ON THE SACHS-GEORGI TEST

as compared with the Wassermann Test.

A study on 2.000 cases

by

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The formation of a precipitate when the serum of a syphilitic individual is brought into contact with the corresponding antigen was known long ago though not used in current practice.

NEUBAUER and ELIAS, SALOMON, PORGES, MEIER and PORGES and also MICHAELIS observed the formation of a precipitate when syphilitic serum and organic antigen where mixed with lecithin or sodium glycocholate.

JACOBSTHAL noticed flocculation in syphilitic serum, brought in contact with antigen obtained from the liver of a heredo-syphilitic foetus. On this fact he established a new test for the diagnosis of syphilis; yet this phenomenon is not constantly observed, as often sera which give an absolutely positive reaction with the WASSERMANN test, fail to flocculate by the method of JACOBSTHAL. This lack of constancy and the irregularity in the appearance of the precipitate are sufficient to render the method unfit for practical use.

The HERMANN PERUTZ test which next appeared, agrees more perfectly with the WASSERMANN test. The antigen used in this case is altogether artificial being a chemical substance. The main difficulty, in obtaining it in good condition, is due to the nature of the chemical substances used, viz. cholesterin and sodium glycocholate.

MEINICKE observed that both positive and negative sera may flocculate in the presence of a special antigen (though with negative sera a precipitate may be wanting); but whereas in the case of negative sera the precipitate dissolves easily and disappears upon adding a saline solution of titrated concentration, in the case of positive sera the precipitate shows no alteration. In this test the antigen employed is made from bullock-heart in various concentrations.

Of the precipitation tests, rapidly described above, none may be used a-

lone without control by the original WASSERMANN process.

SACHS and GEORGI recently described a process of flocculation which in my opinion, is the best hitherto known, and may substitute the WASSERMANN test, if all the component elements are perfectly known and titrated. The technic is much simpler and the results, when compared with those of the WASSERMANN test, agree very well as shown by all the statistics published up to now.

I shall now proceed to give a more detailed description of the preparation of the reactives and of the technic:

Antigen. The antigen used in the SACHS-GEORGI test is bullock-heart. This should be fresh and only the muscular tissue used, cut into small fragments and triturated in a mortar until it becomes a homogeneous pulp; with this an emulsion is made in absolute alcohol, in the proportion of 1 gramme to 5 cc. It is placed, together with glass beads, in a vial, sealed with paraffin and kept in the incubator for a fortnight during which period it has to be shaken twice daily. Finally it is filtered through filter paper and the extract is ready.

In practice the use of only one extract should be avoided, just as one ought not to use one antigen alone in the WASSERMANN test; it is safer to employ three perfectly titrated and controlled extracts. They may be prepared from different fresh bullock hearts or by mixing pieces of various hearts always using the same method.

The antigen thus obtained constitutes the concentrated solution; for use as a reactive it must be diluted with two parts of absolute alcohol, adding cholesterin and finally diluting with physiological solution in the proportion of 1 to 6. The dilution must be made only when the antigen is about to be used. The concentrated solution should be kept well shut in a cool and dark place.

The antigen used in the SACHS test contains much cholesterin but its proportion varies, as neither the antigenic value of the extract nor of the cholesterin are constant. Hence it is necessary to ascertain the optimum of cholesterin to be added to a certain volume of concentrated solution of antigen after diluting in two parts of absolute alcohol. For this purpose a titration has to be made, so as to ascertain the quantity of cholesterin, which, added to the antigen, will cause it to produce flocculation of a known positive serum and will leave a known negative one unaltered.

For this purpose a centesimal solution of cholesterin should be prepared and kept in a sealed vial.

Titration of antigen.

For titration the following is required:

1.—Concentrated antigen solution.

2.—Absolute alcohol.

3.—1% alcoholic solution of cholesterin.

4.—Known positive sera.

5.—Known negative sera.

A series of 10 test tubes is then placed at hand and 1cc. of the concentrated solution of antigen and 2 cc. of absolute alcohol are mixed in each tube. The alcoholic cholesterin solution is then added, beginning with 0,1 cc. and gradually increasing up to 1 cc. in the last tube. Ten solutions of antigen with augmenting doses of cholesterin are thus obtained. Each tube now contains a different antigen, to be tried with positive and negative sera.

Now we take a new series of ten test tubes in which we dilute each antigen in physiologic solution at 0,85 % in the proportion of 1 to 5. For this purpose 1 cc. of antigen is mixed with 1 cc. of physiologic solution, is well

shaken and finally the remaining 4 cc. are added.

The aqueous solution of all the antigens being ready, there only remains to mix it with the positive and negative sera. The blood sera should be diluted with physiologic solution at 0,85 % in the proportion of 1 to 10. To 1 cc. of these serum dilutions 0,5 cc. antigen is added. The same process is used for all the antigens, mixing them with positive and negative sera.

The tubes are kept in the incubator at 37°C during 24 hours, at the end of which time the results are read. One notes the tube in which flocculation is most intense and then one observes if the negative sera remain unaltered with the same antigen. In that case the right antigen is determined and there only remains to note the titre of cholesterin used.

Titration should be made with many sera, both positive and negative, in order to know the antigen well before it is currently used.

We give a table, by which the technic just described is easily understood. It refers to one extract only and also to one positive and one negative serum. Another table gives the test of antigen with physiologic solution only, for controlling if flocculation without presence of serum occurs.

Any other extract is titrated in the same way. I constantly obtained excellent results by following this technic and have used it in 2000 cases under control by the WASSERMANN test.

Titatrion of Antigen

Tubes	1	2	3	4	5	6	7	8	9	10
Concentrated Antigen extact.	1 cc.	1 cc.	1 cc.	1 cc.	1 cc.	1 cc.	1 cc.	1 cc.	1 cc.	1 cc.
Absolute alcohol.	2 cc.	2 cc.	2 cc.	2 cc.	2 cc.	2 cc.	2 cc.	2 cc.	2 cc.	2 cc.
Alcoholic cholesterin solution 1°/°.	0,1	0,2	0,3	0,4	0,5	0,6	0,7	0,8	0,9	1 cc.
	Dilute the contents of each tube with physiologic saline solution at 0,85°/° in the proportion of 1 to 6.									
Diluted extracts.	0,5	0,5	0,5	0,5	0,5	0,5	0,5	0,5	0,5	0,5
Positive serum at 1/10.	1 cc.	1 cc.	1 cc.	1 cc.	1 cc.	1 cc.	1 cc.	1 cc.	1 cc.	1 cc.
	24 hours in the incubator at 37°C.									
Diluted extracts.	0,5	0,5	0,5	0,5	0,5	0,5	0,5	0,5	0,5	0,5
Negative serum at 1/10.	1 cc.	1 cc.	1 cc.	1 cc.	1 cc.	1 cc.	1 cc.	1 cc.	1 cc.	1 cc.
	24 hours in the incubator at 37°C.									

Saline Sol. at 0,85%	1 cc.	1 cc.	1 cc.	1 cc.	1 cc.	1 cc.	1 cc.	1 cc.	1 cc.	1 cc.
Diluted extracts	0,5	0,5	0,5	0,5	0,5	0,5	0,5	0,5	0,5	0,5

24 hours in the incubator at 37°C.

Technic for the use of the reaction

Having obtained the titrated antigens it is easy to perform the reaction The antigen is prepared by diluting the concentrated solution in two parts of absolute alcohol and adding the quantity of cholesterin determined by titration. The next step is to make the dilution of the antigen in the proportion of 1:6, as explained above. The serum for examination is diluted with ten parts of physiological solution and to 1 cc. of the resulting mixture 0,5 antigen is added. Known positive and negative sera are used as checks and the test is made with all of them. The tubes are kept in the incubator at 37°C. for 20 hours when the results are noted. (The temperature must remain constant.)

In the accompanying table the three extracts and the whole technic are registered.

The best way to read results consists in holding the tube against a dark background, so as to perceive the smallest flocculae.

The results are classified by the more or less intense flocculation using the following signs: +++ when the flocculation is very strong and a deposit of precipitate is formed in the tube; ++ positive with less precipitate: + weakly positive when the precipitate is thin and only perceived against a dark background;—negative when there is no precipitate.

In the statistics published in the portuguese text, page 121 and referring to 2000 sera submitted to Sachs-Georgi and WASSERMANN tests the above mentioned conventional signs are used; furthermore, controls are indicated by the word testemunhos and the reactions carried out with physiological saline liquid by the abbreviation: liq.

The process is the same as for the blood serum and for greater sensibitiveness the liquid may be used pure.

SACHS—GEORGI Test

	Extr. A.	Extr. B.	Extr. C.
Serum to be tested diluted at 1/10	1 cc.	1 cc.	1 cc.
Antigens	0,5	0,5	0,5
Positive serum diluted at 1/10	1 cc.	1 cc.	1 cc.
Antigens	0,5	0,5	0,5
Negative serum diluted at 1/10	1 cc.	1 cc.	1 cc.
Antigens	0,5	0,5	0,5
Saline Sol. at 0,85% ..	1 cc.	1 cc.	1 cc.
Antigens	0,5	0,5	0,5

24 hours in the incubator at 37°C.

SYNOPSIS OF STATISTICS

WASSERMANN TEST positive / SACHS-GEORGI test positive	684
WASSERMANN test negative / SACHS-GEORGI test negative	1,178
WASSERMANN test positive / SACHS-GEORGI test negative	74

WASSERMANN test negative SACHS-GEORGI test positive Concordant results — 93,1% Discordant results — 6,9%	64	Unfortunaly I was not able obtain the clinical diagnostic of the patients, which would have been of great value in the case of discordant results.