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BY

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THE PRINCIPLES AND TECHNIQUE OF THE WASSERMANN REACTION AND ITS MODIFICATIONS.*

By HOWARD FOX, M.D.,

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THE subject of the serum diagnosis of syphilis may appear at first glance to be somewhat complicated, owing to the multiplicity of the terms that are used. The principles of the reaction can, however, be easily mastered, whereas the practical knowledge, owing to the many sources of error, can only be acquired after a considerable amount of experience.

In order to understand the Wassermann-Neisser-Bruck reaction, as the serum test for syphilis should properly be termed, it is necessary to understand the principles of hemolysis and of the Bordet-Gengou phenomenon. By hemolysis is meant the power possessed by the serum of one species of animal to dissolve the corpuscles of another species. If, for instance, fresh serum from a rabbit is placed in a test tube, and sheep's corpuscles are added, the serum will be seen to dissolve to a slight extent the corpuscles of the sheep. If the same rabbit is now treated by several injections of sheep's corpuscles, and its serum withdrawn and placed in a test tube, it will be found that this power to dissolve the sheep's corpuscles has been greatly in-

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creased. The rabbit is now said to be immunized against sheep's corpuscles. By this we mean that a certain substance has been artificially formed in the rabbit serum to which the name of hemolytic amboceptor,* or immune body, has been given. Heating to 56° C. for a half-hour does not destroy this substance, therefore it is said to be thermostabile. Hemolysis depends not only upon this hemolytic amboceptor, but also upon another substance called complement, which is present in all normal fresh sera, and which is destroyed by heating to 56° C. for a half hour. It is said to be thermolabile. The three substances, corpuscles + hemolytic amboceptor + complement, constitute what is called a hemolytic system, and hemolysis can take place only when all three are present.

Closely analogous to a hemolytic system is what may be termed a "bacteriolytic system," consisting of (1) a bacterium, corresponding to the corpuscles in the hemolytic system, (2) a bacteriolytic amboceptor (or antibody) corresponding to the hemolytic amboceptor, and (3) complement, similar in both systems. Before proceeding to discuss this so-called bacteriolytic system, it is necessary to explain the terms antigen and antibody. An antigen is any substance (bacteria, corpuscles, body cells, etc.) which, when introduced into an animal, is followed by the formation of antibodies. The sheep's corpuscles in the case of the hemolytic system and the bacteria in that of the bacteriolytic system are examples of antigen. The substances formed by their entrance into the animal are antibodies or amboceptor-

*The term amboceptor means that this substance has an affinity on the one hand for corpuscles, and on the other hand for complement. The complement is so called because it completes the action of the amboceptor.

tors. Strychnine, for instance, though an injurious substance, is not an antigen, as its entrance into the body is not followed by the formation of antibodies. Furthermore, it should be said that the term antibody does not necessarily imply a substance that is protective, such as an antitoxin.

Returning to the bacteriolytic system, and taking a specific instance, we will use for antigen an emulsion of the typhoid bacillus; for bacteriolytic amboceptor or (antibody) the serum of a patient suffering from typhoid fever; and for complement, normal fresh serum, preferably from a guinea-pig. If these three substances, typhoid bacilli + typhoid amboceptor + complement, are placed in a tube for half an hour at body temperature, a union will take place just as it did in the case of the hemolytic system. The bacteria, instead of the corpuscles, are dissolved. Unlike what occurs in the hemolytic system, the union of these three substances will not be followed by any visible change. It cannot be told from the appearance of the tubes whether or not the bacteria have been dissolved. In the case of the corpuscles, solution was most apparent, the tubes changing from an opaque to a clear red color upon hemolysis. In other words, in the hemolytic system there was an indicator to show that solution of the corpuscles had taken place, no such indicator being present in the case of the bacteriolytic system.

Bacteriolytic System consists of	Hemolytic System consists of
1. Bacterium (antigen).	1. Corpuscles (antigen).
2. Bacteriolytic amboceptor (antibody).	2. Hemolytic amboceptor (antibody).
3. Complement.	3. Complement.

The reaction discovered by Bordet and Gengou in 1901 utilizes the phenomenon of hemolysis to show

that a union actually can occur between the three components of a bacteriolytic system. This reaction depends upon the so-called principle of the fixation (anchoring or binding) of the complement, and is carried out as follows: The three components of a bacteriolytic system, such as typhoid bacilli + typhoid antibodies (serum of patient to be tested) + complement, are placed in a test tube and incubated at 37° centigrade for a half hour to allow them to unite. To the same tube are then added two of the elements of a hemolytic system, namely, hemolytic amboceptor and sheep's corpuscles, and the mixture again placed in the incubator. If now there has been an actual union of the first three substances. that is, if the complement has been fixed or used up by uniting with the other two substances, there will be no complement left to unite with the other two parts of the hemolytic system, and consequently no hemolysis will occur. If, on the other hand, no union of the first three substances had occurred, complement will be available to unite with the other two parts of the hemolytic system, and consequently hemolysis will take place. In other words, the absence of hemolysis denotes a positive reaction, *i. e.* the antibodies for which we were testing are present. In the case in question, the patient was suffering from typhoid fever. The occurrence of hemolysis, on the other hand, signifies a negative reaction, *i. e.* the antibodies were not present. In that case the patient was not suffering from typhoid fever.

The principle of the fixation of complement can be illustrated by a swinging pendulum, as shown in Fig. 1. The pendulum representing the complement swings to one side and completes the bacteriolytic system, or to the other side and completes the hemo-

lytic system. In the first case the result is no he
lysis. In the second case hemolysis occurs.

It may be asked why it is necessary to use guin
pig serum for complement when there is com

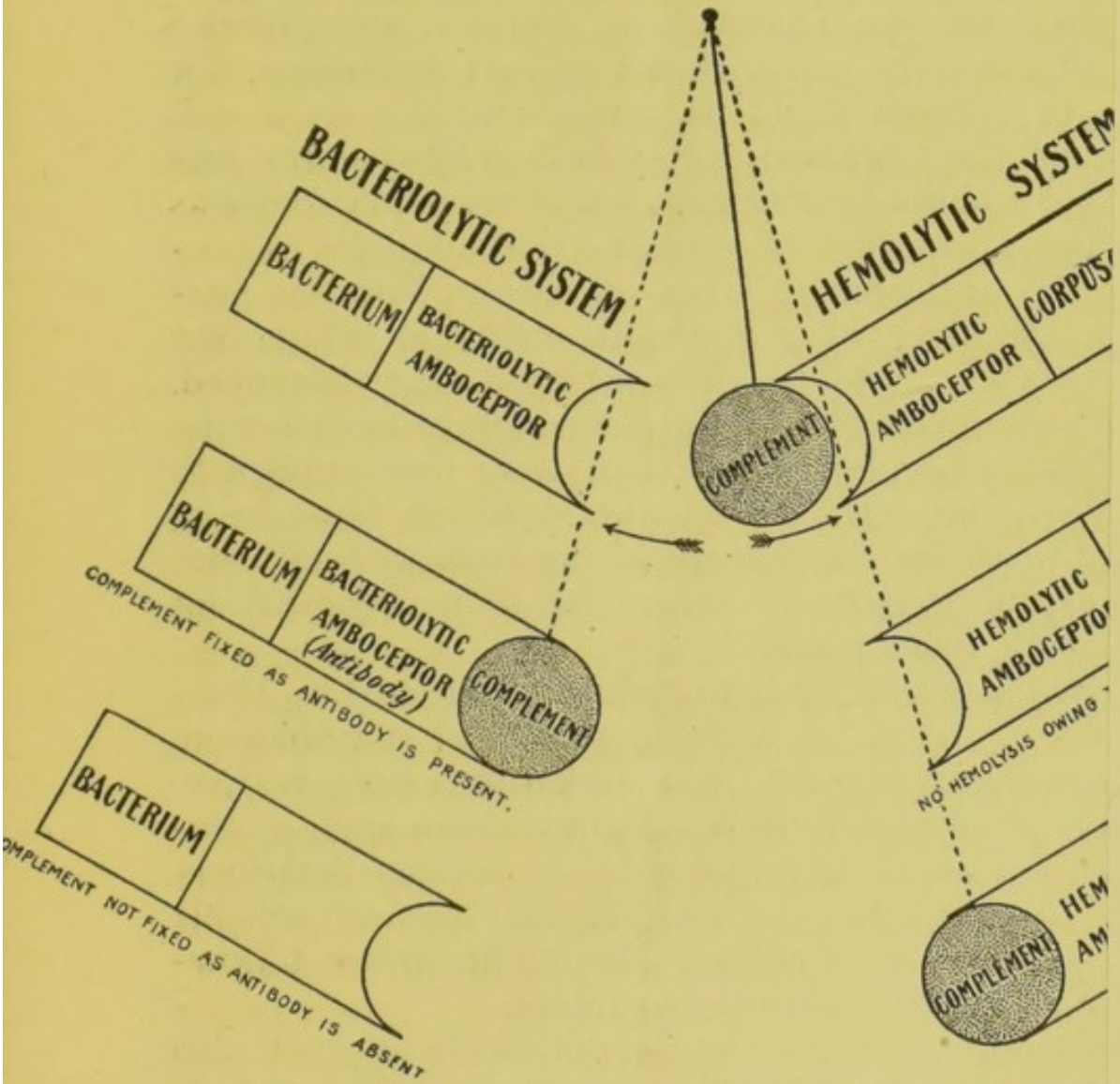


FIG. 1.—Principle of complement fixation illustrated by a free-swi

ment already present in the serum of the patie
be tested. The answer is that the latter is an
known and variable quantity. On this accoun
destroy the complement before beginning the
by heating the patient's serum to 56° C. for a

hour. We then add for complement a measured amount of guinea-pig serum.

The principle of Bordet and Gengou,[1] which has been used since its discovery as a means of diagnosis in many bacterial diseases, was applied to syphilis in 1906 by Wassermann, Neisser, and Bruck.[2] By this test the presence not only of antibodies, but also of antigen could be demonstrated. As a culture of the organism of syphilis (*Spirochæta pallida*) could not be obtained, it was necessary to use as antigen an extract of syphilitic tissue known to contain the organism in large numbers. For this purpose the liver of a syphilitic fetus in which the spirochetes could be demonstrated was employed. The same principles, as explained above to detect the presence of typhoid antibodies, were then applied to syphilis, the extract from the syphilitic fetus being substituted for the culture of the typhoid bacilli and the syphilitic patient's serum for that of the patient suffering from typhoid.

The extract of the fetal liver as first used by Wassermann was made with physiological salt solution, to which carbolic acid was added as a preservative. While good results were obtained from such an extract, it was found to be liable to sudden deterioration in strength. An extract that proved reliable and effective on a certain day might on the following day become entirely worthless.

Not only extracts from syphilitic livers, but also from normal livers (Marie and Levaditi[3]), and, in fact, from a large variety of normal organs were found available as "antigen." Extracts of guinea-pig liver (Landsteiner[4]), and even of tumors (Weil[5]) were found suitable for this purpose. It was later discovered by Porges and Meier[6] Landsteiner, Müller and Pötz,[7] and by Levaditi and

Yamanuchi,[8] working independently, that the active principle could be extracted from the syphilitic liver by alcohol. This showed that the active principle was a lipoid and not an albuminoid substance, as had previously been supposed. Experiments were then made to substitute as the antigen various lipoid substances in place of the syphilitic liver extract. Lecithin was thus first used by Porges and Meier, with results approximately similar to those obtained by using the extract. Numerous other lipoid substances and salts were then tried with more or less good results, among them sodium glycocholate and sodium taurocholate (Levaditi and Yamanuchi), sodium oleate (Sachs and Altman[9]), cholesterin and vaselin (Fleischman[10]), sodium cholate (Noguchi[11]). Cholesterin was, however, found by Levaditi and Yamanuchi and by Noguchi to be inactive. Noguchi found that acetone soluble and acetone insoluble fractions of the alcoholic extract of normal or syphilitic tissues, such as blood serum, blood coagula, liver, kidneys, etc., possessed antigenic properties.

By using an alcoholic extract of guinea-pig heart, in comparison with syphilitic liver extract, Landsteiner and his associates obtained identical results. Even Meier,[12] in a recent communication from the laboratory of Wassermann, admits that the guinea-pig extract of Landsteiner serves as a perfectly reliable substitute for the extract of syphilitic liver. As between the watery and alcoholic extract of the syphilitic fetal liver, it seems certain, as Marchildon[13] has recently shown, that both are equally potent. As the alcoholic extract can be kept for a much longer time, it should for this reason be preferred.

The discovery that various substances in no way

related to syphilis were able to combine with the syphilitic antibodies, showed that the Wassermann reaction could no longer be considered as a true antigen-antibody reaction. While this is admitted by all, including Wassermann himself, it does not in any way lessen the great practical value of the test. The reaction must be considered as a union taking place between certain lipoid substances and the antibodies existing in syphilitic blood.

The five substances used in performing the Wassermann reaction and the steps necessary to prepare them are as follows:

1. *Antigen.*—The liver of a syphilitic fetus is cut in fine pieces, ground in a mortar, and placed in a flask. Alcohol is then added in the proportion of 5 c.c. for each gram of liver substance. The solution is then shaken from time to time, a shaking machine being unnecessary for this purpose. After a few days of extraction the solution is ready to be tested. It is kept at room temperature and will remain for a considerable time (several months) without deterioration. To ascertain the necessary dose of the extract it is tested in varying amounts with a series of cases known to be positively syphilitic. That amount that will give complete inhibition of hemolysis, when 0.2 c.c. of the patient's serum is employed, will be the required dose. At the same time there must be no inhibition of hemolysis in the control tube containing the hemolytic system + extract, even when double the dose of extract is used. In place of the liver extract a suspension of crude lecithin can be used. The latter substance, as prepared and titrated by Noguchi, has been used by me up to the present time as antigen.

2. *Antibody* (patient's serum).—The blood of the patient to be tested can be collected directly from a

vein or from a puncture of the finger or ear. The frequent necessity of repeating a test makes it desirable to have a plentiful amount of serum. For this reason I strongly favor obtaining the blood

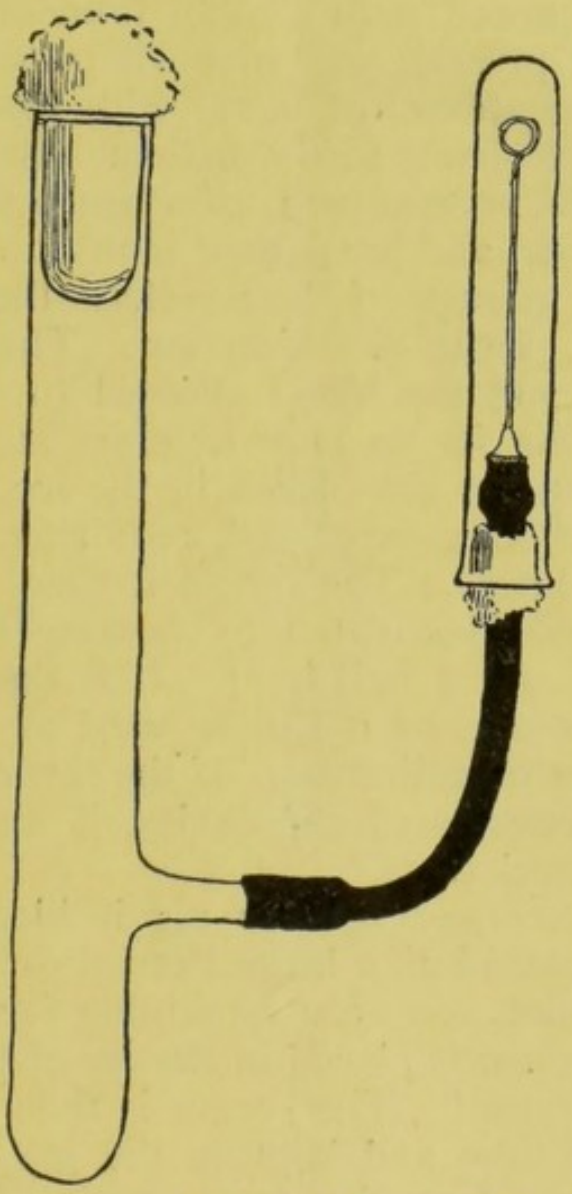


FIG. 2.—Blood-collecting tube; one-half natural size.

from a vein such as the median basilic, which is easily done by using the little apparatus devised by Noguchi, shown in Fig. 2. This consists simply of a test tube having a small outlet at the side connected by rubber tubing with the needle. The lat-

ter is covered by another small test tube for protection. The tubes are then sterilized in the antoclave. The writer uses needles that can be easily detached from the tubing, and advises that the needles be cleansed with alcohol immediately after use. A little practice will make it possible to collect from 6 to 10 c.c. of blood with practically no pain or inconvenience to the patient. Before puncturing the vein, several turns of a bandage are made above the elbow, and the patient is told to clench the fist. A quick plunge of the needle is then made in the direction of the blood stream. The tubes are then slanted, and the blood allowed to clot firmly. After remaining for an hour or more at room temperature, the tubes are placed in the ice chest until the serum has been expressed, requiring, as a rule, about twelve hours. The serum is then pipetted or drawn off and inactivated by heating in a water bath at 56° C. for a half hour. It is then available for immediate use, or it can be used as late as the third day after its collection. If the serum is turbid, due to the presence of corpuscles, it must be centrifugalized until clear.

3. *Complement.*—A guinea pig is bled from the jugular or carotid into a large Petri dish. The blood is allowed to clot, and after remaining for an hour at room temperature is placed in the ice chest until the serum is expressed. The serum is then poured off into a sterile tube and kept in the ice chest until ready for use. Complement is active for two or three days, but is preferably used within twenty-four hours after it has been obtained. The writer's custom is to bleed his animals late on the afternoon of a certain day, using the serum on the following morning or afternoon.

4. *Hemolytic Amboceptor.*—A rabbit is injected

subcutaneously, intraperitoneally or intravenously several successive times with washed, but diluted, sheep's corpuscles. A good plan is to give four injections at intervals of five days in dose of 2, 4, 8 and 16 c.c., respectively. The blood is then withdrawn nine days after the last injection. By this time the serum will have, as a rule, the required amount of amboceptor. The blood is drawn from the carotids into sterile tubes and allowed to clot. The serum is then removed, inactivated and standardized. By standardizing is meant the determination of a so-called *unit* of *amboceptor*. This refers to the smallest amount of the rabbit's serum that will completely hemolyze 1 c.c. of a 5 per cent. suspension of sheep's corpuscles when placed at 37° C. for 1 hour. For the tests two such units are employed. The amboceptor can be kept in the ice box for weeks and months, though it gradually tends to lessen in strength.

5. *Sheep's Corpuscles.*—Blood is obtained either from the jugular vein of a sheep by means of a canula or from a slaughter house when the animal's throat is cut. It is collected in a sterile beaker and defibrinated by stirring with a glass rod. The corpuscles are then washed free of serum by adding normal salt solution and centrifugalizing several times. The salt solution is then poured off and the corpuscles diluted with more normal salt solution to form a 5 per cent. suspension. Of this suspension, 1 c.c. is used for each tube. The corpuscles may also be used in their original volume, *i. e.* after washing no suspension in salt solution is made. One drop of this original mass of corpuscles serves for each tube. The corpuscles can generally be used four or five days if kept in the ice chest. After this they begin to be hemolyzed, as is shown by a dark-

ening of the original bright red color. They are then unavailable for the test.

The practical performance of the Wassermann test requires a laboratory equipped with a thermostat, ice chest, centrifugal machine, water bath, and, in addition, an antoclave, if the tubes shown in Fig. 2 are employed. A large number of small test tubes are needed, and a considerable number of 1 c.c. pipettes (preferably Mohr pipettes graduated in a hundred divisions to the tip). It is of great import-

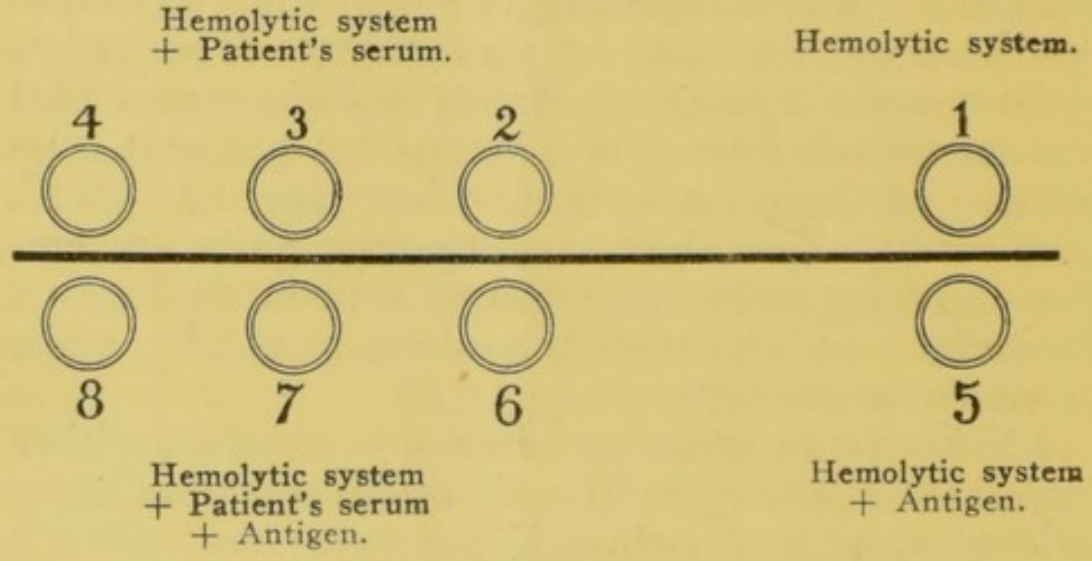


FIG. 3.—Illustrating controls that are used.

ance that both tubes and pipettes should be thoroughly cleansed in running water after use and subjected to a high temperature in the antoclave or other sterilizer before they are again used. It is not essential that the tubes should be plugged with cotton. It is, however, necessary for them to be perfectly clean.

The arrangement of the tubes used for the test, including the necessary controls, is shown in Fig. 3. Tube No. 6 contains serum of a patient to be examined. No. 7 contains the serum of a patient known to give a positive reaction, and tube 8 a

serum known to give a negative reaction. The three tubes 6, 7 and 8 receive all five substances necessary for the reaction. All of the other tubes are controls. Tubes 2, 3 and 4 contain the hemolytic system+the patient's serum, it being of course necessary to have a separate tube for each separate serum. Tube 5 contains the hemolytic system+the antigen (extract or lecithin). Complete hemolysis must take place in all of these control tubes. Tube 5 is necessary to show that the antigen of itself does not bind complement and so prevent hemolysis. In the same way tubes 2, 3 and 4 show that the patient's serum of itself does not bind complement. In case the patient's serum proves to be anti-hemolytic of itself, the test must be repeated and the serum used in a smaller amount, *e. g.* 0.1 c.c. instead of 0.2 c.c. Tube 1 shows that the hemolytic system is in "working order." While it is essential in testing a given serum to employ as controls a known positive and a known negative serum, it is also desirable to test a large series of cases simultaneously. This allows a comparison which aids in making the results more trustworthy.

In performing the test 0.1 c.c. of complement (invariable dose), 0.1 c.c. of serum (usual dose), and antigen (dose varies according to the preparation, average being 0.2 c.c.) are placed in the tubes, and 1 c.c. of physiological salt solution is added. The tubes are then shaken and kept in the thermostat or water bath for three-quarters of an hour. Two units of amboceptor, and either 1 c.c. of the corpuscle suspension or one drop of corpuscles, according to choice, are added. The tubes are then shaken and again incubated and observed at frequent intervals. The final results can be recorded after three hours of incubation. The controls are generally

found to be hemolysed within a half hour, tube No. 1 being the first and tube No. 5 generally the last to be entirely hemolysed. A negative result is denoted by complete hemolysis. A positive result is shown by inhibition of hemolysis, which, according to its degree of intensity, can be classed as positive, strong positive or weak positive. For recording results a chart devised by Dr. Wood McMurtry will be found of very great convenience.

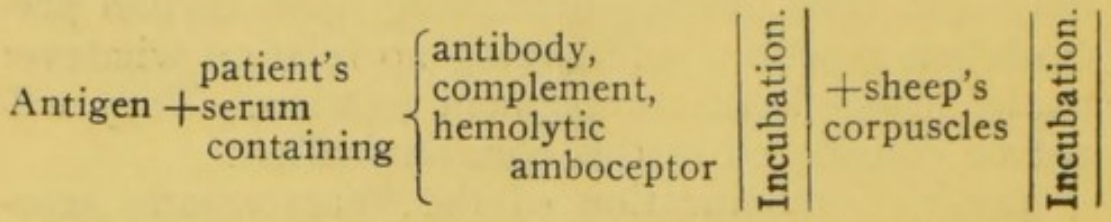
In performing the Wassermann test the body fluids usually employed to show the presence of the syphilitic antibodies are the blood serum and spinal fluid. The presence of antibodies have been reported in the milk by Bab and Plaut,[14] and in the urine by Blumenthal and Wile.[15] The latter observers, in a series of fifty cases in which the serum and urine were tested simultaneously for antibodies, found that a stronger reaction was given by the serum than by the urine. Furthermore, in a series of cases giving a strong reaction, the urine of itself showed anti-hemolytic properties. In a later report of a hundred cases by Wile[16] it was found that 2 per cent. of the cases gave positive reactions with the urine and negative reactions with blood serum. From this the writer concludes that the "diagnostic value of the reaction must for the present be viewed with caution."

As the Wassermann reaction is at best a rather complicated procedure, requiring special laboratory facilities and a considerable expenditure of time, it is not surprising that attempts to simplify the test should have been made. Modifications have been devised by Bauer, Tschernogubow, and Noguchi, which, like the regular Wassermann reaction, depend upon the principle of the fixation of complement. A number of other sero-diagnostic methods

have also been devised depending upon certain precipitation reactions and having no relation whatever to the principle of complement fixation. They will not be considered in this communication.

Bauer's[17] modification of the Wassermann reaction depends upon the fact that there is normally present in human serum a hemolytic amboceptor for sheep's corpuscles. Bauer, therefore, omits the hemolytic amboceptor (rabbit's serum treated with sheep's corpuscles) in his test, and utilizes the amboceptor present in the patient's serum. The advantage gained by this procedure is that instead of the usual five substances, only four are required for the test. The theoretical objection to the method is that the hemolytic amboceptor in the patient's serum is an unknown and variable quantity. Furthermore, the omission of the amboceptor is, after all, not such a great simplification, for it is in the preparation of the other four substances that the chief difficulties and inconveniences are encountered. The test is further unavailable for use in young infants, as no hemolytic amboceptor is contained in their serum. To supply this deficiency, it is suggested by Bauer to add normal human serum from an adult which will contain the necessary amboceptor. In spite of theoretical objections, Bauer's modification appears to have given good results practically in the hands of Bering.[18] In a series of 123 cases of syphilis tested simultaneously by both methods, the results were identical in all except one case. In this case, which was one of positive syphilis, the Bauer test was positive, while the Wassermann was negative.

The steps in performing the Bauer modification are represented as follows:



Tschernogubow, [19] in his modification, uses a hemolytic amboceptor for *human corpuscles* instead of one for *sheep's* corpuscles. The hemolytic system in this case consists of human corpuscles + hemolytic amboceptor (antihuman) + human complement. In performing the test, a single drop of the patient's blood is used. This is relied upon to supply three of the five substances necessary for the Wassermann reaction, namely, antibodies, complement, and corpuscles. Tschernogubow does not inactivate (*i. e.* destroy complement), considering the complement normally present in the patient's blood available for his test. No guinea pig serum is therefore used. This is an advantage claimed for the test, as well as the fact that a drop only of the patient's blood is used and that no inactivation is necessary.

Tschernogubow's assumption that the complement present in the patient's blood is available for the test has been shown by Noguchi to be correct only when 0.1 c.c. of the serum is present in a tube. In other words, human complement as present in such a small quantity of blood as one drop is, as a rule, too weak to produce any considerable amount of hemolysis of human corpuscles. Noguchi obtained no hemolysis upon adding human complement in amounts as large as 0.2 and 0.3 c.c. to 1 c.c. of a weak suspension of human corpuscles + 2 units of antihuman amboceptor. When, however, guinea pig's serum, even in amounts as small as 0.01 c.c. were added, prompt hemolysis resulted.

The disadvantages in general of using human in-

stead of guinea-pig complement for the test are, according to Noguchi, as follows:

1. By using human complement it is necessary to have ten times as much amboceptor as when guinea-pig complement is used. For this reason the latter is economically preferable.

2. Human complement, like that of the rabbit, is less sensitive to fixation than guinea-pig complement, *i. e.* it does not unite as readily with antigen and antibody.

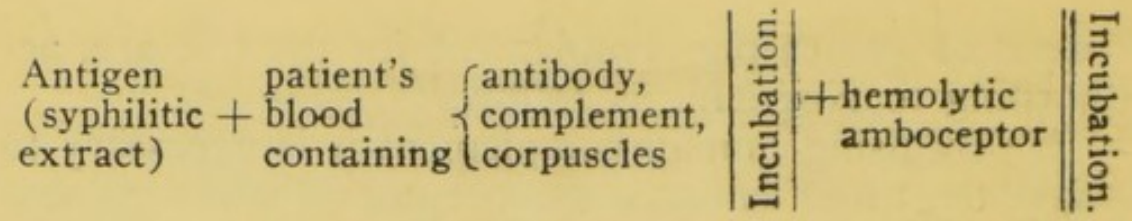
3. The quantity of human complement varies greatly in different sera. If present in excess, the result of the test would always be negative. It is therefore necessary to determine the exact amount of complement present by a preliminary titration, a procedure adding to the difficulty of the test.

4. If human complement is used there is no *direct* way to test the antihemolytic action of the antigen alone, *i. e.* to tell whether inhibition of hemolysis is due to the antigen alone or to the combination of antigen and syphilitic antibody.

5. It is finally impossible by using human complement to test an old specimen of blood, as complement rapidly tends to become inactive. Even the corpuscles begin to disintegrate after a time.

Two of the disadvantages discussed above are mentioned by R. Bauer and Meier[20] as objectionable features in Tschernogubow's test: 1. That human complement varies greatly in amount and 2. That its use necessitates the performance of the test immediately after obtaining the patient's blood. They call attention to the practical point that human corpuscles are not as easy to obtain as sheep's corpuscles.

The step in performing Tschernogubow's modification are as follows:



When Tschernogubow's communication appeared, Noguchi had already completed his antihuman hemolytic system. Noguchi, in his test, employs, as does Tschernogubow, an antihuman amboceptor (*i. e.* a hemolytic amboceptor for human corpuscles), obtained by immunizing rabbits with sheep's corpuscles. Unlike Tschernogubow, he does not depend upon the patient's serum to supply the complement, but uses guinea-pig serum for complement. No inactivation of the patient's serum is necessary as human complement, in the dose used in his test (one capillary drop), is not active against human corpuscles, when 2 units of amboceptor are used. Even in a dosage of 0.1 c.c. it remains without any action at all in the presence of 2 units of amboceptor, and can therefore be entirely disregarded. As indicator, the corpuscles of the patient to be tested or of a normal person are used. The suspension of corpuscles is obtained from the finger or ear and is used in the proportion of one drop to 4 c.c. of physiological salt solution. In this dilution no coagulation of the blood occurs. Noguchi differs from Tschernogubow in using in addition a small amount of serum (containing the antibody). A sufficient amount of serum can be obtained from a half c.c. of blood drawn from the patient's finger or ear. A single drop only from a capillary pipette (which can be easily made in a Bunsen flame) is needed for the test and a second drop for the control. Finally the antigen, complement, and amboceptor have been concentrated, dried, and impreg-

nated in strips of paper, tiny squares of which, representing measured amounts of the impregnated substances are used for each tube. Noguchi has even found it possible to dispense with an incubator by keeping the little tubes in the vest pocket and allowing the body heat to incubate them. In this way he has succeeded in devising a method which is so simple that it can be carried out by any physician without difficulty.

The order of the steps in performing the test is as follows:

1. One drop from a capillary pipette of the serum of the patient to be tested and one drop for the control.

2. One square of paper representing complement or if preferred 0.04 c.c. of fresh guinea pig serum.

3. One c.c. of a suspension of human corpuscles in the proportion of 1 drop of blood to 4 c.c. of physiological salt solution.

4. One square of paper representing antigen, after which the tubes are shaken and incubated in thermostat or vest pocket for a half hour.

5. One square of paper representing the hemolytic amboceptor. The tubes are then incubated again for two hours after which the results are recorded. When examined twenty-four hours later, no change as a rule occurs. The controls are similar to those used in the regular Wassermann reaction.

It the test devised by Noguchi were merely equal in accuracy to the regular Wassermann reaction, its simplicity would mark it as a decided advance in the serum diagnosis of syphilis. That it is actually more delicate and accurate appears from the results obtained thus far by its author. Some rather striking differences in favor of the Noguchi test have been observed among the cases of the writer.

The source of inaccuracy in the Wassermann test according to Noguchi is the presence in the patient's serum of an unknown amount of natural hemolytic amboceptor against sheep's corpuscles, being at times as high as 20 units. The manner in which such as excess of amboceptor may interfere with the test is explained by Noguchi in a critical study to be soon published.

In the first place it is known that increasing doses of amboceptor also increase up to a certain point the activity of the complement. In other words a large amount of amboceptor compensates for a small amount of complement. If, for instance, the complement is not quite fixed in the antigen-antibody combination, the small amount left will not produce complete hemolysis if only 2 units of amboceptor are present. If, however, a large amount of amboceptor is present the effect will be the same as if a larger amount of complement had been present, and complete hemolysis will occur. While one unit of amboceptor + 0.1 c.c. of complement is generally needed to produce complete hemolysis, Noguchi found that if 4, 8, and 20 units were used, hemolysis occurred upon adding 1-3, 1-5, and 1-10 respectively of complement.

In the second place, an excess of amboceptor may bring about dissociation of the complement, *i. e.*, may break up the combination it has formed with the antigen and antibody. If, for instance, a quantity of syphilitic antibody is just sufficient to bind 0.1 c.c. of complement in the presence of 2 units of amboceptor, it will not be able to prevent partial freeing of the complement in the presence of double that amount of amboceptor. For this reason the actual presence of syphilitic antibody may remain unrecognized, as the complement which has been

freed brings about hemolysis and gives a negative reaction, where it should have been positive. When the amount of syphilitic antibody is large, this dissociation of the complement cannot take place, *i. e.* a positive reaction cannot be masked.

It is not my intention to discuss in this communication the many phases of the practical application of the Wassermann reaction. Considering, however, that the subject especially in this country is of such recent development it was thought proper to record the results obtained in examining my first cases. All of these cases were adults, about three-fourths being males.

Table I shows the results obtained in 46 cases of clinically positive syphilis, showing active lesions at the time of examination. Of this number, one was a primary lesion of the lip which had existed sixteen days. It gave a strongly positive reaction. Of the 19 cases belonging to the secondary period (first year) all (*i. e.* 100 per cent.) gave positive reactions. If, however, we add 5 cases from table III, which proved to be secondary syphilis, 4 of which were strongly positive and 1 for some unaccountable reason negative, the proportion of positive cases is reduced to 23 out of 24 or 95 per cent. Twenty-six cases of the tertiary type gave 18 positive, 2 doubtful, and 6 negative reactions, *i. e.* 69 per cent. of the cases were positive. In one case of tertiary syphilis (No. 24) giving a positive reaction, the infection had occurred 23 years previously.

Table II shows the result of testing 21 cases of undoubted syphilis, which at the time showed no clinical evidence of the disease. Most of these cases had been previously seen when lesions were present and a positive diagnosis possible. The others were

TABLE I—CASES OF ACTIVE SYPHILIS.

Case No.	*Stage of Disease	Type of Lesions	Location of Lesions	Time Since First Manifestations	Duration of Lesions	Mercurial Treatment	**Result
1	III	Tuberculo-ulcerative	Buttocks and thighs	Unknown	6 years	Tablets, 3 months	−
2	I	Chancre	Lip	Probably 5 weeks	16 days	None	+
3	III	Tubercular	Forehead and forearm	Unknown	8 months	Medicine, 6 months	+
4	III	Tuberculo-ulcerative	Legs	Probably 3 years	1 year	Medicine, 9 months	−
5	III	Tubercular	Mouth and lips	Probably 1 year	4 weeks	Few inunctions	−
6	III	Tuberculo-ulcerative	Scalp	5 years	1 year	Tablets and medicine, 2½ years	+
7	III	Tubercular	Face, trunk and extremities	Unknown	14 months	Medicine, 14 months	+ +
8	III	Squamous	Palm	Unknown	4 months	Medicine, 1 month	− +
9	III	Tuberculo-ulcerative	Arms and legs	Unknown	5 years	Medicine, 8 months	+ +
10	III	Tubercular	Wrist	3 years	3 months	Medicine, 7 months	+
11	III	Tubercular	Chin	Probably 7 years	1 year	Tablets and medicine, 1 mo.	− +
12	III	Tubercular	Forearms, buttocks, thighs.	3 years	1 year	Tablets and medicine, 3 mo.	+ +
13	II	Papulo-squamous	Forehead, wrists and palms.	Unknown	2 months	None	+ +
14	III	Tuberculo-crustaceous	Abdomen	17 years	5 years	Tablets and medicine, small amount	±
15	II	Papulo-squamous	Palms	Unknown	2 months	Medicine, 6 weeks	+ +
16	II	Macular	General	2 months	5 days	None	+ +
17	III	Gummatous (diffuse)	Knee	Unknown	5 years	Medicine, small amount	+
18	II	Papular (disappearing)	General	Unknown	3 months	Medicine, 3 weeks	+
19	III	Tubercular	Heel	Unknown	5 months	Medicine, 2 years	−
20	III	Gummatous	Heel	7½ years	3 months	Inunctions, 2 years	+
21	II	Papular	General	Unknown	2 weeks	None	+ +
22	II	Papulo-squamous (disap'g)	General	3 months	2½ months	Medicine, 2 months	− +
23	III	Gummatous	Leg	Unknown	1 month	Medicine, 4 months	+ +

24	III	Ulcerative and gummatous (extensive)	Foot	23 years	3 years	Tablets and medicine, 2 yrs.	+
25	III	Gummatous	Wrist	Unknown	6 months	None	±
26	II	Macular	Palm	3 months	2 weeks	None	+ +
27	II	Alopecia syphilitica (dis'g)	Scalp	Unknown	1 year	Medicine, 10 months	+
28	III	Gummatous	Knee	4 years	3 years, at times h'l'd	Medicine, 1 year	+
29	II	Maculo-papular	General	Unknown	6 weeks	None	+
30	II	Maculo-papular (disap'g)	General	Unknown	1 month	Medicine, 2 weeks	+ +
31	II	Macular	General	1 month	10 days	None	+ +
32	III	Ulcerative and gummatous	Leg	Unknown	4 years	Medicine, 2 years	− +
33	II	Papulo-squamous	Face	Unknown	2 months	None	+ +
34	III	Tubercular	Lip	Unknown	4 weeks	Medicine, 10 days	+
35	III	Tubercular	Arm	2 years	1 month	90 injections	−
36	III	Tubercular	Thigh and abdomen	6 years	7 months	Medicine, 4 years	− +
37	II	Papulo-pustular	General	7 months	3 months	None	+ +
38	III	Tubercular	Nose and cheek	Unknown	7 months	Medicine, 1 month	−
39	II	Macular. Chancre persist'g	General	6 weeks	3 weeks	None	+
40	II	Local and gen'l adenopathy		9 weeks		Tablets, 2 weeks	+ +
41	II	Local and general adenopathy. Healing chancre		9 weeks		5 injections Hg. salicylate	+ +
42	III	Gummatous	Leg	14 years	2 years, at times h'l'd	3 years	− +
43	II	Papulo-squamous	General	Unknown	3 months	Tablets and medicine, 3 mo.	+
44	III	Tuberculo-squamous	Foot	Unknown	10 years	Little or none	− +
45	II	Macular	General	9 weeks	5 weeks	Tablets, 1 week	+ +
46	II	Macular. Healing chancre of finger	General	2½ months	6 weeks	None	+ +

*I Primary
II Secondary.
III Tertiary

** + + Strong, positive.
+ Positive.
− + Weak, positive.

± Doubtful
− Negative.

TABLE II—CASES OF SYPHILIS SHOWING NO ACTIVE LESIONS.

Case No.	Stage of Disease	Diagnosis Made When Lesions Were Present	Time Since First Manifestation	Mercurial Treatment	Result
47	III	By writer (private patient)	Probably 4 years	Tablets, 2 years, 10 injections Hg.	±
48	II	By writer (private patient)	1 year	Tablets, 1 year	−
49	III	At clinic. Maculopapular syphilide	3½ months	Tablets, 3½ months	− +
50	II	At Clinic. Chancre of lip and secondaries	5 months	Tablets. 4 months	+ +
51	III	By a colleague	2 years	Inunctions and medicine, 2 years.	+
52	III	At Clinic. Tubercular syphilide a year ago	Unknown	Medicine, 1 year	−
53	II	From patient's statement; pigmentation persisting	1 year	Medicine, 3 weeks	−
54	III	At Clinic. Seven years ago severe ulcerating lesions of scalp.	19 years	Medicine, 7 years	±
55	II	From patient's statement	9 months	Tablets and medicine, 5 months	−
56	III	At Clinic. Thriteen months ago an annular syphilide (early) of chin	Probably 16 months.	Medicine, 4 months	−
57	III	At Clinic	3 years	Tablets, 3 years	−
58	III	At Clinic. Annular syphilide 2 years ago	2½ years	2 years	−
59	III	At Clinic	3 years	Tablets, 3 years	−
60	III	At Clinic. Annular syphilide 2 years ago	2½ years	2 years	−
61	III	At Clinic. Papular syphilide (early) of palm 2 years ago	Probably 2½ years	2 years	−
62	III	By writer (private patient). Chancre lip of 4 years ago	4 years	Tablets, 3 years	+
63	III	By a colleague. Three months ago a possible gumma	12 years	Tablets and medicine, 3 years	−
64	III	From patient's statement	10 years	Medicine, large amount	−
65	III	From patient's statement	18 years	Medicine 1 year at first, 6 months lately	−
66	III	From patient's statement	2 years	2 years	−
67	III	From patient's statement	4½ years	Medicine until 4 months ago, since then injections	−

cases in which a perfectly straightforward history of infection could be obtained. Of these cases 4 gave positive, 2 doubtful, and 15 negative reactions, *i. e.* 19 per cent. only were positive. A glance at the table will show that the majority of these cases had received a fair amount of treatment, tending as is well known to lessen or destroy the reaction. The treatment mentioned in the tables refers only to the use of mercury, iodide of potassium apparently having no effect upon the reaction. The word medicine is conveniently used to indicate that the mercury was taken in a fluid form such as in the customary biniodide mixtures.

Some of the most interesting results were obtained in cases (Table IV) where the diagnosis was in doubt and it will perhaps be of interest to describe a few of these cases at some length.

Table III shows a small series of dermatological cases in which syphilis could be excluded (as far as this is ever possible). All gave negative reactions.

Case 69.—Mulattress, 22 years old, presented recently before a medical society as a case of lupus vulgaris serpigenosus. Shows extensive lesions of face, neck, and legs. Family history of tuberculosis. Previous history of suppurating glands. Cutaneous tuberculosis test strongly positive. Histological preparations favors tuberculosis rather than syphilis. As the case was considered by some to be one of syphilis, a Wassermann test was made, with absolutely negative result. Ten injections failed to cure or even improve the disease. Later generally admitted to be lupus.

Case 76.—Case of syphiliphobia. Patient, a physician, had never shown any symptom or sign of syphilis. Two premature deliveries by his wife made him think he had possibly at some time con-

TABLE III—NON SYPHILITIC CASES.

ALL OF THESE CASES GAVE A NEGATIVE REACTION

98	Acne vulgaris	101	Lichen planus	104	Psoriasis
99	Adenoma sebaceum	102	Lupus erythematosus	105	Scleroderma
100	Dermatitis herpetiformis	103	Lupus vulgaris	106	Xanthoma tuberosum associated with diabetes

TABLE IV—DOUBTFUL CASES.

Case No.	Disease	Mercurial Treatment	Result	Remarks
68	Crusted lesion of lip; syphilis *vs.* impetigo	Inunctions, injections and medicine, 3 months	+ +	Later proved to be undoubted syphilis.
69	Syphilis *vs.* lupus vulgaris	Medicine, 3 weeks	−	
70	Doubtful chancre; possible infection 3 months previously	None	−	Later presented general adenopathy and test became pos've.
71	Peripheral arteritis with symptoms of Raynaud's disease	Numerous injections	−	
72	Tubercular syphilide *vs.* eczema	None	−	Later proved tobe eczema.
73	Previous attacks of iritis; no history of syphilis	Inunctions 2 months	− +	Repeated with same results.
74	Gummata (?); nodules and scars grouped about knee	None	−	
75	Said to have suffered from syphilis ten years previously	Tablets 1½ years, inunctions 6 weeks	±	
76	Syphiliphobia; patient's wife had two premature deliveries; never any other signs or symptoms pointing to syphilis	None	−	
77	Ulceration of nose; history of child born at eight months; no other symptoms.	Medicine, 4 months	− +	Improving under antisphyilitic treatment.

78	Leucoplakia of lip and cheek; chancre (?) 15 years ago	Tablets 1 year, inunctions 6 weeks	−	
79	Syphilis (?); septic metritis	None	−	Impossible to make a second examination.
80	Syphilis *vs.* epithelioma of tongue; no history of syphilis	None	−	Clinical evidence strong in favor of epithelioma.
81	Scabies; possible syphilis	None	+ +	
82	Syphilitic *vs.* traumatic ulcer of lip	None	−	No effect from antisyphilitic treatment.
83	Macular syphilide *vs.* drug rash	None	+ +	
84	Amyotrophic lateral sclerosis; gummata (?) of leg	None	−	Probably not gummata from subsequent course.
85	Miliary papular syphilide *vs.* scabies	2 injections of Hg. salicylate	−	
86	Syphilis *vs.* epithelioma of glans penis	2 injections Hg. salicylate	−	
87	Peripheral endarteritis with symptoms of Raynaud's disease; denies syphilis	80 injections	−	
88	Woman giving history of four children born dead or "mortified"	None	−	Test to be repeated later.
89	Syphilis *vs.* epithelioma of tongue	None	−	
90	Chancre (healing)	None	+	
91	Syphilis of tongue *vs.* leucoplakia	Medicine 3 weeks	− +	
92	Syphilis *vs.* erythematous lupus	Medicine, 3 weeks	+	
93	Syphilophobia	Injections and medicine	−	
94	Syphilitic rhinitis (?); possible chancre 3 years ago	None	−	
95	Possible syphilis; numerous small tumors of leg; gummata (?) 1 year ago; none since antisyphilitic treatment instituted.	Injection 2 months, medicine 5 months.	−	
96	Said to have had condylomata and circinate eruption of penis 1 month ago; general adenopathy at present	Medicine 1 month	+ +	
97	Chancre (?) of clitoris; history of 5 miscarriages and 2 children dying early	None	−	

tracted the disease and infected his wife. The patient's peace of mind was restored by a negative Wassermann reaction.

CASE 81.—Man, 25, genital sore six months ago followed three months later by a second sore. Examination showed typical lesions of scabies, but with the exception of a slight enlargement of inguinal and occipital glands, no sign of syphilis. The writer doubted the existence of syphilis until the blood was tested and showed a very strong positive Wassermann reaction.

CASE 83.—Woman, 42, presented a typical roseola and adenitis. As she had previously taken sajodin, for several weeks, the diagnosis of a drug rash had been made by a colleague of large experience. The Wassermann reaction proved to be strongly positive. In addition the writer found large number of spirochetes, in an ulceration of the vulva, by using the dark field illuminator.

CASE 85.—Man, 24, presented as a case of miliary papular syphilide by an eminent dermatologist. Diagnosis doubted and that of scabies made by many, as signs of severe itching were present. The Wassermann reaction was absolutely negative. The later course of the disease showed that it was not syphilis.

CASE 86.—Case shown as one of epithelioma of the glans penis, considered, however, by a well-known colleague to be syphilis. Wassermann test negative. No improvement from antisyphilitic treatment. Histological examination showed a prickle cell epithelioma.

While the cases quoted above could be easily multiplied, enough has been said to call attention to the practical value of the Wassermann reaction. That the test is now recognized as a diagnostic pro-

cedure of very great value is shown by its well-nigh universal adoption in the hospitals and clinics of Europe.

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