NOTES

Unusual Colonies of *Ureaplasma urealyticum* (T Mycoplasmas) in Primary Agar Cultures of Certain Urine Specimens

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The existence of unusual colonies of *Ureaplasma urealyticum* (T mycoplasmas) in primary agar cultures of certain urine specimens is reported and their morphology is illustrated.

During the course of routine isolations of *Ureaplasma urealyticum* (7) from male nongonococcal urethritis (NGU) patients, we became aware of the presence of small, aberrant, refractile, golden-brown, colony-like bodies in primary agar cultures from certain NGU patients. It was further observed that these refractile bodies developed only in primary agar cultures of urine specimens from these patients and were never observed in cultures of urethral exudates collected at the same visit from the same patients.

The agar medium employed during the time these observations were made was known as agar medium A5C. The basal medium consisted of Trypticase soy broth (BBL), 3%, adjusted to pH 5.5 and solidified with Ionagar no. 2 (or 2S). The medium was enriched with sterile, unheated normal horse serum to 20%, fresh yeast extract (1%), and penicillin G (1,000 U/ml).

Urethral exudates were collected by means of epithelial scraping of the anterior urethra and streaked directly to the surface of an A5C agar culture plate, or placed in 0.5 to 1.0 ml of sterile, pH 6.0, Trypticase soy broth carrying fluid for delayed inoculation of A5C agar and urease color test medium U-9 (6). Urine specimens were collected last, and the first 20 to 40 ml of voided urine was collected in sterile, glass, screw-cap tubes (25 by 100 mm) and centrifuged at 3,000 rpm for 10 min. The supernatant urine was decanted. The sediment was mixed with the remaining few milliliters of urine by means of a Vortex mixer and streaked out on a section of A5C agar medium. The culture plates were incubated 48 h, by the modified Fortner method (4) or in a gaseous mixture of 10% carbon dioxide and 90% nitrogen, at 36 C.

Aberrant, colony-like bodies occurring in primary urine cultures from certain male NGU patients were previously thought to be crystalline structures of chemical origin. Under low-power ($\times 100$) examination, they were characteristically small (10 to 20 μ m in diameter) and dark gray to golden-brown in color. Under higher magnification ($\times 200$ to 400), the bodies displayed a central, refractile core which in turn frequently was surrounded by an indurated, encrusted-looking, granular, irregular border. One of their most characteristic features was their unmistakable "hard-core" look. They sometimes resembled "oval fat bodies" (2). The typical appearance of these aberrant, grayish to golden-brown, colony-like bodies is illustrated in Fig. 1 and 2.

During this same period we were developing a direct test for urease in colonies of *U. urealyticum* for specific identification of this organism (5). In this test, a dark, golden-brown reaction product is produced in and on the surface of agar colonies of this urease-positive *Ureaplasma* species, producing manganese accretion colonies (Fig. 3). There appeared to be more than a casual similarity between manganese accretion colonies of *Ureaplasma* species produced in the direct test for urease and the aberrant, grayish to golden-brown, colony-like bodies which occurred naturally in A5C agar cultures of certain urine specimens.

Occasionally, the coarsely granular peripheral elements of these colony-like bodies showed a slight dye uptake of Dienes stain (1, 3) in wet-stained agar block preparations. The staining, however, was almost always limited to a narrow region of the irregular perimeter (Fig. 1B and 2B). The fact that certain of these

FIG. 1. Characteristic appearance of small, aberrant, golden-brown, colony-like bodies in agar cultures of certain urine specimens. (A) Typical morphology under low power ($\times 85$). (B) Same preparation as in (A), but under medium-low power ($\times 190$); peripheral material surrounding the hard core is showing limited dye uptake (Dienes stain). (C, D, E) Unstained appearance of aberrant structures ($\times 85$). (F) Single aberrant, colony-like body (Dienes stain) showing hard-core morphology with limited dye uptake of peripheral elements ($\times 190$).

FIG. 2. Characteristic morphology of aberrant, golden-brown, colony-like bodies under high and medium magnification. (A) Two aberrant bodies under high magnification ($\times 750$) showing the peculiar nature of the center and the coarse granularity of the peripheral elements (Dienes stain). (B) Three aberrant, colony-like bodies (Dienes stain) under medium magnification ($\times 360$) showing the typical, hard-core morphology and limited dye uptake of the peripheral elements. (C and D) Aberrant colony-like bodies developing in the agar from urinary mucous threads (unstained, $\times 170$).

colony-like, hard-core bodies stained at all with Dienes stain suggested a possible association with mycoplasmas. The true nature of the aberrant colonies observed in urine cultures from certain NGU patients was established by their

successful subcultivation on fresh A5C agar by the agar push-block method. All such aberrant, colony-like bodies produced luxuriant secondary outgrowth of *normal* colonies of *U. urealyticum* when subcultured to fresh A5C agar me-

FIG. 3. Normal agar colonies of *Ureaplasma urealyticum* upon which the direct test for urease (5) has been performed. Manganese accretion colonies are produced as a result of a manganese reaction product which develops in and on the *Ureaplasma* colonies. Such colonies are dark golden brown in color when viewed by direct transmitted light ($\times 100$).

FIG. 4. Secondary outgrowth of normal *Ureaplasma urealyticum* colonies from a single, aberrant, colony-like body which was transplanted to the surface of fresh agar medium. The central, refractile, hard-core body from which secondary, normal growth developed is plainly visible. In this Dienes-stained preparation, the normal, secondary growth was well stained ($\times 750$).

dium (Fig. 4). Thus, these unusual hard-core bodies were shown to be naturally occurring, aberrant colonies of *U. urealyticum* and not inorganic, crystalline bodies which we originally interpreted them to be.

The occurrence of such unusual colonies of *U. urealyticum* in agar cultures of urine specimens from only certain male NGU patients is believed to be associated with elevated levels of certain divalent cations (magnesium and manganese, for example) and increased concentrations of urea. As a consequence of urea hydrolysis by colonies of *U. urealyticum*, the ammonia evolved (as ammonium hydroxide) oxidizes soluble divalent cation salts to insoluble metallic oxides (5). These oxides in turn precipitate within and on the surface of the colonies, producing metallic oxide accretions. The A5G agar medium employed in these studies contained approximately 3.2 mg of magnesium and 0.4 mg of manganese per 100 ml. Although the medium contained approximately eight times more Mg^{2+} than Mn^{2+} , salts of manganese are much more reactive to oxidation by hydroxyl ions than those of magnesium and produce much larger quantities of metallic oxide reaction product. By the simple expedient of increasing the urea content of this medium to 55 mg of urea per 100 ml, we succeeded experimentally in producing unusual *U. urealyticum* colonies which were indistinguishable from naturally

occurring accretion colonies of this organism. Urine specimens from *U. urealyticum*-positive male NGU patients served as test inocula. These small, unobtrusive, unusual, naturally occurring accretion colonies of *U. urealyticum* may easily be overlooked and unrecognized in primary agar cultures of urine specimens from certain male NGU patients.

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