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# NOTES

## Wall-less Microbial Isolate from a Human Renal Biopsy

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An organism was isolated from a kidney biopsy of a patient with renal failure. Electron microscopy revealed an ultrastructure very similar to that of a bacterial L-form or mycoplasma. But macroscopically, its colonial morphology was unusual and distinct from that ascribed to these wall-less organisms. This isolate lacked a rigid cell wall and required a hypertonic medium with serum for growth. Also, a long incubation period was essential for its growth, and use of a hand lens was necessary for detection on solid medium.

Cell wall-defective bacteria and bacterial L-forms have been occasionally isolated from various organs. Also, cell wall-deficient bacterial variants have been demonstrated in kidney tissue by immunofluorescence (3). Recently, Whipple disease has been shown to be caused by a cell wall-deficient form of an alpha-hemolytic streptococcus (5). Other than this one instance, the significance of such organisms in vivo is not known. The primary isolation of fastidious, osmotically fragile, wall-less organisms is an arduous task and probably accounts, in large part, for the paucity of information on their isolation from clinical material.

In a survey of renal (needle) biopsies from five patients with acute renal failure for wall-defective organisms and/or L-forms, tissue was macerated (forceps and knife) and cultured without delay onto a freshly prepared hypertonic culture medium composed of Trypticase soy agar, 10% (vol/vol) horse serum, and 3.0% (wt/vol) NaCl. Duplicate solid medium with oleic acid (2 μg/ml) was also used (4). Sets of plates were incubated aerobically and anaerobically (GasPak, Becton-Dickinson & Co., Rutherford, N.J.) and examined frequently before being discarded after 3 weeks.

One of the biopsies examined was from a 64-year-old black man with cellulitis of the left leg in addition to renal failure. He had been started on cephalothin therapy 7 days before biopsy. The renal biopsy showed acute, proliferative, diffuse glomerulonephritis. Biopsy material, after growth on solid media for approximately 2.5 weeks, yielded 1-mm transparent colonies (approximately 20 per plate; Fig. 1a) which, macroscopically, bore some resemblance to pseudocolonies known to appear on plates containing high serum (30%, vol/vol) concentrations (1). These colonies were easily transferable on the above media but continued to require approximately 2 weeks for visible growth to reappear. However, they were not pseudocolonies because of (i) no growth occurring on solid medium without only serum or osmotic stabilizer or in hypertonic liquid medium with serum, (ii) their absence on plates inoculated with sterile agar blocks, (iii) the appearance of only the typical L-form morphology when a group A streptococcal L-form was inoculated as control, and (iv) the internal structure of these unusual colonies when thin sections were viewed by electron microscopy (see below). Finally, there was no difference in growth on solid medium with or without oleic acid (4) or when plates were incubated aerobically or anaerobically. It should be emphasized that successful isolation of this organism from patient material required the use of (i) a freshly prepared hypertonic, solid medium, (ii) an extended incubation period, and (iii) close examination of the agar surface, with a hand lens, for growth.

As indicated, macroscopically, colonies of this organism were not of the typical L-form or mycoplasma "fried-egg" morphology (Fig. 1a). This unusual colonial morphology plus the great osmotic fragility of this organism suggests that it is probably not a *Mycoplasma*. For electron microscopy, colonies were fixed (glutaraldehyde-cacodylate buffer [pH 7.3]) while still on the agar surface before being scrapped off, postfixed (osmium tetraoxide), and embedded in Epon. Thin sections were cut, stained with uranyl acetate and lead citrate, and viewed with a Hitachi HS-8 electron micro-

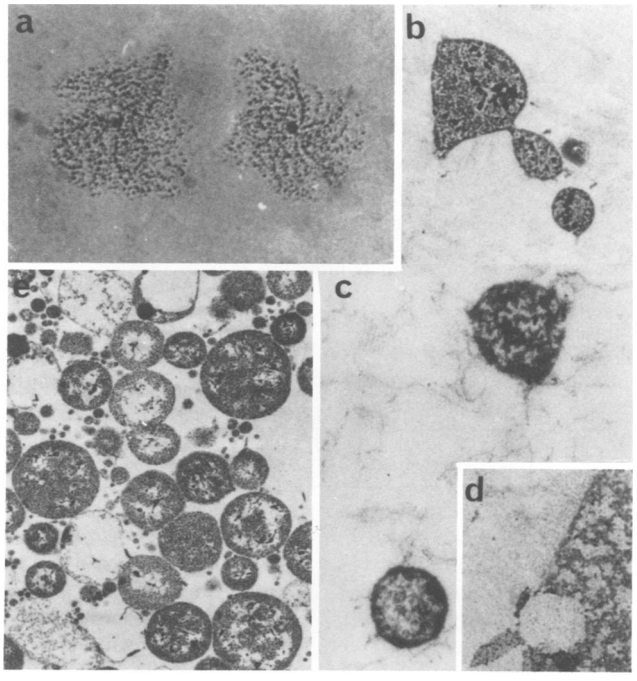


FIG. 1. *(a) Unique colonial morphology of this kidney isolate (×17). Also, electron micrographs of thin sections of this organism compare its cellular morphology and structure at different magnifications: (b) ×14,000, (c) ×9,000, and (d) ×60,000, with (e) that of a similar preparation of a group A streptococcal L-form (×9,000).*

scope. Figures 1b and c are thin-section photomicrographs of cells from such colonies; their condensed nuclear filaments are evident, one cell in the process of budding, and each is surrounded by a unit membrane. Examination of these cells at a higher magnification (Fig. 1d) failed to indicate any rigid cell wall comparable to that of bacteria. However, vesicular structures like those in mycoplasmas and bacterial L-forms were evident. A similar preparation of a stabilized, physiologically isotonic L-form of *Streptococcus pyogenes* capable of surviving in immunosuppressed mice (Fig. 1e) is presented for comparison (control) (2). As is apparent, the cellular morphology of these two "wall-less" organisms is strikingly similar (Fig. 1b, c, e).

This kidney isolate did not cross-react with antisera prepared against this group A streptococcal L-form in the direct fluorescent-antibody technique (2). Also, this organism was catalase negative and was not stained by the Gram reaction or inhibited by penicillin, cycloserine, bacitracin, or the patient's serum.

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