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# Rabbit globulin and antiglobulin factors associated with *Treponema pallidum* grown in rabbits

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The two major confirmatory tests for syphilis are the *Treponema pallidum* immobilization (TPI) test (Nelson and Mayer, 1949), and the fluorescent treponemal antibody-absorption (FTA-ABS) test (Hunter, Deacon, and Meyer, 1964). Since *T. pallidum* cannot be cultivated *in vitro* (Willcox and Guthe, 1966), the organisms used as antigen in both tests are maintained by injecting testicular extract containing *T. pallidum* into the testes of rabbits.

The extent of the immunological response of the rabbit host to the testicular extract containing *T. pallidum* during the 7- to 9-day growth period is not well understood, particularly with respect to an immune response to the rabbit material. Milgrom (1965) reported that antisera from rabbits immunized with altered autologous gamma globulin had all the serological properties of rabbit antisera against human gamma globulin. Reactivity with some human sera may be due to antibody development to testicular material accompanying *T. pallidum* subsequently used as test antigen.

In this paper, evidence is presented that globulin and antiglobulin factors are present in close association with *T. pallidum* when these organisms are obtained from rabbits and that these factors react with human as well as rabbit globulin.

## Material and methods

### *TREPONEMA PALLIDUM* ORGANISMS

The TPI testing unit of this laboratory supplied the fresh lots of *T. pallidum* employed in these studies. These treponemes were harvested from rabbit testicular syphilomas that developed 5 to 10 days after intratesticular injection with *T. pallidum*. These organisms met all the requirements established for the TPI test (Communicable Disease Center, 1964). The suspensions of fresh organisms were stored 1 to 2 weeks at 4°C. in the extraction fluid. Additional lots of *T. pallidum* were lyophilized until reconstituted for use as antigen in the FTA-ABS test (Communicable Disease Center, 1969).

### RABBIT SERA

Sixteen rabbits were inoculated intratesticularly with not less than $2{\cdot}5 \times 10^7$ *T. pallidum* per rabbit. The rabbits were bled before and 8 days after inoculation. Most sera were stored at 4°C., but a few were stored at —20°C. All paired sera were examined in the same test run.

### ASSAY FOR ANTIGLOBULIN FACTORS

Since Milgrom (1965) has reported that rabbit antiglobulin factors may show specificity for rabbit and/or human globulin, each specificity was assayed.

To detect antihuman globulin reactivity, latex particles sensitized with human gamma globulin (RA test, Hyland Laboratories*) were used. The rabbit sera were tested unheated at a dilution of 1 : 20 in glycine buffer, pH 8·0. The undiluted rabbit testicular extracts (containing *T. pallidum*) were tested unheated and after heating at 56°C. for 30 min. Antiglobulin agglutination reactivity was scored on a scale ranging from ± to 4+.

The Rose-Heller test reported by Sonnenwirth (1963) was used to assess reactivity against rabbit globulin. In brief, fluids were tested for their ability to agglutinate sheep erythrocytes sensitized with a subagglutinating dose of rabbit haemolysin. As described in the technique, the rabbit sera were heated at 56°C. for 30 min. and absorbed with normal sheep red blood cells (RBC) before being tested. Standard methods (Communicable Disease Center, 1959) were employed to prepare both the haemolysin and the sheep RBC suspensions.

### INDIRECT FLUORESCENT ANTIBODY (IFA) PROCEDURE

The IFA procedure was modelled on the fluorescent reponemal antibody-absorption (FTA-ABS) test (Communicable Disease Center, 1969), and was performed in the following manner. Approximately ·005 ml. of the *T. pallidum* antigen was applied to a 1 cm. area of a glass slide and air-dried for at least 15 min. The slides were immersed in acetone for 10 min., removed, and air-dried. The fixed antigen smears were covered with phosphate buffered saline (PBS) or rabbit serum diluted 1 : 5 in PBS or sorbent as described by Stout, Kellogg, Falcone, McGrew, and Lewis (1967), placed in a moist chamber, and incubated for 30 min. at 37°C. The slides were then washed for two 5-min. periods in PBS, and at the end of each period were raised and lowered in the PBS for a total of 25 times. They were removed, immersed once in distilled water, and blotted dry. The appropriate

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fluorescein isothiocyanate (FITC) conjugated antiserum was overlaid on the test site, and the slides were incubated and washed as before. Mounting medium (9 parts glycerine 1 part PBS) was placed on the slides, and cover-slips were added. Reactive sera, nonreactive sera, and nonspecific staining controls were included in each test run. All slides were examined with a Leitz Fluorescence Microscope, HBO-200 Osram lamp, primary BG-12 and secondary OG-1 filters, 42 × high dry lens with a dark-field condenser. Fluorescence was scored on an arbitrary scale from 1+ to 4+. Weak fluorescence was indicated by ±. The same observer scored all slides throughout this study.

#### FITC-CONJUGATED ANTISERA

FITC-conjugated antihuman globulin (Sylvana) that met all requirements for the FTA-ABS test (Communicable Disease Center, 1969) was used. The FITC-conjugated goat antirabbit globulin was obtained from Microbiological Associates.

The goat antiserum to rabbit IgG was produced against purified rabbit IgG isolated by the method reported by Julian, Logan, and Norins (1969) at this laboratory. The globulins from this antiserum were conjugated with FITC through the courtesy of Dr. Edward Shanbrom of Hyland Laboratories. By using the immunodiffusion technique reported by Ouchterlony (1968), it was demonstrated that this conjugate contained only IgG.

Working dilutions for use in immunofluorescence assays were determined for all conjugates by standard methods in the FTA-ABS technique.

#### REITER TREPONEMES USED FOR ABSORPTION

Reiter treponemes used for absorbing conjugates and Cohn fractions were grown in thioglycollate broth supplemented with 10 per cent normal rabbit serum that had been heated 2 hrs at 60°C. Before use, Reiter treponemes were washed three times in PBS. Equal volumes of packed Reiter treponemes were mixed well with either FITC-conjugated antiserum or human Cohn fractions II and $III_1$ or rabbit Cohn fractions II and III and incubated at 37°C. for 1 hr and then at 4°C. overnight. The treponemes were removed by centrifugation in a Sorvall RC2-B at 27,138 G for 30 min.

#### COHN FRACTIONS USED FOR INHIBITIONS

Human Cohn fractions II and $III_1$ were obtained from Lederle Laboratories, Division of American Cyanamide Co., Pearl River, N.Y., and rabbit Cohn fractions II and III from Hyland, Division Trevenol Laboratories, Inc., Los Angeles, Calif. Before use, the Cohn fractions were filtered through Sephadex G-200. By using the immunodiffusion technique, Cohn fraction II was shown to contain only IgG, and Cohn fraction III to contain only IgM. Solutions (containing 1 mg. per cent. in PBS) of human Cohn fractions II and $III_1$ and rabbit Cohn fractions II and III were used to inhibit the FITC-conjugated goat antirabbit IgG. The Cohn fractions used for inhibitions were first absorbed with Reiter treponemes to remove antibodies that might react with the reported 'common' treponemal antigen reported by Deacon and Hunter (1962). One volume of each of the absorbed Cohn fractions was added to an equal volume of the fluorescent antiserum and incubated for 30 min. at room temperature. The conjugate was further diluted to its working dilution.

#### OTHER SEROLOGICAL TESTS FOR SYPHILIS (STS)

The Venereal Disease Research Laboratory (VDRL) slide test, the one-fifth volume Kolmer test with cardiolipin antigen, the one-fifth volume Kolmer with Reiter protein antigen (KRP or RPCF), the TPI, and the Rapid Plasma Reagin (RPR) (Teardrop) card test were performed according to standard methods (Communicable Disease Center, 1964, 1969).

## Results

*Reactivity of three different FITC-conjugated antisera with* T. pallidum

Ten fresh and two reconstituted lyophilized lots of *T. pallidum* organisms were tested for their ability to react with FITC-conjugated antisera: antihuman globulin, antirabbit globulin, and antirabbit IgG globulin. The antihuman globulin conjugate (at the 1 : 600 dilution recommended for use in immunofluorescent tests for syphilis antibodies) did not stain any of these lots of *T. pallidum*. On the other hand, either the antirabbit conjugate or the antirabbit IgG conjugate, or both, stained the organisms (Table I). However, with either of the fluorescein-labelled conjugates, some lots of the *T. pallidum* stained strongly, but others stained only weakly or not at all. To determine possible reasons for the observed staining, the FITC-conjugated goat antirabbit IgG was absorbed with Reiter treponemes to remove any 'natural' or 'common' goat antitreponemal antibody from the conjugate. The absorption removed the ability of the antirabbit IgG to stain several lots of *T. pallidum*. However, the conjugate still stained at least four lots of *T. pallidum* (Table I).

*Effect of globulin inhibition on staining of* T. pallidum *by FITC-conjugated antirabbit IgG*

On the basis that the conjugate might be recognizing rabbit globulin associated with *T. pallidum*, other samples of the conjugate were inhibited with Cohn fractions known to be rich in globulins (Table II). After inhibition with rabbit Cohn fractions II and III, the antirabbit IgG produced no more than ± staining on any of the lots of *T. pallidum*. Inhibition of the conjugate with human Cohn fraction II and $III_1$ removed reactivity of the conjugate to some, but not all, lots of *T. pallidum*.

TABLE I *Reactivity of FTIC-conjugated antirabbit globulin antiserum with* T. pallidum *grown in rabbits*

| T. pallidum *lot number* | *Days from inoculation to harvest* | *Staining with FITC-conjugated goat antisera* | | |
|---|---|---|---|---|
| | | *Antirabbit globulin* | *Antirabbit IgG* | |
| | | | *Not absorbed* | *Absorbed with Reiter treponemes* |
| 268F | 7 | 4+ | 4+ | 1–2+ |
| 262F | 8 | 3+ | 4+ | — |
| 665L | 9 | 2+ | 3+ | 2+ |
| 271F | 9 | 2+ | 3+ | 1+ |
| 250F | 8 | 2+ | 3+ | ± |
| 259F | 8 | 1+ | 2+ | ± |
| 258F | 7 | 1+ | 2+ | Not tested |
| 664L | 9 | 1+ | 2+ | 1+–2+ |
| 273F | 8 | 1+ | 1+ | ± |
| 266F | 10 | 1+ | 1+ | — |
| 274F | 6 | — | 1+ | — |
| 267F | 7 | — | 1+ | ±–1+ |

F = Fresh *T. pallidum* suspension
L = Lyophilized rehydrated *T. pallidum* suspension

TABLE II *Effect of globulin inhibition on the staining of* T. pallidum *(grown in rabbits) by FITC-conjugated antirabbit IgG*

| T. pallidum *lot number* | *Staining by FITC-conjugated goat antirabbit IgG* | | | | |
|---|---|---|---|---|---|
| | *Not absorbed* | *Inhibition with Cohn fraction** | | | |
| | | *II (Rabbit)* | *III (Rabbit)* | *II (Human)* | *III-1 (Human)* |
| 268F | 4+ | — | — | — | — |
| 262F | 3+ | — | — | ± | — |
| 665L | 3+ | ± | — | 2+ | 2+ |
| 259F | 2+ | ± | — | 1+ | ± |
| 250F | 2+ | ± | — | — | — |
| 258F | 1+ | — | ± | 1+ | ± |
| 267F | 1+ | — | — | ± | ± |
| 266F | ± | — | — | — | — |

*Before use in this experiment, all globulin solutions were first absorbed with Reiter treponemes

*Assay for antihuman and antirabbit factors in extracts of rabbit testicular syphilomas*

Since it appeared that globulins were associated with *T. pallidum* by the time the organisms were harvested, it seemed of interest to establish if antihuman and antirabbit globulin factors were also present within the extracts of the rabbit testicular syphilomas. Thirteen lots of extracted organisms (eleven freshly harvested for use in the TPI test and two lyophilized rehydrated lots prepared as FTA-ABS test antigens) were tested with latex particles sensitized with human gamma globulin. Seven of the thirteen lots showed reactivity of 2+ or greater when tested undiluted, but heating the extracts (at 56°C. for 30 min.) before testing practically abolished this reactivity (Table III). The thirteen lots of extracted organisms were tested in the Rose-Heller technique for reactivity against rabbit globulin, but gave nonreactive results.

TABLE III *Effect of heating on reactivity of extracts of rabbit testicular syphilomas by using latex particles sensitized with human globulin*

| T. pallidum *lot number* | *Reactivity with human globulin* | |
|---|---|---|
| | *Extract unheated* | *Extract heated* |
| 2602F | 1+–2+ | –± |
| 3167F | 1+ | –± |
| 8367F | ± | –± |
| 676L | 2+ | — |
| 677L | 4+ | — |
| 82267F | — | — |
| 3991F | 2+ | — |
| 3996F | 3+–4+ | — |
| 81767F | 2+ | — |
| 3992F | — | — |
| 3967F | 3+ | 1+ |
| 8567F | — | — |
| 8467F | 2+ | — |

*Presence of antihuman and antirabbit globulin factors in the serum of rabbits inoculated with* T. pallidum

To determine if the anti-globulin factors demonstrable in the extracts of rabbit testicular syphilomas were part of a generalized antiglobulin response, the sera of rabbits in which *T. pallidum* had been grown were tested.

The paired sera from sixteen rabbits were tested for reactivity by using latex particles sensitized with human gamma globulin. All preinoculation sera were nonreactive; however, twelve of the postinoculation samples had significant amounts of antiglobulin reactivity (Table IV).

TABLE IV *Presence of antihuman globulin reactivity in the serum of sixteen syphilitic rabbits 8 days after inoculation with a single lot of* T. pallidum *(RA test, Hyland Laboratories)*

| *Rabbit number* | *Antihuman globulin reactivity* | |
|---|---|---|
| | *Pre-inoculation* | *8 days after inoculation* |
| 3917 | — | 4+ |
| 3919 | — | 3+–4+ |
| 3903 | — | 3+ |
| 3906 | — | 3+ |
| 3913 | — | 3+ |
| 3920 | — | 3+ |
| 3909 | — | 2+–3+ |
| 3911 | — | 2+ |
| 3914 | — | 2+ |
| 3915 | — | 2+ |
| 3923 | — | 2+ |
| 3916 | — | 1+–2+ |
| 3910 | — | — |
| 3912 | — | — |
| 3921 | — | — |
| 3924 | — | — |

The same sixteen paired sera were also tested for reactivity against rabbit globulin by using the Rose-Heller test. One serum with reactivity in the pre-inoculation sample had an increase in titre, and two other samples showed reactivity after 8 days of infection (Table V).

TABLE V *Presence of antirabbit globulin reactivity in the serum of sixteen syphilitic rabbits 8 days after inoculation with a single lot of* T. pallidum. *These are the same rabbit sera shown in Table IV (The Rose-Heller test)*

| | *Antirabbit globulin reactivity* | |
|---|---|---|
| *Rabbit number* | *Pre-inoculation* | *8 days after inoculation* |
| 3903 | — | + (1:7) |
| 3912 | — | + (1:7) |
| 3919 | + (1:7) | + (1:56) |
| 13 other rabbits | — | — |

*Reactivity of sera from rabbits inoculated with* T. pallidum *in standard tests for syphilis*

The appearance of antiglobulin reactivity (Table IV) might be put in better perspective as one of several responses of the host rabbit to the stimulus of a 7- to 9-day growth of *T. pallidum* if it could be shown that reactivity also appeared in other standard tests for syphilis. To examine this possibility, the same preinoculation and 8-day postinoculation serum samples were examined in several nontreponemal and treponemal tests. Table VI shows that a number of the preinoculation samples from apparently 'normal' rabbits were reactive in the VDRL slide, Kolmer, and KRP tests, but not in the FTA 1 : 5 in buffered saline, FTA-ABS, or TPI tests. The postinoculation sera showed development of or increased reactivity in virtually all of the nontreponemal tests. In the treponemal tests increased reactivity was seen in the KRP and FTA 1 : 5 in buffered saline, but no reactions were observed in the TPI and FTA-ABS tests.

## Discussion

To the best of my knowledge, this is the first report that antirabbit and antihuman globulin factors are associated with *T. pallidum* organisms that are routinely obtained from rabbit testicular syphilomas and that similar factors are present in the serum of the donor animals. If the *T. pallidum* organism itself is the stimulus, then the appearance of an antiglobulin response within the 7 to 9 days seems to be more rapid than the appearance of antiglobulin factors which arise in rabbits after intensive immunization with a variety of substances, including bacteria (Milgrom, 1965).

Another possible stimulus is rabbit gamma globulin. Since the organisms injected in the initial inoculum were extracted from rabbits, rabbit serum proteins, including gamma globulin, from the donor animal entered the extraction fluid along with the

TABLE VI *Reactivity of rabbits in serological tests for syphilis before and after intratesticular growth of* T. pallidum *for 8 days*

| | *Non-treponemal* | | | | | | *Treponemal* | | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| *Rabbit number* | *VDRL* | | *RPR card* | | *1/5 volume Kolmer* | | *KRP (RPCF)* | | *FTA 1:5 BS** | | *FTA-ABS* | *TPI* | |
| | *Inoculation* | | *Inoculation* | | *Inoculation* | | *Inoculation* | | *Inoculation* | | *Inoculation* | *Inoculation* | |
| | *pre-* | *post-* | *pre-* | *post-* | *pre-* | *post-* | *pre-* | *post-* | *pre-* | *post-* | *post-* | *pre-* | *post-* |
| 3903 | — | R 4 | — | R | R | R | R | R | — | R | — | — | — |
| 3906 | — | R 1 | — | R | — | R | R | R | ± | R | — | — | — |
| 3909 | — | R 8 | — | R | R | R | R | WR | — | R | — | — | — |
| 3910 | — | R 1 | — | R | — | WR | — | R | — | R | — | — | — |
| 3911 | WR 0 | R 16 | — | R | R | WR | WR | WR | — | R | — | — | — |
| 3912 | WR 0 | R 1 | — | R | R | R | R | R | — | R | X | — | — |
| 3913 | — | — | — | R | — | R | — | R | — | R | — | — | — |
| 3914 | — | — | — | R | R | R | R | R | — | R | — | — | — |
| 3915 | WR 0 | R 1 | — | R | WR | R | — | R | — | R | — | — | — |
| 3916 | — | R 2 | — | R | — | R | — | R | — | R | — | — | — |
| 3917 | — | R 1 | — | R | — | R | — | R | — | R | — | — | — |
| 3919 | — | WR 0 | — | R | — | R | — | R | — | R | — | — | — |
| 3920 | — | R 4 | — | R | — | R | — | R | — | R | — | — | — |
| 3921 | — | R 1 | — | R | — | R | R | R | — | R | X | — | — |
| 3923 | R 1 | R 2 | — | R | R | R | R | R | — | R | — | — | — |
| 3924 | — | WR 0 | — | R | — | R | — | R | — | R | X | — | — |

*2+ or greater was considered reactive in FTA 1:5 BS
X=Quantity not sufficient; not done

organisms. Thus the organisms with which the next rabbit was inoculated were suspended in extraction fluid containing rabbit globulin. Additional rabbit globulin might have been present, attached to the organisms themselves. Milgrom (1965) has shown that rabbits inoculated with homologous or autologous globulin can produce antiglobulin factors and that these factors can react more strongly with human than with rabbit globulin (presumably because human and rabbit globulin have closely related antigenic determinants). Miller, de Bruijn, Bekker, and Onvlee (1966) reported that heating suspensions of *T. pallidum* before they are used as test antigens lowered the apparent titre of reactivity in syphilitic sera. The finding that heat-labile antiglobulin factors, as well as treponemes, are present in the syphiloma extract (Table III) may be an interesting coincidence or it may indicate that antiglobulin factors present with routinely obtained *T. pallidum* play a role in the apparent reactivity of this organism in serological tests. Further studies of this possibility are indicated.

Our interest in whether or not rabbit globulin is associated with *T. pallidum* grown in rabbits was stimulated by our observation that in control slides the FITC-conjugated goat antiserum to rabbit globulin stained *T. pallidum*.

Our first thought was that the reactivity observed in the antibody globulin from the goat in which the antirabbit globulin antiserum was produced was natural or acquired reactivity to treponemal 'group' or 'common' antigens. This explanation was given by Deacon and Hunter (1962). The reactivity is similar to that reported to occur in approximately 20 per cent. of normal humans (Hunter, and others, 1964). Such goat antitreponemal antibodies would have been conjugated with FITC along with the induced goat antirabbit globulin antibodies.

The results shown in Table I indicate that goat antitreponemal reactivity could have been making some contribution to the observed staining, since absorption of the conjugate with nonpathogenic cultivable Reiter treponemes removed or reduced its ability to stain many lots of *T. pallidum*. However, Reiter treponemes routinely grown in this laboratory are grown in media supplemented with rabbit serum, and Gelperin (1951) reported that serum proteins in the growth medium might adhere to Reiter organisms. Thus, there is a possibility that the diminution in staining after absorbing the FITC-conjugated antirabbit IgG with Reiter organisms may result from rabbit globulins or other serum proteins present on the Reiter treponemes and not from the treponeme itself. In any event, after the conjugate was absorbed with Reiter treponemes, it continued to stain certain lots of *T. pallidum*, and this staining must be explained. The variability in the degree to which lots of *T. pallidum* grown in different rabbits were stained with a single antiserum (Table I) suggests that at least some of the factors being recognized by the fluorescent antiserum are present on the *T. pallidum* organisms in different amounts.

This variability could result from unrecognized factors related to storage and handling of the individual lots of *T. pallidum* suspension, but it was also reminiscent of the well-known heterogenicity of the immune response within a group of noninbred rabbits. If the immune response of a rabbit were brisk, then the *T. pallidum* organisms harvested from that animal might be coated with rabbit anti-*T. pallidum* antibody globulin.

The results shown in Table II indicate that rabbit globulin, perhaps antibody in nature, participates in the coating of *T. pallidum* organisms grown in rabbits. Table II also shows that absorption of the antirabbit IgG conjugate with rabbit globulin has sharply reduced or removed its ability to recognize *T. pallidum*. Presumably, goat antibodies directed against treponemal antigens would not have been blocked by the absorption with rabbit globulin.

Syphilitic rabbits show development or enhancement of reactivity in several serological tests for syphilis by the time their testicular syphilomas are harvested at 8 days (Table VI). However, neither the TPI nor the FTA-ABS tests had become reactive, and this may explain why relatively little attention has been given to the early antibody response of rabbits to *T. pallidum*. Our demonstration of a prompt response is in accord with our finding that rabbit globulins can be associated with the *T. pallidum* organisms in the FTA-ABS antigen, since this antigen is, at the present time, either prepared like TPI antigen (except that for use in the FTA-ABS test the *T. pallidum* suspension is lyophilized for storage and reconstituted later) or by the method of Hunter, Creighton, and Lewis (1970). Sub-immobilizing amounts of rabbit antibody globulin may also be present on the *T. pallidum* organisms routinely used as antigen for the TPI test. Although Hunter and others (1970) reported that rabbit globulin was not present after two washings of the *T. pallidum*, as demonstrated by the addition of a 1 : 10 dilution of fluorescein-labelled antirabbit globulin, the authors failed to show the presence of rabbit globulin before washing. In this study two of the lots of *T. pallidum* antigen were nonreactive when overlaid with antirabbit globulin.

These results may have a bearing on the mechanisms by which syphilitic sera are reactive in the TPI and FTA-ABS tests, but further evidence is needed

before definite conclusions can be drawn. Fife (1964) theorized that, because of the reactivity of rheumatoid sera with *T. pallidum* in a particular immunofluorescent test, some rabbit globulin might coat *T. pallidum* even within the usual 7- to 9-day growth period. Wilkinson and Rayner (1966), using immunofluorescent methods, identified rabbit globulin on *T. pallidum* when the organisms grew in rabbits for 14 or more days, that is, for longer than the conventional growth period. They also showed that rheumatoid sera could react with *T. pallidum* if gross amounts of rabbit globulin coated the organisms. Further studies should be performed to ascertain whether syphilitics evidence similar reactivity toward the somewhat smaller amounts of rabbit globulin which may be present on routinely obtained *T. pallidum*. In this connection Kunkel, Simon, and Fudenberg (1958), Peltur and Christian (1959), Dresner and Trombly (1959), Cathcart, Williams, Ross, and Calkins (1961), Williams and Kunkel (1962), Schrohenloher (1967), and Mustakallio, Lassus, and Wager (1967) reported that syphilitics have an increased incidence of antiglobulins, that is, of rheumatoid factors.

The experiments described in this paper do not exclude the possibility that *T. pallidum* itself possesses an antigenic determinant similar or identical to one on the rabbit and human globulin molecule. This possibility, or the possibility that the *T. pallidum* organisms may become coated with host globulin, suggests that recognition of 'self' globulins may be associated with *T. pallidum* grown in rabbits (Hardy and Nell, 1955; Christiansen, 1963; Julian, Portnoy, and Bossak, 1963; Miller, Bekker, de Bruijn, and Onvlee, 1966; Hardy and Nell, 1957; Jones, Nevin, Guest, and Logan, 1968). Thomas, Clark, Cline, Anderson, and Russell (1972) found that repeated centrifugation and re-suspension diminished the reactivity of *T. pallidum* with FITC-conjugated antiserum to rabbit globulin, and they suggested that rabbit globulin might thus be removed from the organisms.

In these studies it was not possible to determine the exact manner in which rabbit globulin and antiglobulin factors are associated with certain lots of *T. pallidum*; if there is union with the treponemes, it may be through antigen-antibody linkages or nonspecific adherence. Moreover, the globulin and antiglobulin factors may also react to varying degrees with each other once either becomes associated with the treponeme. Whatever the situation, the role of rabbit globulins and antiglobulins associated with *T. pallidum* merits further exploration regarding the reactivity of standard tests for syphilis in which *T. pallidum* is used as antigen.


## Summary

Many lots of *Treponema pallidum* organisms extracted, after the usual 7 to 9 days of growth, from rabbit testicular syphilomas stain with fluorescein-conjugated goat antirabbit globulin. Absorbing the conjugate with rabbit globulin removes or greatly reduces this staining. Heat-labile rabbit antiglobulin factors reactive with human globulin are associated with the extracted *T. pallidum*. The sera of rabbits from which *T. pallidum* is extracted contain antiglobulin factors that are reactive with human and rabbit globulin. These sera are also reactive in several serological tests for syphilis but not in the TPI and FTA-ABS tests. These findings that rabbit globulins and antiglobulins can be associated with routinely obtained *T. pallidum* may have a bearing on the mechanisms by which syphilitic sera are reactive with *T. pallidum* in specific serological tests for syphilis.


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## Facteurs globuliniques et antiglobuliniques du lapin associés avec le développement de *Treponema pallidum* chez le lapin

SOMMAIRE

De nombreux lots de *Treponema pallidum* extraits d'orchites du lapin se développant, comme il est usuel, en 7 à 9 jours, se colorent avec un conjugué fluorescent de globuline de chèvre anti-lapin. En absorbant le conjugué avec la globuline de lapin, on empêche ou l'on réduit fortement la fluorescence. On trouve, associé avec le *T. pallidum* extrait, des facteurs thermolabiles antiglobuliniques du lapin qui réagissent avec la globuline humaine. Les sérums des lapins chez lesquels *T. pallidum* est extrait contiennent des facteurs antiglobuliniques qui réagissent avec la globuline de l'homme et du lapin. Ces sérums sont aussi positifs pour plusieurs tests sérologiques pour la syphilis mais non pour le TPI ou le FTA-ABS. Ces constatations que les globulines et les antiglobulines du lapin peuvent être associées avec *T. pallidum* obtenu selon la technique habituelle peut avoir une portée pour éclairer les mécanismes par lesquels les sérums syphilitiques réagissent avec *T. pallidum* dans les tests sérologiques spécifiques.