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The *in vitro* cultivation of *Treponema pallidum* Corroborative studies

JOHN W. FOSTER, DOUGLAS S. KELLOGG, JOHN W. CLARK, AND ALBERT BALOWS

From the Center for Disease Control, Public Health Service, US Department of Health, Education, and Welfare, Atlanta, Georgia

Introduction

Jones *et al.* (1976) reported that they had successfully cultivated *Treponema pallidum in vitro*. Because of the potential theoretical and practical importance of this report, we began studies to corroborate the findings. Our research included the following overlapping phases: (a) performing the methods as published, (b) performing the methods as published with additional advice and counsel (obtained by telephone) from Dr Jones and colleagues, (c) studying the methods for two weeks in Dr Jones's laboratories at the Medical Research Institute (MRI), Florida Institute of Technology, and (d) performing the methods under the scrutiny of a member of Dr Jones's staff, who spent a week in our laboratory. Despite these efforts, however, we could not corroborate the findings.

Materials and methods

The protocol described in the paper by Jones *et al.* (1976) was followed as closely as practicability and content would allow. Our variations and the post-publication instructions that we received are described. We used both the Center for Disease Control (CDC) strain of *T. pallidum* (Nichols, pathogenic) and the strain used by Jones *et al.* (1976) which was obtained previously from CDC. As 'well-developed' orchitis in rabbits is temporally dependent upon inoculum levels of *T. pallidum* and may describe any stage from 10 days after inoculation, we used orchitic testicles ranging from 30 to 60 days, but averaging 49 days, after inoculation. Our selection was based on a comment in the discussion concerning '43-day-old testicular infections' (Jones *et al.*, 1976) and from telephone conversations with the authors.

Reprints from J. W. Foster, Center for Disease Control, Public Health Service, US Department of Health, Education, and Welfare, Atlanta, Georgia 30333, USA

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Ingredients for the preparation of the growth medium were obtained from the sources given in the paper by Jones *et al.* (1976) and, when possible, from the same lots they had used. The amino-acid, vitamin, and Earle's basic salt solution concentrations were obtained from the Biological Products Division at CDC and from the sources cited in the paper. The carbon dioxide concentration in the gas phase was maintained at between 6% and 8%. Our method of enumerating treponemes was different from that of Jones *et al.* (1976), which in turn was not properly described by citing the paper by Chandler and Cannefax (1969). We used a modification of the Morgan-Vryonis' method for enumerating treponemes (Artley and Clark, 1969).

Results

Using the described procedures, we were unable to demonstrate growth, an increase in numbers, or unusual survival of *T. pallidum in vitro* (three to four days with motility as an index, or less than two days with virulence as an index). From telephone and written communications with scientists at the MRI of the Florida Institute of Technology, we became aware of several technical differences between the published and the actual materials and procedures used. A few of the more important differences follow. Testicular tunica, not 'testicular material', was the source of treponemes. This was emphasised as a critical feature in our discussion with the MRI group. Multi-dish tissue culture plates were not necessary, even though it was verbally stated that they were by those at the MRI, as Jones *et al.* had also used 2 oz prescription bottles. The bovine serum albumin (BSA)-sodium stearate complex could not be prepared in accordance with the published formulation and, as the authors later stated, was not prepared as described. The numbers of *T. pallidum* used to initiate testicular infection are not mentioned, although in our later contacts with the MRI group, members placed considerable

importance on an inoculum of 1×10^8 *T. pallidum*/ml. According to oral statements from the authors, but again not in the published paper, freshly prepared glutamine was essential to the proper development of the baby hamster kidney tissue cell monolayers. We found this to be true when we used prescription bottles but not when we used plastic tissue culture flasks.

During the two-week working visit of one of us (JWF) in the authors' laboratory, an apparent increase in the number of motile treponemes was observed after the primary and two serial subcultures of *T. pallidum* on the baby hamster kidney tissue cells. Such results could not be repeated in our laboratory in spite of applying all the newly acquired information to the effort. The reasons for the failure were then sought in the character of the distilled water, the containers for the cell cultures, the source and constituents of the Eagle's MEM vitamin and amino-acid concentrates, the type of pipette (glass versus plastic) and the strain of *T. pallidum* (authors' strain compared with ours). During this examination, a member of the MRI staff spent a week in our laboratory assisting us in our efforts. The results were again negative.

Discussion

We were not able to corroborate the authors' claim of 'successful cultivation and subculturing of . . . virulent *T. pallidum* . . .' by following the procedures described (Jones *et al.*, 1976) or the variations in these procedures acquired later from the authors in verbal form (and emphasis as to their importance). In spite of occasional friction—to be expected under such negative circumstances—the authors were co-operative. The authors inadequately described a complicated system which apparently was still undergoing technical evolution when their publication appeared and, to a certain extent, after we became involved in our corroborative efforts. It may be that their technique for enumerating *T. pallidum* was so inadequate that they and our staff visitor to their laboratory were misled by their findings. In our opinion, their method was not described correctly by citing the paper by Chandler and Cannefax (1969). Multiplication times for *T. pallidum* derived from some of the enumeration data (Jones *et al.*, 1976) are much shorter than those that we and others (Magnuson *et al.*, 1948) have observed. Although it would not be proper or useful to question the validity of the authors' data, we believe that our inability to corroborate even the first steps casts serious doubt on the reproducibility of their findings. So far they have not further documented their work.

References

Artley, C. W., and Clark, J. W., Jr (1969). Statistical approach to evaluating the method of Morgan and Vryonis for enumerating *Treponema pallidum*. *Applied Microbiology*, **17**, 665–670.

Chandler, F. W., Jr, and Cannefax, G. R. (1969). Evaluation of dark-field and immunofluorescent techniques for demonstrating *T. pallidum* in fluids with small numbers of organisms. *British Journal of Venereal Diseases*, **45**, 1–5.

Jones, R. H., Finn, M. A., Thomas, J. J., and Folger, C. (1976). Growth and subculture of pathogenic *T. pallidum* (Nichols strain) in BHK-21 cultured tissue cells. *British Journal of Venereal Diseases*, **52**, 18–23.

Magnuson, H. J., Eagle, H., and Fleischman, R. (1948).The minimal infectious inoculum of *Spirochaeta pallida* (Nichols strain), and a consideration of its rate of multiplication *in vivo*. *American Journal of Syphilis, Gonorrhea, and Venereal Diseases*, **32**, 1–18.