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Immobilization of Certain Cultured Treponemes in Sera from Syphilitic Humans

W. J. GUEST, T. A. NEVIN, M. L. THOMAS, AND J. A. ADAMS
Venereal Disease Research Laboratory, National Communicable Disease Center, Atlanta, Georgia

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The loss of motility by protozoa, certain eubacteria, and certain spirochetes upon incubation with immune sera has been recognized for some time (A. Laveran and F. Mesnil, Ann. Inst. Pasteur **15**:673, 1901; R. Rossle, Arch. Hyg. Bakteriol. **104**:1, 1903; T. Smith and A. L. Reagh, J. Med. Res. **10**:89, 1903; H. G. Beyer and A. L. Reagh, J. Med. Res. **12**:313, 1904; D. Zabolotny and D. Maslakowetz, Zentr. Bakteriol. Parasitenk. Abt. I Orig. **44**:532, 1907; R. A. Nelson and M. M. Mayer, J. Exptl. Med. **89**:369, 1949; G. D'Alessandro and P. Zaffiro, Riv. Ist. Sieroterap. Ital. **36**:203, 1961). However, except for the Reiter organism, similar studies of cultured spirochetes in sera from syphilitic humans have not been reported, although several of these strains have been described as variants of *Treponema pallidum*. Therefore, the Reiter, English Reiter, Noguchi, Nichols, and Kazan-2 strains (purportedly *T. pallidum*) and the FM, N-39, and MRB strains of *T. microdentium* were cultivated in NHI Thioglycollate Broth (Difco) with a 10%, heated (60 C, 2 hr), rabbit serum enrichment. The cultures were incubated at 35 C until direct cell counts with Petroff-Hausser chambers reached 30×10^6 to 35×10^6 per milliliter. For testing, suspensions were adjusted with fresh medium to contain about 15×10^6 to 17×10^6 cells per milliliter (15 per high dry field; × 450).

Human sera were obtained from cases of primary, secondary, and latent syphilis, and from nonsyphilitics [H. N. Bossak et al., Public Health Rept. (U.S.) **75**:130, 1960]. *T. pallidum* immobilizing levels, or lack thereof, in the sera were verified by the standard *Treponema pallidum* Immobilization (TPI) reaction (*Serologic Tests for Syphilis*, U.S. Public Health Serv. Publ. 411, 1964).

Preliminary tests with cultured treponemes demonstrated that the TPI techniques and controls were also useable with the substitute medium. However, the incubation time was shortened, since immobilization was essentially completed in 1 hr. During prolonged incubation (5 to 6 hr) under TPI conditions, some of the strains were lysed. Guinea pig serum, added as complement, caused this reaction, which will be described separately.

Each strain was tested at least 10 times with sera from primary and secondary syphilitics, 6 times in sera from latent syphilitics, and 10 times in sera from nonsyphilitics. The averaged data are presented in Table 1. The Nichols, Noguchi, Kazan-2, and English Reiter strains were immobilized to greater or lesser degrees in all sera from syphilitics, whereas the strains of *T. microdentium* and the Reiter organism were comparatively less affected by the sera from secondary and latent syphilitics and seemed unaffected by the sera from primary cases. None was greatly affected by sera from nonsyphilitics.

TABLE 1. *Percentage of immobilization of cultured spirochetes in sera from nonsyphilitic and syphilitic humans after incubation for 1 hr*

Spirochete	Serum category			
	Non-syphilitic	Primary	Secondary	Latent
Kazan-2	14	62	68	77
Nichols	13	46	57	69
Noguchi	7	27	66	72
E. Reiter	7	27	67	71
FM	6	6	28	44
Reiter	5	6	15	14
N-39	6	11	29	25
MRB	4	7	22	23

TABLE 2. *Immobilization of the Noguchi treponeme in a mixture of serum from a secondary syphilitic human, anti-Noguchi serum, and complement*

Serum	Immobilization
	%
Anti-Noguchi + secondary syphilis	25
Anti-Noguchi	76
Secondary syphilitic	72

The immobilizing action of a selected serum from a secondary syphilitic against any reactive strain could be absorbed (1 volume of packed cells/3 volumes of serum, 2 hr, 4 C) by that strain. In addition, the sample adsorbed by the Noguchi strain lost activity against the other reactive strains and virulent *T. pallidum*. Absorption of rabbit anti-Noguchi serum with *T. pallidum*, however, failed to remove anti-Noguchi activity. Blocking experiments (J. R. Preer, Jr., J. Immunol. **83**:276, 1959) were then conducted in which a mixture of Noguchi spirochetes, anti-Noguchi serum, and the selected serum was incubated for 10 min, followed by the addition of complement. Controls, in which a saline solution replaced either of the sera, were carried in parallel. When both sera were included, antitreponemal activity was markedly less than that of either control (Table 2). It seems probable, therefore, that antitreponemal substances were bound at different sites on the Noguchi organism, and that when both sera were included the stereochemical configuration of the resulting complex was such that complementary activity was reduced.