RECOVERY OF *TREPONEMA* AND *BORRELIA* AFTER LYOPHILIZATION

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Hampp (J. Am. Dental Assoc. **34**:317, 1947) reported the successful recovery of six of seven spiral organisms from lyophilized material with the use of a complex medium unavailable through commercial sources. Annear (J. Bacteriol. **83**:932, 1962; J. Gen. Microbiol. **27**:341, 1962) recovered Reiter *Treponeme* and four strains of *Leptospira* from dried material by use of a prolonged and complex method. Earlier reports by Turner et al. (Am. J. Med. Sci. **202**:416, 1941) and Stavitsky (J. Bacteriol. **50**:118, 1945) indicate that attempts to dry spiral organisms from the frozen state with maintenance of viability and pathogenicity were unsuccessful.

The following species or strains of spiral organisms used in this study were tested for viability, both microscopically and culturally, at 24 hr and at 30-day intervals for 1 year after drying from the frozen state: *T. pallidum* strains Kazan 2, 4, 5, and 8, Reiter, English Reiter, Nichols, and Noguchi; *T. microdentium* strain FM; *T. phagedenis; Borrelia vincentii* strain N-9; *T. zuelzerae;* and *B. refringens.* These organisms were grown in the following medium, which was used as the lyophilization menstruum: 45% spirolate broth (BBL), 45% Brain Heart Infusion broth (BBL) with 0.05% added sodium thioglycolate; 0.025% asparagine (Difco); 0.025% Tryptone (BBL); 5.0% gelatin (BBL); and 10% normal inactivated rabbit serum. Three transfers were made at 24-hr intervals with the use of heavy inocula in freshly prepared medium and incubation at 35 C. After incubation of the third transfer, 2 ml of the growth were transferred to a sterile, 5-ml, hard-glass lyophile ampule. The suspensions of organisms were shell-frozen in an alcohol-Dry Ice mixture, and dried from the frozen state on a cryochem apparatus by the method of Flosdorf and Mudd (J. Immunol. **34**:469, 1938). When the material was dry (18 to 20 hr), the ampules were sealed with the interior under vacuum and stored at 8 to 10 C. Upon completion of the test intervals, the dried material was rehydrated with the lyophilization medium without the gelatin. The rehydrated organisms were inoculated into the same medium containing 0.08% agar. Cultures were incubated at 35 C, and were checked for motility and growth at 0-, 24-, 48-, and 72-hr intervals.

Large numbers of organisms with typical morphological characteristics were observed, with no evidence of fragmentation. There was languid motility observed in the freshly rehydrated cultures of the Kazan and Reiter strains, Nichols, *T. microdentium,* and *B. vincentii* organisms.

Growth obtained in 72 hr from lyophilized cultures after prolonged storage for 1 year was equivalent to that obtained from organisms preserved by storage at −70 C for the same length of time.

This method of preservation of *Treponema* and *Borrelia* strains or species was found to provide a convenient and inexpensive method of maintaining viability for a minimum of 1 year.