DIAGNOSTIC REAGENTS AND THEIR USES IN THE DIAGNOSIS OF INFECTIOUS DISEASES (*Continued*).*

BY F. E. STEWART, PH.G., M.D., PHAR.D.

As stated in my former paper, the complement-fixation test may be applied to other diseases than syphilis, such, for example, as gonorrhœa, typhoid fever, glanders, contagious abortion, tuberculosis, etc. In fact, gonorrhœa was one of the first infections to be studied by means of the complement-fixation technic, but the results were not generally satisfactory until it was found that the antigen must be polyvalent.

Complement-fixation Test in Gonococcus Infections.—As a rule, the antisheep hemolytic system is employed. Kolmer and Brown [1] have compared the comparative value of the antisheep and antihuman hemolytic systems in the examination of a number of systems, and while it is true that some of the reactions were somewhat stronger and yield slightly better results when the latter were used, the former is considered preferable by the authors.

As already stated, the antigen must be polyvalent, that is, it should be prepared of many different strains of gonococci. Owing to the difficulty of isolating the gonococci and the constant care required in sub-culturing and keeping a large number of strains alive, this antigen is best prepared in large laboratories where the cultures are handled and preserved by specially trained persons.

"The gonococci are well-grown on a salt-free veal agar, neutral in reaction to phenolphthalein, and to which a few drops of sterile hydrocele fluid may be added. After culturing for from twenty-four to forty-eight hours, the growths are washed off with distilled water, and the emulsion is heated in a water-bath for two hours at 56°C. It is then centrifugalized and passed through a Berkefeld filter. A small amount of preservative, as, *e. g.*, 0.1 Cc. of a 1:100 dilution of phenol to each cubic centimeter of antigen, may be added. The antigen is then well preserved in small amounts in ampules that are sealed and heated to 56°C. for half an hour on three successive days. Just before being used the antigen is made isotonic by adding 1 part of a 10 percent salt solution to 9 parts of antigen. I preserve the antigen in ampules containing 1 Cc., and after removing the antigen from the ampule to a large test-tube, add 1 Cc. of 10 percent salt solution, and dilute the whole 1 : 10 with the addition of 8 Cc. of normal salt solution, after which the anticomplementary titration is made." [2]

"*The Test.*—The serums should be fresh and clear, and heated to 56°C. for one-half hour. For each serum use four test-tubes (12 by 1 cm.), arranged in a row. Into each of the first three place the dose of antigen and increasing doses of serum—0.05 Cc., 0.1 Cc., 0.2 Cc.; the fourth tube is the serum control, and into this is placed the maximum dose of serum (0.2 Cc.), but no antigen; 1 Cc. of complement diluted 1:20 is added to each tube. The following *controls* are included:

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[1] Complement Fixation in Gonococcus Infections, by John A. Kolmer and Claude P. Brown. Jour. Infect. Diseases, vol. xv, No. 1, July, 1914, pp. 6–21.

[2] John A. Kolmer: A Practical Text-Book of Infection, Immunity and Specific Therapy.

" 1. A positive control with an antigonococcus serum or with the serum of a patient who reacted positively on a former occasion.

" 2. A negative control with the serum of a healthy person.

" Both of these controls may be set up with but the maximum dose of serum (0.2 Cc.).

" 3. The serum control of each serum is conducted in the fourth tube of each series. At the completion of the test this tube should show complete hemolysis and thereby indicate that the serum was not anticomplementary.

" 4. The antigen control at this time includes the dose of antigen and complement.

" 5. The hemolytic system control at this time receives the dose of complement.

" 6. The corpuscle control receives 1 Cc. of the corpuscle suspension.

" To each tube sufficient saline solution is added to bring the total volume up to about 2 Cc. The tubes are shaken and incubated for one hour at 37° C., when 1½ units of antisheep amboceptor and 1 Cc. of sheep corpuscle suspension are added to each tube except the corpuscle control. The tubes are gently shaken again and reincubated for an hour or longer, depending upon the hemolysis of the controls, after which the results are recorded. This secondary incubation may be omitted and the tubes placed in a refrigerator overnight and the results read the next morning. Under these conditions hemolysis occurs slowly, and according to some workers in this field the reaction becomes more delicate." [3]

The limits of this paper will not permit a more extended reference to the complement-fixation tests. The reader is referred to a " Practical Text-Book of Infection, Immunity and Specific Therapy," by John A. Kolmer, M.D., Dr.P.H., published by W. B. Saunders Company, 1915, for more complete information.

The practical value of the gonococcus complement-fixation test is there recorded, also details concerning the complement-fixation test in glanders, contagious abortion, dourine or horse syphilis, typhoid fever, tuberculosis and echinococcus disease. Reference is also made to the complement-fixation test in the standardization of immune serums, also protein differentiation by complement fixation, under which is described the complement-fixation test for the identification of blood stains, the identification of meats, the identification of bacterial antigens, and, finally, under the head of " complement-fixation test in cancer," reference is made to von Dungern's method which he claimed to yield 90 percent of positive reactions in known cases of cancer.

These tests open up a field of work for the skilled bacteriologist and point to a field in which the pharmacist who has become skilled in that branch of science and practice may look forward to vocational work, profitable in application and also valuable from the viewpoint of professional reputation.

Agglutination Tests.—When a small quantity of immune serum is added to a suspension of the corresponding bacterium the organism becomes agglutinated into clumps and motility is suspended or destroyed. For example, if typhoid-immune serum from an immunized animal or a patient suffering with typhoid fever is added to an emulsion of typhoid bacilli in a test-tube and the mixture placed in an incubator, the bacteria which previously formed a uniform emulsion

[3] John A. Kolmer, *ibid.*

clump together into little masses, settle at the sides of the test-tube, and gradually fall to the bottom, the fluid becoming almost clear. This phenomenon is assumed to be due to the presence of an antibody in the blood to which the term "*agglutinin*" has been applied, and the reaction is known as "*agglutination.*"

Kolmer [4] defines agglutinins as "antibodies that possess the power of causing bacteria, red blood-corpuscles, and some protozoa (trypanosomes) suspended in a fluid to adhere and form clumps." In 1896 this phenomenon was applied practically by Widal and Grunbaum to the diagnosis of typhoid fever. At the present time this test is known as the Gruber-Widal reaction. It has proved of great value, not only in making diagnosis of typhoid fever, but also in other infections. The true nature of the phenomenon of agglutination is unknown. The presence of some salt is necessary for its occurrence. For a time after its discovery the reaction was regarded as strictly specific; that is, typhoid-immune serum would agglutinate only typhoid bacilli and no other. It is now known that immune serum will frequently agglutinate other closely related organisms, although not usually to so high a degree.

Agglutination reaction is employed for several purposes: (1) For the diagnosis of disease, by identifying the bacteria of infection from which the patient is suffering. (2) As an aid to the identification of a micro-organism that has been cultivated from a patient. (3) To aid in determining whether in a case in which more than one micro-organism has been cultivated and the condition at hand is a single or mixed infection. (4) For measuring the immunizing response that a patient is making to his infection, or to artificial immunization by the use of bacterial vaccines, for example.

For the diagnosis of disease agglutination reaction is limited chiefly to typhoid and paratyphoid fevers. It is also used, but is of less value, in diagnosis of cerebrospinal meningitis and bacillary dysentery.

According to Park, a positive reaction is obtained about the seventh or eighth day. "About 20 percent give positive reaction in the first week; about 60 percent in the second week, about 80 percent in the third week; about 90 percent in the fourth week, and about 75 percent in the second month of the disease." Kolmer says, "That in about 90 percent to 95 percent of cases in which repeated examinations are made a positive reaction is to be found at some time during the patient's illness. Also occasionally the reaction appears first during the stage of convalescence, and at times it may even be absent, the diagnosis being confirmed by cultivating typhoid bacilli from the blood. The possibility of a given case reacting strongly one day and weakly or entirely negative a day or so later, has been emphasized. Usually the reaction is strongest during convalescence, remains positive for several weeks and then gradually returns to the normal. Occasionally the reaction remains positive for months, or even years, after an attack of typhoid fever. Many such cases are 'carriers' and harbor the bacilli in the gall-bladder although the person appears to be quite well." [4]

"Positive reactions occasionally occur in other diseases, such for example as acute miliary tuberculosis, malaria, malignant endocarditis, and pneumonia. It is also well to bear in mind the possibility of a patient having been vaccinated against typhoid fever at some early date, with resulting agglutinin formation." [4]

[4] John A. Kolmer, *ibid.*

Agglutination reaction is also used in the diagnosis of dysentery, although the absence of the reaction does not exclude dysenteric infection.

Two methods may be employed, namely: The microscopic method, and the macroscopic method, the former being the one ordinarily used for diagnosing typhoid fever, and the latter the method of choice in scientific research.

The technic used in the various agglutination tests is very fully described in all of the modern text-books, to which the reader is referred. One of the simplest is that suggested by Dr. John H. Borden, of New York. The outfit is as follows: A, 30 Cc. stock bottle Typhoid Suspension; B, Cc. stock bottle Salt Solution; C, 10 Cc. dropping flask used for dropping Typhoid Suspension; D, 10 Cc. dropping flask used for dropping Salt Solution; E, 6 graduated test-tubes used in making tests; F, 1 accurately graduated pipette; G, 12 small capillary bulbs or tubes to collect the blood serum; H, 1 needle.

Directions.—1. Prick lobe of ear briskly with the needle and collect the blood at

FIG. 1.

small end of the small tube (G) until two-thirds full; hold the bulb in a slanting manner for two minutes, then close the vent hole with the rubber band attached. The point is sealed by the drying of the blood in it. Put aside for one hour to permit the serum to separate from the blood.

2. Half fill one dropping flask (C) from the stock bottle of Typhoid Suspension and the other with salt solution as labeled. Remove the rubber cap at small end of the dropping flask and drop the salt solution into one of the graduated test-tubes up to the mark 1 Cc.

3. File a mark near the large end of the tube containing the blood to be tested, break off this end and carefully draw up through the graduated pipette (F) the serum from the blood tube until 4–100 Cc. is registered, then mix this with the 1 Cc. Salt Solution in test-tube and thoroughly wash out with the Salt Solution. This will make a final dilution of 1–50.

4. Add enough Typhoid Suspension to bring the mixture in the test-tube up to the graduation marked 2 Cc., close mouth of tube with plug of non-absorbent cotton, invert twice to mix, and set aside in a dark place in test-tube rack.

Within one-half hour to twenty hours, if typhoid reaction is positive,[5] depending upon the dilution of the serum used and its agglutinating power, a marked granularity of the fluid in the tube will be noted. Following this there will be seen distinct clumps beginning to sink toward the bottom, and at the conclusion of the reaction the fluid above will be limpid and free from clumps and the point of the test-tube will contain a small, white, flocculent mass of agglutinated bacilli.

The dilutions may be increased or diminished at will of operator. They should never be less than 1.50. Pipette and tubes should be thoroughly cleansed after using, with water, alcohol and ether.

This outfit contains sufficient Typhoid Suspension and Salt Solution to make at least thirty (30) tests. The suspension permanently retains its susceptibility to agglutination if kept from extreme of temperature.

Another simple test has been suggested by Prof. C. C. Bass, of Tulane Uni-

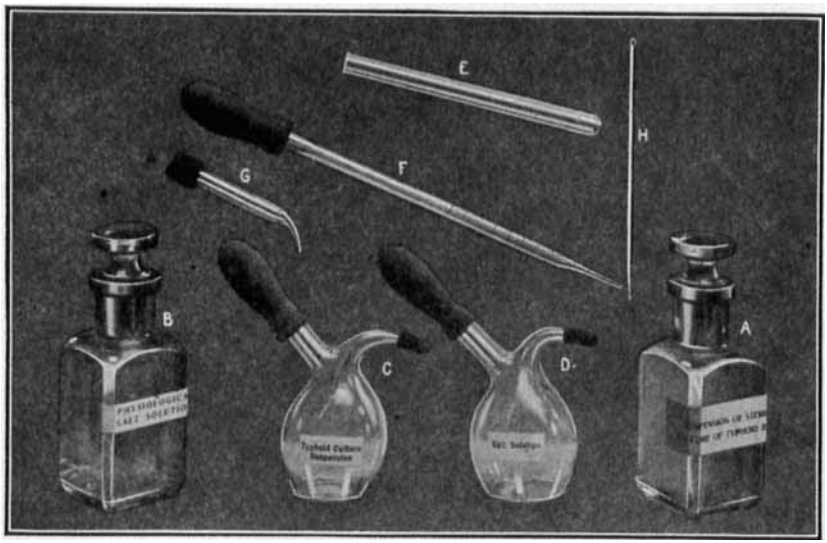

FIG. 2.

versity, New Orleans, La., and is known as "The Bass Watkins Macroscopic Typhoid Agglutination Test."

Any specimen of blood, wet or dry, fresh or old, may be diluted with approximately four times as much water as original volume of blood appears to have been fresh. One drop of this diluted blood is mixed with one drop of the test fluid (typhoid emulsion) on a slide. The slide is tilted from side to side or end to end so as to mix and agitate the mixture and thereby hasten agglutination.

Still another and more practical way is to make a smear on a slide of approximately one-fourth of a drop of the patient's blood. This quantity (one-fourth drop) is welled up by squeezing the ear or finger after pricking with a surgical needle or other instrument. Sufficient blood is taken up by touching the spot with a microscopic slide. The blood is then spread out on the slide with the end of another slide furnished for that purpose.

[5] Should the reaction be negative, a stronger solution may be used if it is advisable to duplicate the test.

The blood is dissolved by adding one drop of water, which makes the dilution approximately 1 to 5. The second slide may again be used, so as to thoroughly dissolve the specimen with the water. After the blood has been thoroughly dissolved, one drop of typhoid emulsion is added on the slide containing the diluted blood to be tested and the reaction determined in the usual way.

The reaction, when positive, occurs within one or two minutes and consists of the appearance of a fine grayish sediment composed of agglutinated bacilli, visible to the naked eye. This sediment becomes coarser and coarser as the agglutination is continued. Where the amount of agglutination is very small the reaction may be weak or doubtful. When the test is negative no such sediment is formed and the mixture remains clear and unchanged.

Two glass slides are provided in the outfit on one of which is a dried smear of active blood for demonstrating the test so that the physician may familiarize himself with the technic and reaction before putting the test to practical use. These slides should be afterwards cleaned and preserved for spreading and testing the smear of patient's blood as above directed.

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CORRECTIONS IN DR. F. E. STEWART'S PAPER, SEPTEMBER NUMBER, PAGES 978–983.

Page 976, fourth line from the bottom should read, "of the red corpuscles, liberating hemoglobin into the surrounding fluid."

Eighth line from the bottom should read, "immune serum to which the name specific *hemolysin* has been given."

Page 977, third line from the top should read, "precipitate" instead of "precipitin."

Seventeenth and eighteenth lines from the top should read, "and complement in a test-tube in the incubator."

Page 978, twelfth line from the bottom should read, "(*spirochæta pallida*)" instead of "(*spirochæta pallidum*)."

Page 980, third line from the top should read, "the capsule to the blood as it oozes from the puncture" instead of "the needle as it oozes from the puncture."