TREPONEMA PALLIDUM AGGLUTINATION TESTS

HAROLD J. MAGNUSON, M.D.
CHARLOTTE P. McLEOD, D.Sc.
Venereal Disease Experimental Laboratory,
United States Public Health Service,
School of Public Health, University of North Carolina,
Chapel Hill, N.C., USA

SYNOPSIS

One approach to obviating some of the difficulties and complexities of the Treponema pallidum immobilization (TPI) test is to be found in the use of T. pallidum as an antigen in agglutination tests. The four main representative techniques of such tests are compared with respect to: strain of organism used, treatment of rabbits, time of harvesting, extracting medium, method of inactivation, preservative, and reagent. The results obtained with the different techniques are then reviewed and the various difficulties met in each are discussed.

The authors consider that the T. pallidum agglutination tests do not yet have a diagnostic value equal to that of the TPI test; the technical difficulties have not yet been overcome, and the immunological interpretation of results is by no means clear. Much still has to be done, and indeed it is possible that entirely different methods may even supersede techniques using whole, virulent T. pallidum as antigen.

The development of the Treponema pallidum immobilization (TPI) test reawakened interest in the use of treponemal antigens in the study of syphilis and related treponemal infections. During the many years that have elapsed since the discovery of the treponeme, the use of this pathogenic organism as an antigen has been described for several types of serological test. The failure of those techniques to attain a perfection warranting their use as diagnostic tools made it seem likely to many observers that the successful employment of pathogenic T. pallidum as a serological antigen would have to await the successful in vitro cultivation of the organism. That such conclusions were unduly pessimistic was clearly shown by Nelson & Mayer’s brilliant achievement.9 The success of the Treponema pallidum immobilization test has led many investigators to re-examine the problems involved in the use of pathogenic T. pallidum as a diagnostic antigen.

Such re-investigation is dictated by many needs. Some have their origin in the practical requirements of day-to-day serological operations, while
others arise from unanswered problems regarding the underlying immunological phenomena. The TPI test has proved a tool of the first magnitude, permitting the resolution of many diagnostic problems that have heretofore defied other serological techniques. Unfortunately, it has had certain obvious practical limitations for the serological laboratory. Even the most experienced laboratories have had obscure periodic difficulties in performing the test. The physical requirements have precluded its performance by most serological laboratories. The variability inherent in a test that must employ an antigen of unknown and variable sensitivity for

### Table I. Comparison of Agglutination Techniques

<table>
<thead>
<tr>
<th>Author</th>
<th>Tani (^{11,13})</th>
<th>Cain (^{1})</th>
<th>McLeod (^{6,7})</th>
<th>Hardy (^{4,8})</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Strain of (T. \text{ pallidum})</strong></td>
<td>Various Japanese</td>
<td>Nichols</td>
<td>Nichols</td>
<td>Nichols</td>
</tr>
<tr>
<td><strong>Treatment of rabbits</strong></td>
<td>None</td>
<td>X-ray (600 r)</td>
<td>None</td>
<td>Cortisone</td>
</tr>
<tr>
<td><strong>Time of harvesting after inoculation</strong></td>
<td>3rd-4th week</td>
<td>Early orchitis</td>
<td>8th-10th day</td>
<td>12th-16th day</td>
</tr>
<tr>
<td><strong>Extracting medium</strong></td>
<td>NaCl</td>
<td>Nelson's basal</td>
<td>NaCl</td>
<td>Sodium citrate</td>
</tr>
<tr>
<td><strong>Method of inactivation</strong></td>
<td>0.5-0.7% antiformin</td>
<td>Heating at 56°C for 30 minutes</td>
<td>Heating at 56° C for 40 minutes</td>
<td>Heating at 65° C for 120 minutes; occasionally re-heated at 100° C for 60 minutes</td>
</tr>
<tr>
<td><strong>Preservative</strong></td>
<td>0.5% phenol</td>
<td>2-4°C; no chemical</td>
<td>4°C; no chemical</td>
<td>0.01% Merthiolate</td>
</tr>
<tr>
<td><strong>Volume of reagents in test:</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>serum</td>
<td>0.01 (0.1 ml in 1:10 dil)</td>
<td>0.5 ml</td>
<td>0.1 ml</td>
<td>0.01 (0.1 ml in 1:10 dil)</td>
</tr>
<tr>
<td>antigen</td>
<td>0.1 ml</td>
<td>0.5 ml</td>
<td>0.1 ml</td>
<td>0.1 ml</td>
</tr>
<tr>
<td>other</td>
<td>Serum diluted to 0.5% phenol in 0.85% NaCl</td>
<td></td>
<td>steer serum in M/7 NaCl</td>
<td></td>
</tr>
<tr>
<td><strong>Maximum concentration of serum</strong></td>
<td>1:20</td>
<td>1:2</td>
<td>1:3</td>
<td>1:20</td>
</tr>
<tr>
<td><strong>Incubation period</strong></td>
<td>3-24 hours at 37°C</td>
<td>6-24 hours at 37°C</td>
<td>2-23 hours at 37°C</td>
<td>18 hours at 37°C</td>
</tr>
</tbody>
</table>
each day's series is such as to give the serologist considerable uneasiness. From a practical standpoint, there thus remains a need for a specific serological test which can be performed in the average serological laboratory, is highly reproducible, can be used on sera collected by routine methods, and can be performed with a minimum of materials. Practical considerations such as these have led many laboratories to seek other treponemal tests which might employ antigens prepared at a central source and which would eliminate many of the complexities of current TPI procedures.

There is a second motivating factor: the need for a more adequate understanding of the nature and significance of the antigen-antibody reactions being measured. Treponema pallidum would be a unique organism were it to have a single antigenic component and were a single antibody the only one that could be demonstrated. As will be noted later, there is increasing evidence that T. pallidum does present a complicated antigen mosaic, only part of which is detected by the immobilization technique. Little of the clinical and immunological significance of these various antigen-antibody reactions has been defined.

One of the approaches to these problems under current investigation in a number of laboratories involves the use of killed T. pallidum as antigen in agglutination tests. The early history of agglutination tests has been reviewed by Chesney 2 and by Tani 18 and will not be considered here. The first of the more recently developed agglutination tests was described by Tani in 1940.12 With the dislocations encountered in the Second World War, Tani's work was neglected by other authors, and Tani himself was forced to suspend his work in 1942. The strains of T. pallidum he employed were lost, and no further work was done on the problem.

Current re-investigations of agglutination technique, utilizing the knowledge gained from the TPI test, were first described by Cain,1 followed by McLeod & Magnuson 6 and by Hardy & Nell.5 The present discussion of different agglutination techniques will be confined to the four methods outlined in Table I. It is believed that these are representative of techniques currently being evaluated, and that they illustrate certain points of difference, the relative merits of which have not been resolved.

### Strains of Organisms Used as Antigens

Authors other than Tani have employed the Nichols strain of T. pallidum as antigen. Tani used several Japanese strains of T. pallidum, which strains, as noted above, were lost during the Second World War. Although it is not possible to determine the immunological relation of these strains to the Nichols strain, it would appear that many strains of T. pallidum could be employed. Turner at a discussion on the TPI test held in Paris in 1954 noted, for example, that satisfactory agglutination antigens can be made with T. pertenue. A priori one would assume that strains which have been well
adapted to the rabbit and are capable of rapidly producing orchitis would be best suited as antigen material.

Treatment of Rabbits

Whether rabbits should be pre-treated—for instance, with X-rays, as done by Cain,\(^1\) or with cortisone, as done by Hardy\(^8\)—is related to two problems: first, that of possible in vivo sensitization of the organism; and secondly, that of obtaining maximum yields of antigenic material. Neither Tani nor McLeod pre-treated their rabbits. Tani’s experiments were performed before it was suggested that \(T. pallidum\) might be so sensitized. McLeod\(^8\) relied upon the rapid evolution of acute orchitis and early harvesting of organisms to obviate the possibility of sensitization. The hypothesis that in vivo sensitization of \(T. pallidum\) may occur during the development of the orchitis has much appeal, and the work of Nelson\(^8\) would tend to substantiate this view. It is not universally accepted that in vivo sensitization of \(T. pallidum\) occurs during the early phases of orchitis. Portnoy and co-workers,\(^11\) in work performed at the Venereal Disease Research Laboratory, Chamblee, Ga, were unable to demonstrate convincing evidence of sensitization of the treponemes in vivo. In the technique proposed by Cain, in vivo sensitization is accepted as a fact and an attempt is made to prevent it by pre-treatment of the rabbits with 600 r of X-irradiation prior to inoculation of the treponemes. Cain notes that treponemes obtained from such treated rabbits did not show spontaneous agglutination, whereas organisms from rabbits not so treated made unsatisfactory antigen.

The use of cortisone as proposed by Hardy may or may not have a suppressive effect on in vivo sensitization. It is known, of course, that cortisone has a profound influence upon the immune mechanisms of the body, but the effect of cortisone on the development of various antibodies to \(T. pallidum\) is only partly understood. There is little question, however, that the use of cortisone will frequently increase the yield of organisms from acute testicular syphilomata. If such use does not change the antigenic composition of \(T. pallidum\), and there is little reason to believe that it does, the use of cortisone as recommended by Hardy is of considerable value in obtaining large lots of homogeneous antigens from a minimum number of infected animals. Hardy starts cortisone treatment on the third day after inoculation, giving daily intramuscular injections of 6.0 mg per kg of body-weight until the animals are sacrificed.

Time of Harvesting

The probable optimum time for harvesting is, of course, related to the rate of development of the orchitis, which in turn is a function of the
strain of organism inoculated, environmental temperature, use of pre-treatment (such as cortisone or X-ray), size of the infecting inoculum, etc. According to Tani’s description, the orchitis in the rabbits infected with his Japanese strains of *T. pallidum* did not reach suitable intensity until three to four weeks after inoculation. This is considerably longer than the period conventionally taken in the more recent American techniques. McLeod, relying on the rapid evolution of the Nichols lesion to obviate possible *in vivo* sensitization, harvested her organisms at 8 to 10 days after inoculation. Although the size of the inocula used in inducing infection in these animals was not precisely calibrated, the inocula were of the order of $10^7$ organisms per testis. It is recognized that the pre-treatment of animals with X-rays will delay somewhat the appearance of clinical orchitis, and it is not possible to determine from Cain’s paper the exact time when these organisms were harvested, except that they were obtained when the orchitis was in an early phase. It appears that the lesions employed by Hardy are somewhat more advanced than those employed by McLeod, harvesting being made 12 to 16 days after inoculation at a time when the animals have a “firm orchitis”.

**Extracting Medium**

Both Tani and McLeod employ isotonic sodium chloride as the initial extracting medium. Cain employed Nelson’s basal medium as used in the TPI test. Cain recognized that this medium may have been more complex than necessary and noted that other workers had used sodium chloride, but he was unable to investigate the matter fully, being forced to conclude his work in its preliminary phases. At present, there appears to be no appreciable advantage in using a medium as complex as Nelson’s basal medium for the extraction of these organisms. The 0.075 M sodium citrate proposed by Hardy has an attractive advantage over sodium chloride. With sodium citrate, the formation of fibrin clots is inhibited, and it appears that the yield of organisms from the testes is increased through the suppression of this coagulation.

**Method of Inactivation**

The unique feature of Tani’s technique was the use of 0.5%-0.7% antiformin as an inactivating agent. It is to these dilute solutions of sodium hypochlorite that Tani attributed his success in developing antigens that did not spontaneously agglutinate. The use of antiformin presented some difficulties. Tani found the product by no means uniform, and a considerable amount of experimentation was required for each lot of antigen.

Although it was not possible to obtain the same brand of antiformin that Tani used, McLeod & Magnuson,⁶ employing antiformin of American
manufacture, were able to duplicate Tani's results. The writers understand that the workers at the International Treponematosis Laboratory Center, Johns Hopkins University, were not successful in repeating Tani's observations. At the time when McLeod repeated Tani's observations, she also investigated other methods of inactivating the treponeme. Various heat treatments, 1/4000 Mapharsen, 0.2% phenol, and 0.1% formalin were all studied. It was found that agglutination characteristics similar to those obtained with the antiformin-treated antigens could be observed with non-inactivated treponemes, treponemes heated at 56°C for 40 minutes, or Mapharsen-killed organisms. All the phenol-killed antigens showed some degree of spontaneous agglutination, and the formalin-killed organisms failed to agglutinate. In view of the variability of various types of antiformin, McLeod decided to rely on the heat treatment of the antigens. It was felt that 56°C for 40 minutes represented the optimum time, and in McLeod's hands the use of higher temperatures resulted in a greater tendency to agglutination by normal serum and by reagin. Cain also employed heat inactivation at 56°C for 30 minutes. The heat inactivation employed by Hardy was somewhat more intense. The antigens were inactivated at 65°C for 120 minutes and, in some instances where these antigens failed to show sufficient reactivity, the antigens were reheated at temperatures as high as 100°C for an additional 60 minutes. Subsequent personal communication from Hardy has indicated that the optimum temperature of heat inactivation of the antigens appears to be related to the storing characteristics of the antigens themselves. Hardy finds that after the antigens have been prepared, there is a gradual increase in their favourable-reactivity characteristics over a period of 4 to 6 months. Some time after this period, the antigens become less satisfactory. The use of higher temperature apparently accelerates this aging process and, in Hardy's hands, results in an antigen with optimum characteristics very early in the aging process. It is the opinion of Hardy that this increased sensitivity, contrary to the views expressed by McLeod, represents an increased sensitivity to non-reagin antibodies. The possible relationship of this antibody to the TPI antibody will be discussed below.

Preservatives

Neither Cain nor McLeod used any type of chemical preservative in storing their antigens, relying on ice-box temperature and sterility alone. Tani employed 0.5% phenol and Hardy employed 0.01% Merthiolate. Apparently, the Merthiolate does not interfere with the reactivity of the treponeme suspensions. This is fortunate since it may permit the rapid evaluation of agglutination techniques through the use of merthiolated serum collections that have been made in the past on well-documented clinical material. Such sera could not be used in the TPI test.
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Reagents

The various volumes of reagents in the four test techniques are given in Table I, but there are certain important differences that merit additional comment. In the technique employed by Tani and by Hardy, the highest concentration of serum tested represents a final concentration of 1:20. The technique suggested by Cain employs a highest concentration of 1:2 and that proposed by McLeod of 1:3. All other things being equal, one would assume that the two latter techniques might offer a somewhat more sensitive test, but this is not necessarily true. Both Tani and Hardy, with their antigens, observed a significant agglutination by normal serum more concentrated than 1:20. For that reason, they have adopted that level as the starting point. All authors incubate their tests at 37°C, there being variations in the time of incubation as noted below.

There are important differences in the diluting agents. Tani employs a 0.5% solution of phenol diluted in M/7 NaCl as a diluent for the sera. This is the same concentration of phenol as that used in the preservation of his antigens. The 0.005M EDTA (ethylene-diamine-tetracetate) employed by Hardy and his colleagues has a particular significance. In their early experiments with agglutination techniques, they found that approximately 35% of normal sera produced agglutination of the pathogenic organisms. The use of EDTA, which is a powerful chelating agent, obviated these difficulties, presumably through the removal of divalent cations from the reaction mixture.

The use of steer serum in the test proposed by McLeod has a very different function. The evolution of this has been adequately described in McLeod's early papers. The steer serum appears to enhance the specific agglutination of the treponemes. It is believed that this favourable effect of steer serum is a function of the conglutinin present in the serum of the bovine species. Since various steer sera vary in their content of natural antibodies agglutinating T. pallidum, it is necessary to titrate various lots of steer serum to determine the dilution which will not agglutinate the organisms and yet be sufficiently concentrated to retain significant conglutinin activity. With most steer sera the optimum dilution ranges from 1:7 to 1:5, but McLeod has encountered some sera that are devoid of conglutinating activity.

The writers understand that some European laboratories have been unable to verify this conglutinin effect, and the technique obviously needs further confirmation.

Incubation

Although all the techniques here discussed involve incubation at 37°C, it appears from recent work of McLeod that temperature in the range 20°-37°C is not a critical factor, although the reaction time may be of
considerable importance. Hardy uses a uniform 18-hour incubation period, while other authors have used both shorter and longer incubation periods. Tani employed a short incubation period, when it was observed that in some sera the organisms disappeared during the longer incubation period. Cain felt that a 6-hour incubation would be adequate even though most of his experiments ran for 24 hours. Some of McLeod's recent studies suggest that the kinetics of the various antigen-antibody reactions occurring in the agglutination of *T. pallidum* may differ. It is her feeling that reagin reacts more rapidly and that comparison of the 2-hour and 24-hour reactions may be a means of differentiating various antibodies.

**Reading of Results**

All the techniques so far proposed have had to rely on darkfield examination of samples from the agglutination tubes to determine the degree of agglutination of the organisms. It has been noted by both Cain and McLeod that in serum showing the most active agglutination, the treponemes may be bound in such tightly grouped clots as to leave the surrounding field essentially devoid of treponemes. Indeed, the tightness of some of these clumps may be misleading to the point that one is apt to conclude that actual lysis of the organisms has occurred. For that reason, two things must be kept in mind in attempting to evaluate these agglutination reactions: first, the degree of clumping; and, secondly, the number of organisms observed in the field. The technique described by Hardy is apparently not quite so susceptible to this complete disappearance of the organism, and he is able to base his readings on the degree of clumping alone. It is in the microscopic reading of results that the agglutination test presents some of the greatest difficulties. While there is little difficulty in differentiating between a serum which is strongly positive and a serum which is frankly negative, the intermediate zone of reactivity is considerable and difficult to interpret. This is particularly true in attempting to perform quantitative agglutination tests. The precise end-point is often difficult to determine. This disadvantage of agglutination techniques is not confined to the agglutination of pathogenic *T. pallidum* but is shared by most agglutination tests. Although this drawback is not prohibitive, it seems that it will not be easily overcome, and it may make other techniques more attractive in spite of the simple mechanics of the agglutination test.

**Results**

The agglutination tests performed by Tani were, of course, done before the TPI test was developed. In view of the limited clinical data presented and the absence of TPI testing, it is rather difficult to evaluate the potentialities of Tani's technique. It would appear that he was successful in develop-
It appears that patients who were seropositive as measured by the Wassermann, Meinicke, and Murata tests. All these 86 serum specimens were positive by the agglutination test, with titres ranging from 1:40 to 1:320. One hundred and thirty-one serum specimens negative to those three tests were similarly tested; 99, or 75.6%, of these were found negative in the 1:10 solution, 19 samples, or 14.5%, faintly positive at the same dilution, and 13 samples, or 10%, positive in dilutions greater than 1:20. Of 33 samples of Wassermann-positive spinal fluids, all were agglutination positive at titres of from 1:4 to 1:80. Thirty-nine Wassermann negative spinal fluids were tested, and of these 38 were negative on the agglutination test and only 1 was positive in a 1:4 dilution.

The first comparison of agglutination tests with TPI tests was performed by Cain. It was the extraordinary correlation between the agglutination results and the TPI results that prompted him to suggest that the agglutination techniques warranted further re-investigation. Unfortunately, Cain was forced to terminate his work in the preliminary stages. Serum specimens were drawn apparently from the United States Navy TPI studies. The place of performance of the lipoidal tests and the type of test done are not stated. It appears that all the patients reported in his paper were seropositive with one or more of the lipoidal tests. Of 109 sera presumably positive with one of the lipoidal tests, in which the history and physical examination showed no evidence of syphilis, 49 were TPI positive, and 60 were TPI negative. All the 49 TPI-positive specimens gave positive agglutination tests. Of 60 TPI-negative specimens, 59 were agglutination negative and one was agglutination positive. The latter was the only specimen in which Cain felt there was a serious discrepancy between the TPI and the agglutination results in his experiments. A group of 13 treated syphilitic patients is reported on. Of these, 8 were TPI positive and agglutination positive as well. Two were TPI doubtful, of which one was agglutination positive and the other agglutination negative. Of 3 TPI-negative patients, one was agglutination positive and two were agglutination negative.

The first comprehensive series involving normal individuals and clinically proved syphilitic patients in which the results of the usual serological tests and those of the TPI and agglutination tests were compared was that published by McLeod & Magnuson. These results are summarized in Table II. The results in this series showed an excellent correlation between the TPI and agglutination results both in the normal individuals and in syphilitic individuals. Among the normal individuals, only one out of 151 specimens tested was agglutination positive, and 3 were agglutination doubtful. In the patients with syphilis, it appeared that the agglutination
test was considerably more sensitive than the TPI test in primary syphilis and that the sensitivity was equal to or better than that of the lipoidal test. The results with patients suspected of having biologically false positive reagin tests are also shown. Of 43 such patients on whom definitive TPI results could be obtained, 28 were TPI positive and 15 were TPI negative. Of the 28 TPI-positive patients, 27 were agglutination positive and one was agglutination negative. Of the 15 TPI-negative individuals, 4 were agglutination positive and 11 were agglutination negative.

Hardy has done parallel agglutination and TPI testing on an extensive series in which the agglutination results usually, but not always, agree with the results of the TPI tests. Unfortunately, these studies have not yet been published.

**Difficulties**

To the casual reader, it might appear that one or more of the agglutination tests proposed presents a ready solution to many of the problems encountered in testing sera with treponemal antigens. Such is far from the case. Reports from many European laboratories attempting to duplicate the American findings indicate clearly the difficulties in obtaining satisfactory antigen suspensions. At the moment, it does not seem possible to relate these difficulties to whether or not X-rays or cortisone have been employed. It is difficult to relate these unsatisfactory antigens to the age of the orchitis at
the time the treponemes are harvested. It has been difficult to predict which lots of antigen will be satisfactory and which unsatisfactory. It is possible that Hardy's recent observation on the aging effect of these antigens may contribute materially to the solution of some of these difficulties. Several laboratories have had difficulties in repeating McLeod's observations. Part of these difficulties may be due to differences in the conglutinin activity of the sera employed, since McLeod & Stokes have recently found that some steer sera may be completely devoid of conglutinating activity.

Over and above the difficulties involved in the preparation of suitable antigen and in mastering the problems of reading end-points, there looms an even larger problem. That is related to the type and significance of the antibodies being measured. With the excellent correlation of TPI and agglutination results in the early reports, it seemed likely that the antibody measured in the agglutination techniques was similar to, if not identical with, the \textit{T. pallidum} immobilizing antibody. However, further work by McLeod & Stokes clearly demonstrated that reagin was capable of agglutinating the pathogenic organism. The most convincing demonstration of this was found in the fact that reagin-containing serum produced by immunizing rabbits with lipoid reagin aggregates could produce agglutination of \textit{T. pallidum}. It was also shown that the absorption of syphilitic sera with VDRL (Venereal Disease Research Laboratory) antigen removed some, but by no means all, of the agglutinating activity of that serum for pathogenic \textit{T. pallidum}. It should be noted that reagin serum does not produce immobilization of the treponemes in the TPI test and that absorption of syphilitic sera with VDRL antigen produces little, if any, change in the TPI titre.

That reagin and TPI or similar antibodies are not the only ones reacting with pathogenic \textit{T. pallidum} is further suggested by some unpublished experiments performed by Dr W. E. Vannier of our laboratory and reported at the 1954 Symposium on Recent Advances in the Study of Venereal Diseases, held in Washington, D.C. Dr Vannier employed electrophoresis convection to fractionate the antibodies in the syphilitic rabbit serum. It was a fairly simple matter to separate TPI antibody from reagin in distinct serum fractions. However, there was no such clear separation of antibodies producing agglutination to pathogenic \textit{T. pallidum}. There was a spread of agglutinating activities across many serum globulin fractions suggesting that several antibodies may be involved in the agglutination of the organisms and not simply reagin and TPI-like antibodies.

It is a moot question at the present time whether the component reacting with reagin in the \textit{T. pallidum} agglutination test is actually a component of the organism itself or whether it may be a function of some tissue lipoid absorbed on the pathogenic organism. Since all the present agglutination tests must rely upon treponemes obtained from tissues, it is not an easy matter to resolve this dilemma. It seems likely that a final
solution must await the chemical fractionation of the pathogenic organism itself. It may be noted, however, that a lipoid similar to that obtained from mammalian tissue has been demonstrated in the Reiter strain of cultured *T. pallidum* by D’Alessandro,9 Puccinelli,10 and other Italian workers. One cannot easily dismiss the possibility that a similar lipoid may, indeed, be an integral part of the pathogenic organism.

The reactivity of *T. pallidum* agglutination antigens with reagin presents many problems from the diagnostic and immunological standpoint. Hardy & Nell 5 have recently proposed eliminating this reagin limitation by retesting agglutination-positive sera after they have been absorbed by VDRL antigen to remove reagin. It remains to be seen how practical this solution is, and whether it begs the question whether reagin is an antigen component of the treponeme. Although Hardy reports that he has had excellent results with a single-stage absorption, it seems to us that the total removal of reagin from serum in a one-stage absorption process, in which the same technique is applied to all sera regardless of reagin titre, may be fraught with difficulties. Although we have not had an opportunity to employ Hardy’s technique, it has been our experience that there is considerable variation in the ease with which reagin may be removed from different sera. In many instances, we have been forced to employ repeated absorptions in order to achieve complete removal. For that reason, a one-stage removal would seem open to criticism unless each serum is tested after absorption to make sure that all the reagin has been removed.

Pending the solution of the problem whether multiple antigen-antibody reactions may be involved, there is a very insidious danger in evaluating the efficacy of all new treponemal tests against the TPI results. This was very aptly put by T. B. Turner in a conference on agglutination techniques in Baltimore, Md., in April, 1954, in which he pointed out that the clinician has now changed his criteria for biologically false serological tests. The clinician now requires that the patient also be TPI negative in addition to all the conventional and older criteria for a biologically false positive test he now insists on. From the practical clinical management of the patients, that can be recognized as a sensible procedure. From the immunological standpoint, it may not be quite so acceptable. It would seem far better to insist that these techniques be ultimately evaluated against the total clinical findings. For obvious reasons, whenever it is possible, these comparisons should be made with simultaneous TPI or TPIA (*Treponema pallidum* immune adherence) testing, but to make the TPI results the ultimate authority involves the use of a secondary rather than a primary standard.

At the moment, it appears to the writers that the agglutination tests do not have a diagnostic value equal to that demonstrated by the TPI test. The technical difficulties have not been overcome, and the immunological interpretation is by no means clear. It is to be hoped that the practical
pressures of developing truly specific serological tests employing killed antigens will not force the premature widespread clinical use of these procedures. Much remains to be done, and it is entirely possible that before this work is accomplished, alternative approaches such as that employing chemical fractionation of the organism may supersede all the techniques currently employing whole, virulent *T. pallidum* as antigen.

**RÉSUMÉ**

Le test d’immobilisation du tréponème (TPI) a permis de résoudre plusieurs problèmes de diagnostic, jusqu’alors rebelles à d’autres méthodes. Malheureusement, ce test aussi a ses inconvénients et ne peut satisfaire encore aux exigences des laboratoires qui réclament un test pratique, facile à répéter, applicable à des sérum prélevés par les méthodes courantes et demandant de petites quantités de matériel seulement. Ces considérations ont engagé plusieurs laboratoires à élaborer d’autres tests à base d’antigène tréponémique. La recherche a porté en particulier sur l’agglutination du tréponème tué. Quatre tests principaux (Tani, Cain, McLeod et Hardy) ont été proposés. Les différences entre ces tests portent sur la souche de tréponème utilisée, le traitement des laps avant l’inoculation, le liquide d’extraction, la méthode d’inactivation, l’agent conservateur, le volume des réactifs, la concentration maximum du sérum et la durée de la période d’incubation. La comparaison, faite sur des séries complètes de sérum syphilitiques et non syphilitiques, a montré une concordance étroite entre les résultats du TPI et ceux des tests d’agglutination du tréponème.

L’adoption des tests d’agglutination n’apporterait cependant pas la solution du problème: certains chercheurs n’ont pu reproduire de façon satisfaisante les résultats obtenus dans d’autres laboratoires, et cela sans que la cause de l’échec puisse être déterminée. Mais au-delà de la question de la préparation des antigènes ou de la lecture des résultats, surgit un problème plus général: celui de la nature des anticorps mesurés. La concordance entre le TPI et les tests d’agglutination du tréponème suggérait que les anticorps en jeu étaient analogues, sinon semblables, dans les deux types de réactions. Or, des travaux ultérieurs ont montré que la réagine agglutinait l’agent pathogène, même après absorption des sérum par l’antigène du VDRL, mais que, en revanche, elle ne provoquait pas l’immobilisation du tréponème dans le test TPI. Des expériences récentes (non publiées) ont porté sur le fractionnement par électrophorèse des anticorps du sérum de lapin syphilitique. La séparation de la réagine des anticorps TPI a été assez facile; il n’en a pas été de même de la séparation des anticorps agglutinant *T. pallidum*. La propriété agglutinante était répartie sur plusieurs fractions de la globuline sérique, indiquant que plusieurs anticorps indépendants de la réagine et des anticorps immuns participaient à la réaction d’agglutination. Il n’est pas possible de dire actuellement si le composé réagissant avec la réagine dans le test d’agglutination appartient au siprochète lui-même ou si c’est un lipotide tissulaire provenant de l’animal sur lequel ont été prélevés les tréponèmes. Le fractionnement chimique du tréponème fournirait des éléments permettant de répondre à cette question. En attendant que soit résolu le problème des anticorps multiples, il est dangereux d’apprécier, par rapport au test TPI, la valeur de nouvelles épreuves. Actuellement, le clinicien exige que le sérum du sujet examiné soit négatif au test TPI — en plus des autres épreuves réglementaires — avant de conclure à une fausse réaction positive. Du point de vue immunologique, cette exigence est discutable. Il serait préférable d’évaluer l’ensemble des données de ces techniques par rapport à l’ensemble des données cliniques. Il y aurait lieu, au moins, d’employer simultanément le test TPI et le test d’immunoadhérence (TPIA), car le test TPI seul ne représente guère qu’une référence de deuxième ordre.
De l’avis des auteurs, les tests d’agglutination du treponème n’ont pas une valeur diagnostique égale à celle du test TPI. Les difficultés techniques inhérentes à ces tests ne sont pas vaincues et l’interprétation immunologique est loin d’être claire. Il est à espérer que l’emploi des tests à antigène tué ne se généralisera pas prématurément. Il reste beaucoup à faire et il est possible que, avant qu’aient abouti les recherches visant à perfectionner ces tests, d’autres méthodes — par exemple celles qui feraient intervenir le fractionnement chimique des treponèmes — relèguent à l’arrière-plan les techniques employant comme antigène le T. pallidum virulent, dans son intégralité.

REFERENCES