago, 1949, v64, No. 6.

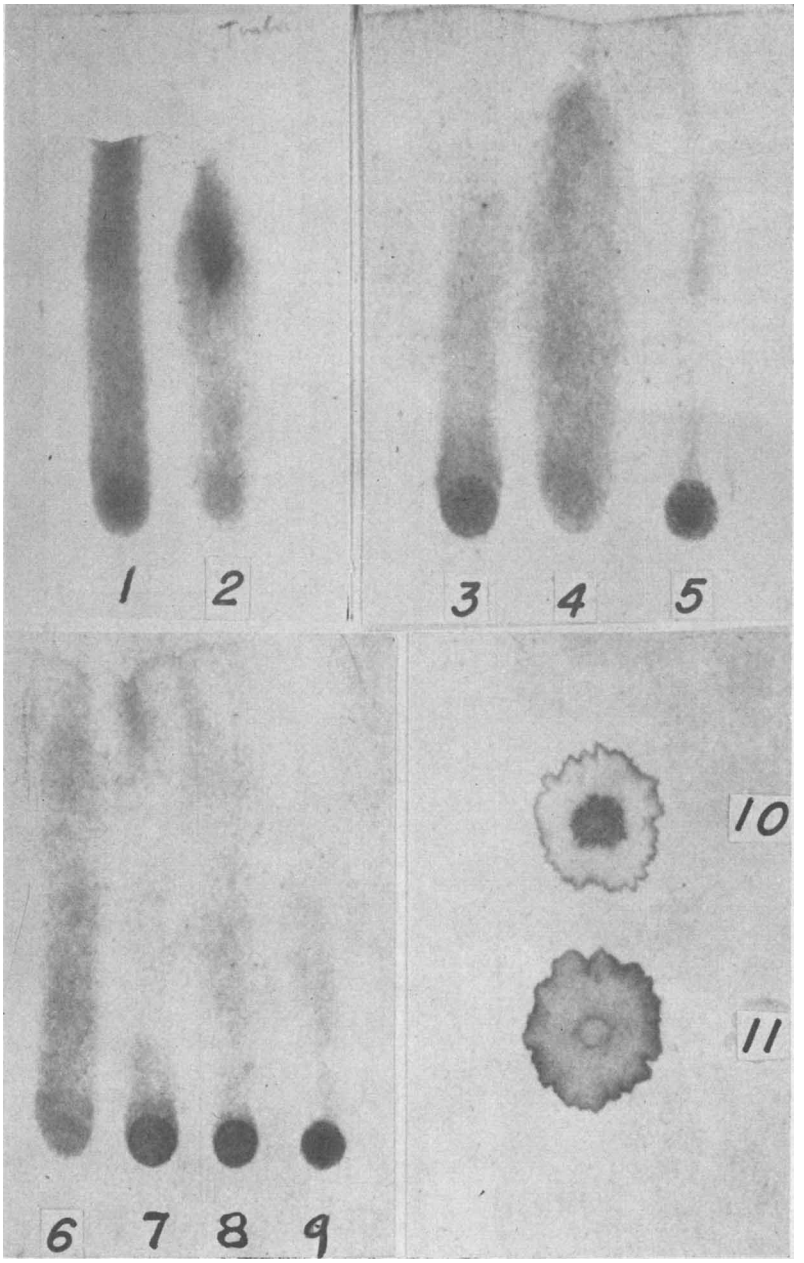

FIG. 1. Mixture of diluted Parker No. 51 ink with isotonic saline solution. Notice that the ink, in spite of being washed upwards in part, remains largely at the bottom of the paper.

FIG. 2. Same ink mixed with human serum. Little or no ink left at the bottom.

FIG. 3. Brucella antigen in a mixture with isotonic saline solution. Remains largely at the bottom.

FIG. 4. Same antigen in a mixture with "normal" human serum. Notice that it is washed upwards.

FIG. 5. Immune serum and antigen mixture. Becomes attached to the spot where it was placed.

FIG. 6 to 9. This shows various intensities of the reaction starting with a negative in 6 to a strong serum in 9.

FIG. 10. Spot test using serum from a case of brucellosis. Notice that fixation of the antigen has occurred in the center of the spot. FIG. 11. Spot test with a negative serum.

and serum from a person known to have brucellosis in evolution, the immune serum antigen mixture leaves a black spot practically unaltered at the place where it was set. A control mixture containing normal serum is washed upwards. The different behavior of both mixtures is very striking and takes place in less than 1 minute (Fig. 5).

The intensity of the test varies in degree as shown in Figs. 7, 8, and 9. Fig. 6 is a normal serum control.

The main difference in the action of normal and immune serum seems to depend on the fact that normal serum acts as a protective colloid upon the antigen (as it acts upon the ink), preventing its fixation in the paper from which it may be readily washed away by the ascending fluid. When immune serum is used in the test the protective effect fails to act upon a "colloid" previously modified in its electrical condition because of the attachment of antibody. Under such conditions the reaction becomes similar to that of a mixture of saline and antigen though considerably reinforced by the action of the specific globulin.

Because of this affinity to the filter paper by the immune serum-antigen mixtures, we believe that the designation of "surface fixation" may be explanatory of this phenomenon.

The union of the antigen and antibody seems to be very rapid which is to be expected from the findings on agglutination and precipitation reactions by the authors cited herewith and a very demonstrative addition to this view may be observed using method (b). When the antigen is placed in a previously arranged wheal of normal serum the addition of saline will push the antigen to the periphery producing a more or less uniform spread of stained organisms (Fig. 11). This occurs in a fraction of a second. If the antigen is set on a spot of immune serum, the addition of saline is without noticeable effect upon the antigen which remains at the place where it was placed (Fig. 10).

The intensity of the fixation phenomenon depends on the antibody content of the serum. It is demonstrable with serum from patients suffering from brucellosis in evolution and remains active even when agglutinins have become of very low titres. The application of this method as a test for doubtful cases of brucellosis has not been investigated *in extenso*. So far, only antigens prepared with Brucella have been studied. All organisms do not have the same ability to spread with the ascending fluid, which shows certain relationships to phenomena studied in paper chromatography.

Summary. A new method of demonstrating the union of antigen and antibody is described. The test is performed on the surface of filter paper and submitted to the washing effect of a stream of isotonic NaCl solution. While normal serum is washed together with the antigen, the immune serum produces the fixation of the antigen to the paper at the place where it was deposited.

Regarding its specificity, it has been observed that this test, studied only in brucellosis, produces responses which so far have agreed with the usual agglutination reactions.

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Disappearance of Hyaluronidase from the Blood of Albino Rats. (17571)

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Following the intravenous injection of hyaluronidase into the albino rat, edema of the extremities, elevation of the blood hematocrit and a decrease of the concentration of circulating plasma proteins result.(1,2) These effects are most marked in 15-45 minutes and

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