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Comparison of Major Protein Antigens and Protein Profiles of *Treponema pallidum* and *Treponema pertenue*

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The protein profiles of *Treponema pallidum* and *Treponema pertenue*, the causative agents of syphilis and yaws, respectively, were compared by one- and two-dimensional gel electrophoresis. One-dimensional gels showed essentially no differences in the protein patterns of these treponemes. On two-dimensional gels most radiolabeled protein species were shared; however, variations were noticed in several minor protein species. Antigenic comparison by radioimmunoprecipitation and Western blotting also demonstrated similarities between these spirochetes. However, lactoperoxidase-catalyzed iodination of *T. pallidum* and *T. pertenue* suggested differences in their surface proteins.

Treponematoses are a significant worldwide human health problem. There are four distinct and clinically dissimilar diseases, which are caused by three species of treponemes (7). *Treponema pallidum* is the etiological agent of venereal syphilis as well as the nonvenereal endemic syphilis (bejel). *Treponema pertenue* is the agent responsible for yaws, and *Treponema carateum* causes pinta. Although the clinical syndromes are distinct, biological differences between the causative agents have never been adequately described. In contrast to syphilis, which is sexually transmitted, yaws and pinta appear to be transmitted by close personal contact. This transmission occurs under conditions in which abrasions of skin or mucous membranes permit entry of treponemes shed from exuding lesions. Until recently (5, 15), none of the pathogenic treponemes has been cultured in vitro; however, *T. pallidum* and *T. pertenue* are routinely passaged in rabbits. (*T. carateum* cannot be passaged in rabbits.) Upon isolation of these spirochetes from infected rabbit tissue, they so closely resemble each other that they cannot be distinguished either morphologically or serologically (21). The fluorescent antibody and treponemal immobilization tests exhibit no differences when yaws sera or syphilitic sera are reacted against either *T. pallidum* or *T. pertenue* (3, 6). However, the type of lesion and the period required for development of orchitis differ for these two microorganisms. *T. pallidum* requires 8 to 12 days to establish orchitis in the intratesticular infected rabbit, whereas *T. pertenue* requires 26 to 30 days (8).

Since serological testing has failed to distinguish between these two treponemes, biochemical studies have been undertaken. When DNA from *T. pallidum* and *T. pertenue* were examined by Cot analysis of DNA-DNA hybridization, they were found to be indistinguishable (12).

Thus, there is an inconsistency between the clinical data, presenting two distinct diseases, and the biochemical data, which indicate that these microorganisms are virtually indistinguishable. We therefore compared the antigenic peptides and the intrinsic and surface-labeled protein profiles of *T. pallidum* and *T. pertenue*.

MATERIALS AND METHODS

Animals. Adult New Zealand white rabbits (3 to 4 kg), obtained locally, were used for passage of treponemes. Rabbits were inoculated intratesticularly with 5×10^7 treponemes and treated with 6 mg of cortisone acetate (Merck Sharpe & Dohme) per kg on days 3 to 7 after infection (10). With *T. pallidum*, peak orchitis occurred about days 10 to 12 postinoculation, and with *T. pertenue*, peak orchitis was observed at days 26 to 30.

Treponemes. *T. pallidum* Nichols and *T. pertenue* Gautier were obtained from the Centers for Disease Control, Atlanta, Ga., and were maintained by routine passage in rabbits. Stock solutions of treponemes were stored at −80°C as described previously (14). Treponemes were harvested from infected rabbit testes as previously outlined (1).

Intrinsic radiolabeling of treponemes. After sterile extraction of treponemes from infected rabbit testes, the spirochetes were carefully layered over a Metho-cel-Hypaque (32:20) solution (1) and centrifuged at 800 × *g* for 20 min to remove contaminating host tissue. Treponemes were then centrifuged at 12,000 × *g* for 20 min and suspended in 2 ml of modified basal reduced medium (5) containing 0.5 mCi of [^{35}S]methionine. After incubation at 34°C overnight, the treponemes

were harvested by centrifugation at 12,000 × *g* for 20 min and washed three times in phosphate-buffered saline (PBS). Radiolabeled treponemes were stored frozen at −20° until use.

SDS-polyacrylamide gel electrophoresis. Treponemal proteins were electrophoresed on 7.5% polyacrylamide slab gels in the discontinuous Tris-glycine system described by Laemmli (9). After one-dimensional electrophoresis, gels were fixed in methanol-acetic acid-water (45:45:10) and either processed for fluorography with [^{35}S]methionine-labeled preparations (4) or dried directly and exposed to X-ray film for ^{125}I-labeled samples. Two-dimensional gels were run by the method of O'Farrell (16). The first dimension was isoelectric focused for 16 h at 350 V in 5% polyacrylamide gels, followed by electrophoresis on a 7.5% sodium dodecyl sulfate (SDS)-polyacrylamide gel. After the second dimension, the gels containing [^{35}S]methionine were fixed and processed for fluorography.

Radioimmunoprecipitation (RIP). [^{35}S]methionine-labeled treponemes were solubilized in SDS-Triton buffer as described previously (2). Samples of detergent-solubilized, [^{35}S]methionine-labeled treponemes were incubated with samples of normal or syphilitic rabbit sera for 2 h at 23°C in a total volume of 250 μl. Then 100 μl of a 10% suspension of protein A-bearing *Staphylococcus aureus* cells were added, and incubation continued overnight at 4°C. The protein A-bearing *S. aureus*-precipitated immune complexes were washed four times in Tris-buffered saline containing 0.05% Triton X-100. After solubilization of the immune complexes in sample buffer, the radioactive proteins were electrophoresed on 7.5% SDS-polyacrylamide slab gels.

Western blots. Samples of unlabeled treponemes were solubilized in 0.063 M Tris containing 2% SDS, 2% β-mercaptoethanol, and 10% glycerol and electrophoresed on a 7.5% polyacrylamide slab gel. After electrophoresis, the proteins were electrophoretically transferred for 16 h at 380 mA to nitrocellulose paper (Bio-Rad Laboratories) by the technique of Towbin et al. (19). One lane was stained with amido black to determine that both high- and low-molecular-weight proteins were sucessfully transferred to the nitrocellulose sheets. Nonspecific protein-binding sites were blocked by incubation in 3% bovine serum albumin for 6 h. The nitrocellulose strips were incubated in a 1/50 dilution of antisera in PBS overnight with constant rocking. The strips were rinsed in PBS–0.01% SDS or PBS alone and incubated with bovine serum albumin-PBS containing ^{125}I-labeled protein A-bearing *S. aureus*. The strips were rinsed as before, dried, and exposed to X-ray film (Kodak XR5) with an enhancing screen (18).

Monoclonal antibody. BALB/c female mice (3 to 6 weeks old) were used for immunization. Freshly extracted *T. pallidum* purified by Methocel-Hypaque gradient centrifugation as described above were centrifuged at 17,000 × *g*. The treponemes were then washed once with PBS and again pelleted at 17,000 × *g*. The treponemes (6×10^8 *T. pallidum* in 0.5 ml of saline) were emulsified 1:1 (vol/vol) in Freund complete adjuvant. Organisms were administered intramuscularly (0.1 ml), subcutaneously (0.1 ml), and intraperitoneally (0.3 ml). Each mouse received a total of 3×10^8 *T. pallidum*. On days 7 and 21 mice were boosted with 3×10^8 treponemes emulsified in Freund incomplete adjuvant as above. Four days after the final immunization, the spleens were removed, and spleen cells were isolated. These cells were fused with SP2/0 cells (17). Hybrid cells were selected by growth in hypoxanthine-aminopterin-thymidine medium, and antibody secreting clones were detected by an enzyme-linked immunosorbent assay (20) against whole *T. pallidum* (13) and also by RIP (2, 3) against [^{35}S]methionine-labeled *T. pallidum*.

Lactoperoxidase-catalyzed surface iodination. Treponemes were surface iodinated by a modification of the method of Marchalonis et al. (11). Briefly, whole treponemes were resuspended in 100 μl of PBS, and then 0.1 mCi of carrier-free ^{125}I, 0.5 mg of lactoperoxidase, and 3.0×10^{-4} M H_2O_2 were added. After 30 min on ice, the reaction mixture was quenched by the addition of $Na_2S_2O_7$ and NaI, and the treponemes were washed three times with PBS. Samples of *T. pallidum* and *T. pertenue* (ca.100,000 cpm) were electrophoresed on a 7.5% SDS-polyacrylamide slab gel and processed for autoradiography.

RESULTS

Protein profiles of *T. pallidum* and *T. pertenue*. Tissue-harvested treponemes were intrinsically radiolabeled as described above. Overnight incubation of approximately 10^9 treponemes with 0.5 mCi of [^{35}S]methionine permitted incorporation of approximately 3.5×10^6 to 7.0×10^6 cpm into trichloroacetic acid-precipitable mate-

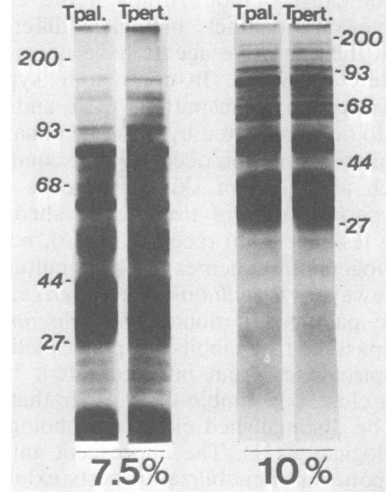

FIG. 1. Comparison of the protein profiles of *T. pallidum* and *T. pertenue*. Samples of [^{35}S]methionine-labeled *T. pallidum* and *T. pertenue* (30,000 to 40,000 cpm) were separated by molecular weight on either 7.5 or 10% SDS-polyacrylamide gels. The gels were processed for fluorography, dried, and exposed to X-ray film. Molecular weight markers (200 to 27) represent the following protein standards: myosin, 200,000; phosphorylase B, 93,000; bovine serum albumin, 68,000; ovalbumin, 44,000; and α-chymotrypsinogen, 27,000.

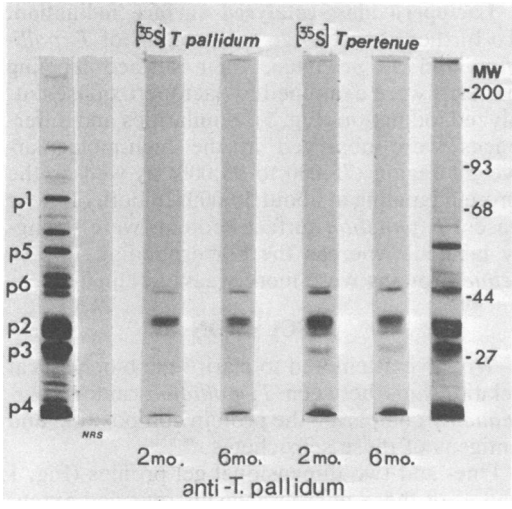

FIG. 2. Comparison of *T. pallidum* and *T. pertenue* antigenic proteins by radioimmunoprecipitation. Samples of detergent-solubilized [^{35}S]methionine-labeled *T. pallidum* and *T. pertenue* (300,000 to 500,000 cpm) were incubated with 1/100 dilutions of normal or syphilitic rabbit sera for 2 h at 23°C in a total volume of 250 μl. The samples were processed as described in the text. Radioactive proteins were electrophoresed on a 7.5% SDS-polyacrylamide slab gel and prepared for fluorography. Molecular weight (MW) markers are given in the legend to Fig. 1. The nomenclature (p1 to p6) in the left margin refers to previously identified *T. pallidum* proteins (1).

rial. As shown in Fig. 1, the protein profiles of *T. pallidum* and *T. pertenue* on 7.5 and 10% gels appear identical. These proteins range in molecular weight from approximately 160,000 to <10,000, and, as reported by Lukehart et al. (10), most protein bands fall in the range between 30,000 and 90,000.

RIP of treponemal antigens. Detergent treatment of radiolabeled treponemes resulted in approximately 50 to 60% solubilization of total radioactivity. The solubilized peptides are representative of an extensively washed Coomassie blue-stained gel profile of *T. pallidum* (1). After solubilization, treponemal antigens were examined by RIP. Normal rabbit serum controls did not precipitate labeled treponemal antigens (Fig. 2); however, when rabbit syphilitic sera obtained either 2 or 6 months postinfection were incubated with solubilized *T. pallidum* or *T. pertenue* samples, similar low-molecular-weight treponemal protein antigens were demonstrated after fluorography.

Western blotting of treponemal antigens. Antigenic analysis by Western blotting revealed essentially the same antigenic pattern as did RIP (Fig. 3). However, some differences in the antigenic patterns existed with the different techniques. By Western blot, the most readily visualized antigen of *T. pallidum* and *T. pertenue* was a 45,000-dalton protein, in contrast to a 32,000-dalton protein defined by RIP. Shared immunogenic proteins also appeared in regions having molecular weights of 59,000, 25,000 to 30,000 and approximately 20,000. With increased time of X-ray film exposure, several shared antigens of higher molecular weights were also identified by Western blotting and RIP (data not shown; 2, 3). Again, no significant differences were observed between *T. pallidum* and *T. pertenue*. A monoclonal antibody (Fig. 3, $13F_3$) raised against a 45,000-dalton *T. pallidum* protein reacted by Western blot analysis with an apparentically identical protein from *T. per-*

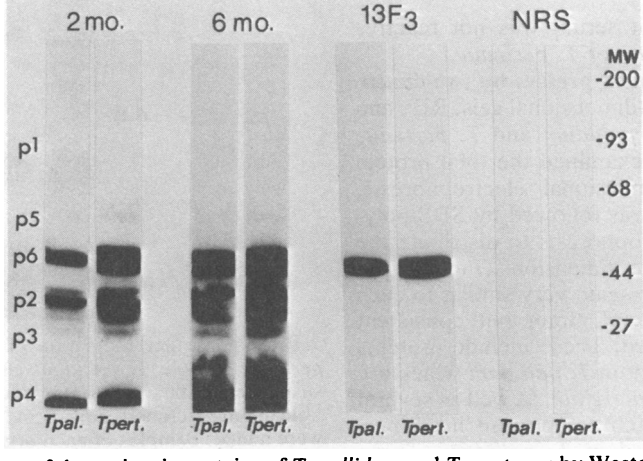

FIG. 3. Comparison of the antigenic proteins of *T. pallidum* and *T. pertenue* by Western blotting. Samples of unlabeled *T. pallidum* and *T. pertenue* representing about 7×10^7 and 10^8 organisms, respectively, were solubilized and electrophoresed on a 7.5% polyacrylamide slab gel. Molecular weight (MW) markers and reference to proteins p1 to p6 are given in the legends to Fig. 1 and 2. NRS, Normal rabbit serum.

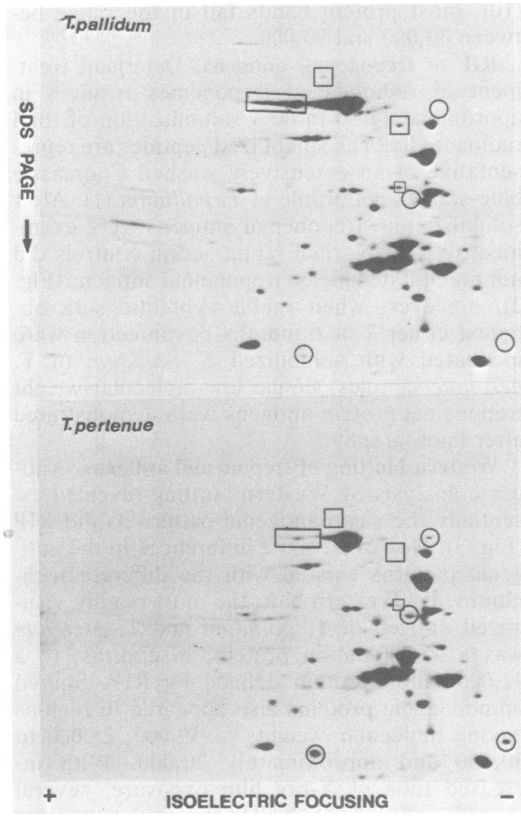

FIG. 4. Comparison of the two-dimensional protein profiles of *T. pallidum* and *T. pertenue*. Samples of [^{35}S]methionine-labeled *T.pallidum* and *T. pertenue* (100,000 to 120,000 cpm) were isoelectric focused in 5% polyacrylamide gels (horizontal direction), and then the focused proteins were separated by molecular weight on a 7.5% SDS-polyacrylamide slab gel (vertical direction) and processed for fluorography.

tenue. Normal rabbit serum was not reactive with either *T. pallidum* or *T. pertenue*.

Comparison of protein profiles by two-dimensional gels. Since one-dimensional gels, RIP, and Western blots of *T. pallidum* and *T. pertenue* were so similar, we examined the total protein patterns by two-dimensional electrophoresis. Isoelectric focusing was followed by SDS-polyacrylamide gel electrophoresis to distribute the protein profiles in two dimensions (Fig. 4). The protein patterns were again very similar to each other; however, several minor but consistent differences were noted. These include proteins (square boxes) present in *T. pallidum* which are greatly reduced in *T. pertenue*, as well as several proteins (circles) which are present in *T. pertenue* but are reduced in *T. pallidum*. These reproducible differences probably represent quantitative differences between the two microorganisms.

Lactoperoxidase-catalyzed surface iodination. To further characterize the proteins of *T. pallidum* and *T. pertenue*, their surface labeling patterns were examined by lactoperoxidase-catalyzed iodination (Fig. 5). Similarities and differences were observed in the high-molecular-weight region (75,000 to 95,000) as well as the protein banding at about 50,000. In both of these cases, *T. pallidum* surface proteins were strongly labeled, whereas the corresponding *T. pertenue* proteins were more weakly iodinated.

DISCUSSION

We have attempted to clarify the biochemical relationships between *T. pallidum* and *T. pertenue* by comparing the protein composition and antigens of these spirochetes.

One- and two-dimensional gel profiles (Fig. 1 and 4) of these microorganisms revealed extensive protein homology. The reproducible differences observed in the two-dimensional protein patterns appear to be quantitative rather than qualitative and apparently represent minor treponemal molecules. Although the biosynthetic activities of *T. pallidum* and *T. pertenue* were measured under nonpermissive growth condi-

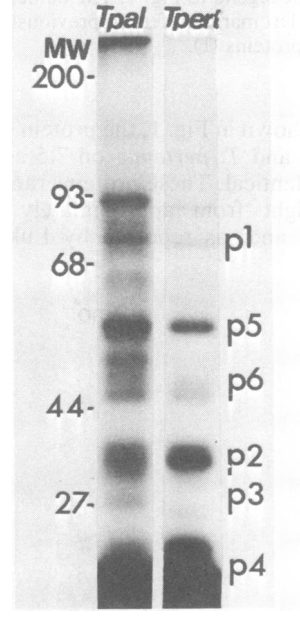

FIG. 5. Surface iodination of *T. pallidum* and *T. pertenue*. Whole *T. pallidum* and *T. pertenue* were suspended in 100 μl of PBS, and 0.1 mCi of carrier-free ^{125}I, 0.5 mg lactoperoxidase, and 3.0×10^{-4} M H_2O_2 were added. Samples of each organism (100,000 cpm) were electrophoresed on a 7.5% SDS-polyacrylamide slab gel and processsed for autoradiography. Molecular weight (MW) markers and reference to proteins p1 to p6 are given in the legends to Fig. 1 and 2.

tions and other physiological activities were probably not fully functional, the DNA isolated from these treponemes is indistinguishable by Cot analysis of DNA-DNA hybridization (12). Therefore, the commonality of protein expression is not surprising.

Consistent with RIP and Western blot analyses of *T. pallidum* and *T. pertenue*, none of the polypeptides identified on two-dimensional gels appears to be a unique antigenic species. Immunological analysis of *T. pallidum* and *T. pertenue* reinforces the antigenic similarities between the two spirochetes (3, 6, 21). Radioimmunoprecipitation of *T. pallidum* and *T. pertenue* antigens by rabbit syphilitic sera demonstrated that the polypeptide-directed immunoglobulin G antibodies present at 2 and 6 months postinfection reacted with *T. pallidum* and *T. pertenue* in a similar fashion. Western blot analysis was consistent with these findings.

Examination of the surfaces of these spirochetes by radioiodination revealed potentially distinguishing characteristics. These surface differences might be used to discriminate between *T. pallidum* and *T. pertenue*. The host obviously differentiates between these treponemes by presenting different clinical manifestations of disease, correlating with the varied lifestyles of these microorganisms. Nonetheless, the differences observed are far overshadowed by the extensive biological, chemical, and immunological similarities of these two treponemes, suggesting that these spirochetes are variants of a single prototype species that have been adapted by host and/or environmental factors.

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LITERATURE CITED

1. **Alderete, J. F., and J. B. Baseman.** 1979. Surface-associated host proteins on virulent *Treponema pallidum*. Infect. Immun. **26**:1048–1056.
2. **Alderete, J. F., and J. B. Baseman.** 1981. Analysis of serum IgG against *Treponema pallidum* protein antigens in experimentally infected rabbits. Br. J. Vener. Dis. **57**:302–308.
3. **Baseman, J. B., and E. C. Hayes.** 1980. Molecular characterization of receptor binding proteins and immunogens of virulent *Treponema pallidum*. J. Exp. Med. **151**:573–586.
4. **Bonner, W. M., and R. A. Laskey.** 1974. A film detection method for tritium-labeled proteins and nucleic acids in polyacrylamide gels. Eur. J. Biochem. **46**:83–88.
5. **Fieldsteel, A. H., D. L. Cox, and R. A. Moeckli.** 1981. Cultivation of virulent *Treponema pallidum* in tissue culture. Infect. Immun. **32**:905–915.
6. **Garner, M. F., J. L. Backhouse, G. Daskalopoulos, and J. L. Walsh.** 1974. Use of *T. pertenue* in the fluorescent and immobilization tests. Br. J. Vener. Dis. **50**:264–266.
7. **Hackett, C. J.** 1963. On the origin of the human treponematoses. Bull. W.H.O. **29**:7–41.
8. **Hardy, P. H.** 1976. Pathogenic treponemes, p. 107–119. *In* R. C. Johnson (ed.), Biology of the parasitic spirochetes Academic Press, Inc., New York.
9. **Laemmli, U. K.** 1970. Cleavage of structural proteins during the assembly of the head of bacteriophage T4. Nature (London) **227**:680–685.
10. **Lukehart, S. A., S. A. Baker-Zander, and E. R. Gubish.** 1982. Identification of *Treponema pallidum* antigens: comparison with a non-pathogenic treponeme. J. Immunol. **129**:833–838.
11. **Marchalonis, J. J., R. E. Cone, and V. Santer.** 1969. An enzymatic method for trace iodination of immunoglobulins and other proteins. Biochem. J. **113**:299–305.
12. **Miao, R. M., and A. H. Fieldsteel.** 1980. Genetic relationship between *Treponema pallidum* and *Treponema pertenue*, two noncultivable human pathogens. J. Bacteriol. **141**:427–429.
13. **Morrison-Plummer, J., J. F. Alderete, and J. B. Baseman.** 1983. Enzyme-linked immunosorbent assay for the detection of serum antibody to outer membrane proteins of *T. pallidum*. Br. J. Vener. Dis. **59**:75–79.
14. **Nell, E. E., and P. H. Hardy.** 1972. The use of freeze-preserved treponemes in the *Treponema pallidum* immunobilization test. Cryobiology **9**:404–410.
15. **Norris, S.** 1982. In vitro cultivation of *T. pallidum*: independent confirmation. Infect. Immun. **36**:437–439.
16. **O'Farrell, P. H.** 1975. High resolution two-dimensional electrophoresis of proteins. J. Biol. Chem. **250**:4007–4021.
17. **Oi, V. T., and L. A. Herzenberg.** 1980. Immunoglobulin-producing hybrid cell lines, p. 351–372. *In* B. B. Mishell and S. M. Shligi (ed.), Selected methods in cellular immunology. W. H. Freeman and Co., San Francisco.
18. **Swainstrom, R., and P. R. Shank.** 1978. X-ray intensifying screens greatly enhance the detection by autoradiography of radioactive isotopes ^{32}P and ^{125}I. Anal. Biochem. **86**:184–192.
19. **Towbin, H., T. Staehelin, and J. Gordon.** 1979. Electrophoretic transfer of proteins from polyacrylamide gels to nitrocellulose sheets: procedure and some applications. Proc. Natl. Acad. Sci. U.S.A. **76**:4350–4354.
20. **Voller, A., D. Bidwell, and A. Bartlett.** 1976. Microplate enzyme immunoassays for the immunodiagnosis of virus infections, p. 506–512. *In* N. R. Rose and H. Friedman (ed.), Manual of clinical immunology. American Society for Microbiology, Washington, D.C.
21. Yaws or Syphilis? 1979. Br. Med. J. **1**:912.