As has been pointed out by others, there is no certainty, even if portal thrombosis is the only lesion found at autopsy, that it has caused the condition or even preceded the onset of splenomegaly. Even if it has done both we are still left in doubt as to what has been the cause of the thrombosis.

On the whole I am inclined to look upon this as a case of "portal thrombosis." Possibly the further course of the case may show more definitely the underlying pathological condition. Meanwhile the question arises, Should splenectomy be advised now? Considering the very large size of the spleen, the probable existence of considerable adhesions in the abdomen, and the fact that he is practically free from symptoms, I think the answer should be in the negative.

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THE RECORD OF A BRIEF EXPERIENCE WITH THE SACHS-GEORGI TEST.

BY

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*With an Historical Account of the Development of Flocculation
Tests for Syphilis,*

BY

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OUR only excuse for placing the following notes on record is the fact that hitherto comparatively few collated results of parallel Wassermann and Sachs-Georgi tests have appeared from laboratories in this country. It will doubtless be the policy of the near future to institute carefully planned investigations on the great scale dealing with parallel complement-fixation and flocculation tests with a view to the selection of the best working methods and the correlation with clinical experience of what may prove to be the future routine serological test for syphilis. Pending the appearance of such massed statistics it may be of interest to record the capabilities of a flocculation test performed with material sent from outside sources to the diagnosis department of this institute accompanied by clinical data of varying completeness. Our series comprises only 265 serums forwarded to the institute during the period July to October, 1921. One of us (B.) carried out the routine Wassermann tests, while the other (P.) undertook, solely as a matter of research, the parallel flocculation tests.

METHODS EMPLOYED.

Flocculation Tests.

Two antigens were employed throughout ("D" and "L"), and the results with both are summarized. Antigen "D" was that of Bordet and Ruelens (1919), with the addition of cholesterolin as recommended by Dreyer and Ward (1921), and the latter's technique was strictly adhered to so far as qualitative diagnosis was concerned, no attempt being made in this short series to probe the possibilities of quantitative evaluation. A dropping apparatus was used, and for the first 122 serums five tubes containing dilutions of serum varying from 1 in 1.25 to 1 in 26.4 in a total volume of 25 drops were put up. Later, as the results from the first tube were indefinite, and as a large quantity of serum was required, the first tube was dispensed with. The mixture of antigen, saline, and serum was incubated at 37° C. for seven hours in a water-bath, and the results then read. One batch of antigen was employed throughout, and was preserved in a cool incubator at a temperature of about 22° C. Antigen "L" was simply an alcoholic extract of guinea-pig's heart muscle, to which was added a 1 per cent. solution of cholesterolin in the proportion of 9 parts of heart extract to 1 of the cholesterolin solution.

For the first 132 cases four tubes were put up, containing in a total volume of 26 drops a constant quantity of pure serum (6 drops) and 20 drops of a dilution of antigen varying from 1 in 20 to 1 in 80. For the remaining half of the series the antigen was kept constant in the four tubes (20 drops of a 1 in 40 suspension) and the serum was varied from 1 drop to 6, the total volume being made up to 26 drops.

The dilutions of serum in the tubes were therefore 1 in 3, 1 in 6.5, 1 in 13, and 1 in 26. The serums had been previously heated at 54° C. for 90 minutes. The tubes were incubated for five hours in a water-bath at 37° C. and the results were read 20 minutes after

removal from the bath. They were reread after standing at room temperature for twelve to fourteen hours.

One batch of this antigen "L" was used throughout and experience showed that preservation in the cold (about 4° C.) was most satisfactory. Results with both antigens were recorded as "standard" (easily visible to the naked eye), "trace" (visible with lens), and "negative." At each test controls were used—namely, one Wassermann-positive serum, one Wassermann-negative serum, also previously tested positive and negative Sachs-Georgi samples. The antigen was also put up alone to exclude any non-specific precipitation.

Wassermann Tests.

These were performed with the antigen employed here—namely, an alcoholic extract of guinea-pig's heart muscle, to which is added before the test a 1 per cent. cholesterolin solution in the proportion of 5 parts of extract to 4 parts of the cholesterolin solution. Of this antigen a 1 in 90 dilution was used.

RESULTS.

Total number of serums tested	...	...	...	265
Of these, Wassermann positive	...	...	...	91
Positive flocculation ("L")	...	...	...	86
Positive flocculation ("D")	...	...	...	74

The degree of agreement may be otherwise expressed for the two antigens used, thus:

W+ L+ ...	...	83	W+ D+ ...	...	71
W- L- ...	...	171	W- D- ...	...	171
W+ L- ...	...	8	W+ D- ...	...	20
W- L+ ...	...	3	W- D+ ...	...	5
Total ...	...	265	Total ...	...	265

Thus, comparing the Wassermann and the flocculation tests by "L" antigen the total number of discrepancies in the series was 11, or 4.1 per cent., and in the case of "D" 23, or 8.6 per cent. The 91 positive Wassermann tests are classified as 70 "full positive," 10 "partials," and 11 "slight partials." Corresponding to this grouping the results with "L" and "D" are as follows:

	Total Positive.	Full Positive.	Partial Positive.	Slight Partial.
W ...	91	70	10	11
"L" ...	83	67	9	7
"D" ...	71	61	5	5

Thus 5 of the 8 missed positives with "L" antigen correspond to partial and slight positive Wassermann reactions, and half of those missed by "D" antigen are similarly accounted for. Only three serums yielded positive results with both "L" and "D" antigens, while the corresponding Wassermann tests were negative. In all three the flocculation tests were recorded as "trace" reactions. One was from a latent case under treatment and in which the Wassermann test had been positive seven months previously; another was untreated and was stated to have a rash on trunk and perforation of palate (certainly a suggestive history); while the third came with no particulars.

Treated and Untreated Cases.

One hundred and fifteen of the series were stated to be treated cases, while 150 were entered as untreated or "no particulars." They are thus grouped:

	Total.	W+.	"L"+.	"D"+.
Treated ...	115	31	28	23
Untreated ...	150	60	55	48
Total ...	265	91	83	71

Primary Cases.—There were 20 cases of primary sore, in 9 of which spirochaetes were found. They yielded 9 positive results by all three methods.

Only 5 cases of nerve syphilis appear in the list, with a yield of one positive result with the Wassermann test, the corresponding flocculation tests proving negative.

Discussion.

It has been customary to take the discrepancy percentage as a measure of the efficiency of a flocculation test, and on this basis the results from our small series compare favourably with those recorded by other workers in this country who have published results within the last eighteen months. The discrepancy percentage, however, must in the meantime be regarded solely as a provisional measure of efficiency. The ultimate interpretation of discrepancies between complement-fixation and flocculation tests must, as Lesser (1919) has wisely pointed out in a paper dealing with results by three different methods (Wassermann, Meinecke, and Sachs-Georgi), rest with the clinician, whose record of the past, present, and future clinical course of the case and response to treatment will materially assist the serologist to appraise with some confidence the results from different methods and to eliminate those which prove unsatisfactory. The flocculation test may indeed prove, as Dreyer and Ward suggest, to fit the clinical facts even better than the Wassermann. Even now, with some sixteen years' experience of the Wassermann test, doubtful reactions are constantly occurring (especially in treated cases), and the correlation of these with parallel flocculation tests may serve to place the latter on a proper footing with respect to the clinical condition in the diagnosis of which it is designed to participate. So far, the agreement

between Wassermann and flocculation tests carried out under the best auspices is remarkably good, and there is every reason to expect that further extensive experience of parallel tests in relation to syphilitic disease will lay a sure foundation for the flocculation test as the future test of election for syphilis.

THE DEVELOPMENT OF FLOCCULATION METHODS.

It is, we think, of prime importance that the profession should be familiar with the historical development of a test which, now that the time seems favourable, may have a chance of establishing itself as the method of choice.

The Wassermann reaction dates from 1906, when the paper by Wassermann, Neisser, and Bruck appeared. This test, which at first was regarded as a true antigen-antibody reaction, was made possible by the demonstration in 1901 by Bordet and Gengou that, in the reaction between antigen and antibody, complement, if present, was fixed and consequently rendered unavailable for completion of haemolysis in a haemolytic system. In the following year (1907) it was shown by Landsteiner, Müller and Pötzl, Levaditi and Yamanouchi, and Porges that the effective substance which reacted with syphilitic serum was not present solely in organ extracts of luetic cases, but also in extracts of normal organs. Further, this effective substance was shown by them to be contained almost solely in the alcohol-soluble fraction, its association therefore with a lipoidal substrate being thus demonstrated. In this same year Michaelis noted in one single instance the formation of a visible precipitate when the extract of a luetic liver was brought in contact with a luetic serum. He concluded wrongly that this observation supported Wassermann's view of the specific character of the Wassermann test as a true antigen-antibody reaction. However, chance observation as it was, it gave the impetus to those attempts which have followed each other down to the present time to substitute for the complicated system of the Wassermann reaction a straightforward flocculation test involving two ingredients only. Following this isolated observation of Michaelis, Porges and Meier (1907, 1908) were the first to probe the possibilities of practical application, and, curiously enough, they chose to work, not with alcoholic organ extracts, but with commercial lecithin preparations (from egg yolk), the capacity of which to act as antigen in the Wassermann reaction had already been demonstrated. For many reasons, however, and more especially the instability of the lecithin suspensions and the occurrence of flocculation with normal serums, the use of lecithin was discontinued, and in later work by Porges, in collaboration with Elias, Neubauer, and Salomon (1908), resort was had to the use of sodium glycocholate, a salt possessing colloidal properties in solution, and which Levaditi and Yamanouchi had previously found to function as an antigen in the Wassermann test. Porges states that with this salt as antigen results were achieved which approximated in accuracy to those yielded by the Wassermann test; but it would appear that no thorough testing of flocculation methods was possible or practicable at this period, the Wassermann test in all its many modifications and improvements claiming the undivided attention of serologists and clinicians alike.

In the meantime the properties and mechanism of action of lipoids in serological reactions were being minutely investigated by numerous workers. Among them Sachs and Rondoni (1909), in the course of a study of the antigenic powers of lecithin in the Wassermann reaction, demonstrated an increased action of lecithin in the presence of sodium oleate, and in the following year (1910) Browning, Cruickshank, and McKenzie demonstrated the remarkable action of cholesterol in increasing the amount of complement absorbed in the presence of a mixture of lecithin and luetic serum. Taking advantage of this discovery, Herman and Perutz (1911) mixed cholesterol with sodium glycocholate with the object of obtaining a more visible flocculum in the presence of syphilitic serum. This modification proved in Herman and Perutz's hands more sensitive than the original technique of Elias, Neubauer, Porges, and Salomon with the glycocholate alone, and yielded positive results in certain luetic cases when the original method gave no visible flocculation whatever. Meanwhile the flocculation of these lecithin suspensions again drew attention to the study of the inner mechanism of the Wassermann reaction. Was the complement really fixed by virtue of a precipitin reaction? No visible precipitation, apart from chance observation, was noted, which might account for the fixation of complement

in the presence of alcoholic organ extract and luetic serum. Jacobsthal (1910), however, succeeded in demonstrating by the ultra-microscope the occurrence of floccular masses, the development and growth of which could be followed. He even attempted to employ the method practically, and stated that absolutely negative results occurred with non-luetic serums, but the percentage of positive results was not quite so high as in the Wassermann-positive series.

This work was followed up by Bruck and Hidaka (1910), who found that, by the aid of the centrifuge, precipitates, small indeed in bulk, could be obtained after some prolonged contact of alcoholic liver extract from foetal lues with luetic serum at a low temperature. With the object of making this precipitate more bulky, and consequently more visible, they added mastix to the system, and with this technique it was possible to obtain results approximating to the Wassermann, but a fair percentage of discrepancies occurred. The outcome of this form of study was to support strongly the view that the Wassermann reaction depends primarily on fixation of complement by an invisible precipitate (nascent precipitate) taking place in the earliest stage of the interaction between the alcoholic organ extract and the luetic serum. Apparently the only difficulty now was to secure an extract sufficiently sensitive to show visible precipitation in the presence of luetic serum. Hecht (1916) had definitely successful results, but the most recent and definitive stage in the development of flocculation methods as practical diagnostic aids was reached towards the end of the war, when Meinecke (1917) and Sachs and Georgi (1918) came forward with tested procedures, the latter of which (by Sachs and Georgi) is likely, either in its original or more possibly in some modified form, to become the method of election.

Meinecke's method is known as the two-stage method, and in its ordinarily practised form (the so-called salt method) is carried out on the following lines, purely technical details being omitted:*

An ordinary Wassermann extract in distilled water dilution, and containing no cholesterol, acts as antigen. Mixtures of inactivated serum and antigen in this salt-free medium are kept at incubator temperature for twenty-four hours. At the close of this period all tubes should show flocculation, whether they contain normal or luetic serum. The second stage now commences. A definite amount of a sodium chloride solution of previously determined strength is run into each tube. After an hour's further sojourn in the incubator the tubes are read, when it is found that in the Wassermann-negative series the flocculi are dissolved up, while the Wassermann-positive series remain permanently flocculated.

The salt concentration determined from previous experiment is that which will in one hour just completely dissolve the flocculi in the normal controls, so that differences can be elicited as between strongly positive, weakly positive, doubtful, and negative series.

The Meinecke reaction appears to be a perfectly straightforward and feasible test. It has been tried by many workers in Germany, generally in conjunction with the Wassermann and with the Sachs-Georgi reaction presently to be described. On the whole, however, it has found much less favour than the latter.

With their experience of complement-fixation technique, and their intimate knowledge of the comparative values of simple alcoholic organ extracts and purified lipoids as antigens, Sachs and Georgi (1918) chose as antigen an alcoholic extract of bullock's heart (hearts of other animals are, however, admittedly suitable), to which cholesterol as an absolutely indispensable adjunct is added. For the Wassermann test it is of course not indispensable, though sufficiently advantageous to make its use almost universal, but for the flocculation test its presence is essential for visible precipitation. The cholesterolized alcoholic extract is diluted with five parts of physiological saline. One c.c.m. of a 1 in 10 dilution of the inactivated patient's serum is mixed with 0.5 c.c.m. of the diluted extract along with the necessary controls. The tubes are left either for two hours at 37° C., and then overnight at room temperature, or they are kept continuously at 37° C. for eighteen to twenty hours. Positive reactions are read as +++, ++, and +, while the control serums remain clear or slightly opalescent. Both Meinecke's and Sachs and Georgi's reactions have been extensively tested in large series, and a summary of results in over 12,000 cases by various workers who compared the Sachs-Georgi with the Wassermann reaction in parallel series yielded a conformity

* In flocculation procedures technical details are all-important, but those interested must refer to the original papers cited below.

percentage with the Wassermann of no less than 92.44 per cent. (Sachs and Georgi, 1920).

The most recent advance is that of Dreyer and Ward (1921), who follow in principle the method of Sachs and Georgi, but prefer as antigen, on the ground of greater stability, one recommended by Bordet and Ruelens (1919) for Wassermann tests. To this antigen, which, according to Bordet and Ruelens, can itself be flocculated in the presence of luetic serum, Dreyer and Ward add cholesterol. They claim that with a stable antigen and a refined technique involving the quantitative expression of all degrees of flocculation, the test so conducted can lend itself to accurate standardization in terms of standard units.

Time and experience on the large scale will show how far this claim is justified and compatible with practicability in the hands of average workers, whose power to translate visual impressions into arithmetical language is notoriously variable. In treated cases particularly, the superiority of quantitative readings in flocculation tests over the purely qualitative evidence of the Wassermann would be warmly acknowledged, and it is to be hoped that the extensive parallel series which we understand are in contemplation may furnish important information on this point.

The above sketch is concerned solely with methods based on the mutual interaction of organic colloids and takes no account of the various and, on the whole, unsatisfactory tests which involve merely the precipitation of serum globulins by electrolytes.

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RESIDUAL VACCINES IN ACUTE BACTERIAL DISEASES.

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WHEN a dose of any vaccine is administered the extent of the resulting general reaction is beyond control. In chronic disease and where prophylaxis is intended it is possible to adjust the dose so that the reaction, if any, is small and therefore negligible. But cases which are acutely ill may, and usually do, react violently to a dose which would cause only trivial malaise in the other types of cases. The reason for the lack of control lies in our inability to gauge the effect before administration.

Another objection less frequently heard is to the effect that the patient is already "full of toxins," and any addition is not likely to improve his state. The argument is so plausible that its hypothesis is overlooked and suffered to pass unquestioned. The patient is not "full of toxins" until he is moribund; and since that is a clinical estimation, the lethal amount of toxin may have been reached before or after the patient is estimated to be in that condition. The objection also appears to presume that the human organism is a kind of pot into which a fixed amount of toxin may be poured. In any case the criticism misses the mark as far as residual vaccines are concerned, for they contain neither toxin nor endotoxin. It is evident that lack of control is the real bugbear, and I believe that the system about to be described is one which goes some way towards meeting the difficulty.

The treatment of acute sepsis does not permit delay, consequently it is not justifiable to wait for the preparation of

an autogenous vaccine. A stock vaccine may be below an autogenous in efficiency, but the prospect of success in a given case would be placed in far greater jeopardy by two or three days' delay before commencing treatment.

To be generally useful a stock vaccine must contain the organisms that are most likely to cause such conditions as septicaemia, pyaemia, peritonitis, cellulitis, and large carbuncles associated with grave toxic disturbance. Two vaccines were therefore prepared: the first contained equal numbers of the streptococcus, pneumococcus, staphylococcus, and diphtheroid bacillus; the second contained the colon bacillus instead of the pneumococcus—in other respects it was the same as the first vaccine. A certain degree of discretion is needed to decide which of the two is the more suitable in a given case, but as a simple guide it may be said that the first is intended for all purposes except septic conditions of the abdomen and pelvis, in which the second will be found more generally useful.

A stock vaccine was frequently required in the out-patient department, and the opportunity was taken to note the largest dose that most walking chronic cases could tolerate without a general reaction. A minority showed a reaction with this dose, but they were ignored because it is impossible to assess individual idiosyncrasy on a graduated scale.

The vaccine was next tried with acute cases, starting with very small doses, and increasing them as experience demonstrated their lack of effect. A dose was ultimately reached which was capable of producing marked clinical improvement without causing any discernible clinical reaction. This was true of the great majority; a small minority reacted with undesirable intensity, and the problem they presented still awaited solution. The dose was one-third that of the corresponding dose in the chronic cases, and was approximately 80 millions of the mixed organisms. It will be observed that the vaccines were standardized upon a physiological basis. This method is, in the author's opinion, more exact than the counting methods, which are notoriously only accurate, as far as can be discovered, to within about 40 per cent. This dose, suitable for acute cases, was termed the standard dose.

The solution of the difficulty presented by the minority was found in a close study of the clinical manifestations of the effects of the vaccines. The following facts emerged:

(a) A general reaction in an acute case will be seen, if at all, in six to ten hours after injection.

(b) An improvement will make itself evident in nine to eighteen hours.

(c) It will be admitted that, if two doses of a vaccine are given within a few minutes of each other, the effect will be as if one dose equal to the sum of the previous two had been given. It was found that this rule invariably held good for all periods up to twenty-four hours, and was commonly true up to forty-eight hours.

These three items taken together offer a simple method of dealing with the matter.

Since the lengths of time in (a) and (b) are appreciably shorter than in (c) it is possible to give a fraction of the standard dose, then observe its effect, and if neither reaction or improvement occurs the second fraction can be administered next day. Any reaction following this second fraction is either very mild or, more frequently, so small as not to be recognizable clinically. After the first dose has been completed, in one or two portions, no further vaccine should be given for at least four days; it is better to wait five or six.

The system is very simple in operation and gives such reliable results that there is some danger of it generating a belief that the clinician is working with a remedy of very feeble potency, and that he can do as he pleases upon occasion. When a first fraction produces no result of any sort and the second a marked improvement without visible reaction there is a temptation to "continue" the benefit by giving a third fraction on the third day. If this is done the resulting general reaction may be very disconcerting, and although permanent harm has not ensued, for a few hours the aspect of the patient has been such as to deter me from any further excursions in the same direction.

It is not every case that needs a second dose, or a second fraction of the first dose. The first dose is frequently sufficient to be a turning point in the course of the disease, enabling the patient to recover without further stimulation.

The rapidity with which improvement can show itself throws an interesting light upon the question of a "negative phase" in connexion with this type of vaccine. The patient's tolerance to a second dose is not established for some four or five days after the first dose, and during that time he is