

Passage of *Treponema pallidum* Through Membrane Filters of Various Pore Sizes

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The passage of *Treponema pallidum* through commercially available Millipore membrane filters of various pore sizes was examined. No microscopically detectable organisms passed through a filter with a pore diameter of 0.22 μm . As pore size was increased, progressively more organisms passed through. Motile organisms passed through filters to a greater extent than nonmotile ones; however, 22% of the motile and 50% of the nonmotile *T. pallidum* organisms did not pass through the largest pore diameter tested (14.0 μm). Filtration of *T. pallidum* suspensions through membrane filters may offer a way of separating the organisms from larger particles of debris which accompany their extraction from rabbit testicular syphilomas.

Chandler recently reported an immunofluorescent technique for the identification of *Treponema pallidum* after entrapment of the organisms by filtration of suspensions through membrane filters having a mean pore size of 0.22 μm (F. W. Chandler, Jr., Brit. J. Vener. Dis., *in press*). No microscopically detectable treponemes passed through the 0.22- μm pores. In the present study, we examined the effect of larger pore sizes on the passage of *T. pallidum* through commercially available membrane filters.

MATERIALS AND METHODS

Preparation of *T. pallidum* suspension. *T. pallidum* (Nichols strain) organisms were harvested from 7- to 9-day rabbit syphilomas, as described for the *Treponema pallidum* immobilization (TPI) test (5), with 80% 0.15 M NaCl and 20% normal rabbit serum, heated at 56 C for 30 min, being substituted for the usual TPI extracting medium. The extracted material (treponemes and tissue debris) was adjusted in phosphate-buffered saline (PBS), containing 2% Tween 80 (polyoxyethylene sorbitan monooleate) to give a final concentration of approximately 1.5×10^7 *T. pallidum* per ml. This suspension was used as the stock test suspension for filtration and recovery studies.

Portions of the test suspension were passed through the various filters within 1 hr after the death of the donor rabbit while the treponemes were 96 to 100% vigorously motile. Another part of the suspension was filtered after being left at room temperature for approximately 24 hr, by which time the treponemes were no longer motile.

Enumeration of *T. pallidum*. Throughout these studies, the Artley and Clark (1) modification of the dark-field enumeration method of Morgan and Vryonis (3) was used to calculate the number of

treponemes per unit volume of suspension, based on the number of organisms per microscopic field.

Preparation of glassware, syringes, and filter assemblies. To minimize the possibility of organisms adhering to glass, rubber, or plastic surfaces, all materials which were to come in contact with *T. pallidum* during the filtration and recovery procedures were immersed for 10 min in a water-soluble silicone concentrate (Siliclad, Clay-Adams, Inc., New York, N.Y.) at a dilution of 1:100 for glassware and 1:20 for plastic and rubber materials. They were then rinsed thoroughly in distilled water and dried at 100 C for 10 min.

Equipment and experimental procedure. Nine different membrane filter pore sizes were investigated. After agitation of the test suspension to maximize chances for an even distribution of organisms, 10, 1-ml samples were aspirated into 2.5-ml plastic syringes. Each syringe was firmly fitted to a Swinnex-25 filter assembly, each assembly containing a 25-mm white, plain membrane filter (Millipore Corp., Bedford, Mass.) of one of the following pore diameters: 0.22, 0.45, 0.65, 0.80, 1.2, 3.0, 5.0, 8.0, and 14.0 μm . (Filters were kindly supplied by Norman Hunt of Millipore Corp.) As a control, the 10th syringe was fitted to a Swinnex-25 assembly which did not contain a filter. By slowly depressing the syringe plunger, 1 ml of suspension was forced into and partially through each filter assembly. The syringe was then detached, filled with 2 ml of PBS containing 2% Tween 80, and reattached to the assembly. The saline solution was then slowly forced through the filter to increase chances for treponemal recovery in the filtrate.

The filtrates were agitated to promote a uniform dispersion of treponemes, and a 0.01-ml sample was pipetted from each. Each 0.01-ml sample was then placed on a glass microscope slide, covered with a cover slip (22 by 30 mm), and enumerated.

TABLE 1. Recovery of motile and nonmotile *Treponema pallidum* in filtrate after filtration of 1-ml (1.5×10^7 organisms/ml) suspensions through selected filter pore diameters

Filter pore size (μm)	Average no. of organisms counted per 50 fields		Range of counts per 50 fields		Avg no. of organisms counted per 50 fields (% of control)	
	Motile	Nonmotile	Motile	Nonmotile	Motile	Nonmotile
Control (no filter)	196.35 ^a	202.35 ^a	132-327	156-312	100.0	100.0
14.00	152.85 ^b	102.00 ^b	105-252	78-141	77.84	50.40
8.00	121.35 ^b	6.90 ^b	102-144	0-12	61.80	3.40
5.00	96.00 ^b	3.15 ^b	69-123	0-9	48.89	1.55
3.00	38.25 ^b	2.55 ^b	27-57	0-12	19.48	1.26
1.20	22.05 ^b	0.15 ^b	12-36	0-3	11.22	0.07
0.80	21.00 ^b	0.15 ^b	9-33	0-3	10.69	0.07
0.65	2.55 ^c	0.15 ^c	0-12	0-3	1.29	0.07
0.45	0.90 ^c	0 ^c	0-3	0	0.45	0
0.22	0	0	0	0	0	0

^a The observed difference between motile and nonmotile groups is not statistically significant since such differences would occur by chance more than 5% of the time (χ^2 with one degree of freedom and $\gamma = 0.05$).

^b The observed difference between motile and nonmotile groups is statistically significant, since such differences would occur by chance less than 1/1,000 (χ^2 with one degree of freedom and $\gamma = 0.001$).

^c The difference is not considered statistically significant, since such differences would occur by chance more than 5% of the time (Poisson distribution assumed and modified "t" test performed, $\gamma = 0.05$).

Ten slides were prepared from each filtrate and two counts of 50 random fields were recorded per slide, for a total of 20 observations from each filtrate.

RESULTS

The principal results are summarized in Table 1. Confirming our earlier work, no *T. pallidum* was microscopically detectable in the filtrates of 0.22- μm filters. As filter pore diameters were increased, the number of organisms detected in the filtrates also increased. However, only 78% of the motile and 50% of the nonmotile treponemes were recovered from the largest pore size tested (14.0 μm).

A chi square test indicated no significant difference ($P > 0.05$) for the number of treponemes counted in the motile and non-motile groups of controls. For pore sizes of 0.80 μm and above, tests of significance (chi square) indicated that the passage of motile *T. pallidum* through the filters was greater ($P < 0.001$) than the passage of non-motile organisms. No difference was noted between motile and nonmotile *T. pallidum* for the 0.65- and 0.45- μm pore sizes.

DISCUSSION

The advent of commercially produced membrane filters in a range of defined pore sizes allowed us to reexamine the filterability of *T. pallidum* through openings of various diameters. In the 1930's, Hindle and Elford (2) and Tilden (6) failed to pass *T. pallidum* through collodion

membranes with a pore size of 0.40 μm and concluded that the narrowest diameter of the organism is approximately 0.2 μm . We observed *T. pallidum* microscopically in filtrates of membrane filters (Millipore Corp.) with a pore size of 0.45 μm , but not in filtrates of 0.22- μm filters.

It was of interest to note that passage of *T. pallidum* was not an "all or none" phenomenon at any pore diameter greater than 0.22 μm . Even at the largest pore size tested, 14.0 μm , 22% of the motile and 50% of the nonmotile organisms did not pass through the filters. Many factors could contribute to this heterogeneity of filtration. (i) The organisms are not of uniform size; as far back as 1912, Noguchi (4) observed that *T. pallidum* organisms vary in diameter and length. (ii) During filtration the organisms approach the pores at random angles of orientation; the smaller the pore diameter, the more critical it would seem to be that the organisms approach at 90° to pass through the pores. (iii) Active, motile *T. pallidum* cells may succeed in "corkscrewing" their way through pores which block the passage of non-motile organisms; this possibility was supported by our finding that motile organisms passed the filters to a greater extent than nonmotile ones.

The present study may also point the way toward a method for reducing the amount of particulate debris in *T. pallidum* suspensions extracted from rabbit testicular syphilomas. Subjective observations made in the course of the

research suggest that, by filtration of the *T. pallidum* suspension through a membrane of given pore size, it may be possible to let a substantial number of organisms through, yet screen out the particulate debris of a diameter greater than the pore size. Whether this impression can be objectively documented and whether the procedure would be efficient are matters under continuing study.

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