Effect of Carbohydrates on Growth of *Ureaplasma urealyticum* and *Mycoplasma hominis*

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We examined the effect of 31 carbohydrates on the growth of *Ureaplasma urealyticum* and *Mycoplasma hominis*. Arbutin and its breakdown product, hydroquinone, inhibited growth of both species; the other substrates did not alter the extent of growth. Volatile and nonvolatile end products of carbohydrate metabolism were not detected by gas chromatography.

Ureaplasma urealyticum and *Mycoplasma hominis* are the two mycoplasma species most commonly isolated from the human genital tract. *U. urealyticum*, one of the etiological agents of nongonococcal urethritis, has been associated with other genitourinary tract disorders, whereas *M. hominis*, long believed to be a saprophyte, is now known as a potential pathogen in a variety of situations (16). Despite their importance in human medicine, the basic metabolic functions of these organisms are not understood. For instance, although the abilities of *U. urealyticum* to degrade urea and of *M. hominis* to break down arginine are well documented and accepted as means of energy production (6, 15), neither mechanism has been defined. Whereas it is generally believed that the usual carbon sources are not utilized by either species (6, 11, 13, 15), examination of their ability to degrade carbohydrates has been limited to few of these compounds. The primary study (2) of carbohydrate utilization by ureaplasma refers only to the inability of the species to ferment glucose and to the absence of hexokinase activity in strains 58 (serotype IV) and T960 (the initial serotype VIII standard). However, the pH of the broth medium used for that study was 7.0, which is outside the 5.5-to-6.5 range considered optimal for this organism (12). Furthermore, the broth contained 1% (wt/vol) glucose, a concentration reported by another worker as inhibitory (8). The absence of hexokinase activity in *U. urealyticum* has been confirmed by others (3, 5). Cocks et al. (3) have found low activity for only some of the enzymes of glycolysis in a strain of *U. urealyticum* designated Belacosa H and have suggested their association with nucleotide synthesis or degradation (or both). The effect of *M. hominis* on carbohydrates other than glucose and mannose (6) appears not to have been addressed.

We are interested in the role of mycoplasmas in the human genital tract. We know that both *U. urealyticum* and *M. hominis* coexist in vivo with *Neisseria gonorrhoeae* and have shown that this association in vitro may be correlated with *Neisseria* auxotype modification (1). To find out whether either species could affect patterns of carbohydrate metabolism of procaryotic or eucaryotic cells, we examined their ability to degrade a wide range of these substrates.

The sources of the two strains used for these studies (the type species *U. urealyticum* T960-CX8 and *M. hominis* ATCC 14027) have been previously described, as has the bromothymol blue (B) broth medium used for their propa-

gation (9), a complex medium with a significant (10% [vol/vol]) serum supplement. Logarithmic-phase cultures of the two species were sonicated to disperse cell clumps (9), and 0.1-ml volumes were used to initiate duplicate serial 10-fold dilutions in bromothymol blue broth (pH 6.0). Made as previously described, the broth was used for control cultures; after supplementation with each carbohydrate substrate, it was used for the test cultures. These substrates were adonitol, amygdalin, arabinose, arbutin, cellobiose, dulcitol, erythritol, esculin, fructose, galactose, glucose, glycerol, glycogen, inositol, inulin, lactose, maltose, mannitol, mannose, melezitose, melibiose, raffinose, rhamnose, ribose, salicin, sorbitol, sorbose, soluble starch, sucrose, trehalose, and xylose. The sources of these were commercially available reagent grade chemicals or equivalents. The final concentrations were 0.5% (wt/vol) at 22°C except for compounds of lower solubility: esculin (0.1%), glycogen (0.25%), and salicin (0.3%). These included all of the substrates used by Cowan (4) and Holdeman et al. (7) for the differentiation of bacterial species. The cultures were incubated at 36°C and, on the basis of color changes of the pH indicator from ureolysis or arginolysis, scored for growth twice daily. Final titers were expressed as color-changing units per milliliter. None of the substrates increased either the growth rate or the maximum titer, nor did the addition of 27 mM glucose (half of the 1.0% [wt/vol] concentration reported to inhibit ureaplasma growth [8]) have any apparent effect on the biomass of the cultures. Surcrose, raffinose, and inositol slowed the growth of both species but did not reduce the final titers. Of the other substrates that we examined, only arbutin (at 2.2 but not 1.1 mM) sometimes showed a clearly inhibitory effect on both the growth rate and final titers of both species. This inhibition was not demonstrable with freshly prepared arbutin. Because arbutin would be hydrolyzed in weak acid (14), such as fresh B broth, to glucose and hydroquinone, we examined the effect of the latter on the growth of the two mycoplasma species. After 2 days of incubation, the control cultures of *U. urealyticum* and *M. hominis* showed titers of 10^7 and 10^9, respectively, but the color change indicative of growth was absent in test cultures containing 2.0 mM hydroquinone. When 0.1 ml of the 10^{-1} culture of either species was diluted into 5 ml of fresh broth, the inoculum failed to give rise to growth, indicating the mycoplasmacidal effect of hydroquinone. Arbutin has been used as a urinary antiseptic (14), perhaps on the basis of the bactericidal activity of hydroquinone.

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Because alkalinization from urea or arginine degradation by *U. urealyticum* and *M. hominis*, respectively, could mask acidic products, we determined the pH of spent cultures in each type of broth. No significant changes (i.e., 0.3 pH unit or more) were noted between test cultures and controls. The spent cultures in each of the 31 media were then examined for the presence of volatile and nonvolatile acids by the gas chromatographic procedures established by the Anaerobe Laboratory of the Virginia Polytechnic Institute and State University (7). By the height of the peaks obtained from the standard chromatograms, the procedure was shown capable of detecting acetic acid at a concentration of 0.5 mM, which is between <1 to 17% of the concentrations of the substrates tested except, perhaps, for inulin and starch, for which the precise concentrations were unknown. The end products sought were not detected in either fresh or incubated broth controls or in any of the test cultures. If we assume that metabolic activity is related to biomass, we can consider the sensitivity of the gas liquid chromatography method for detecting end products of mycoplasma growth. Without difficulty, we have detected lactic acid in cultures of the glycolytic mollicute *Acholeplasma laidlawii*. The cellular volume of this species, calculated from published dimensions of the organisms (17), is similar to what we found for *M. hominis* by morphometry (10). Both species reach similar density in cultures, i.e., 10^9 organisms per ml. Although *U. urealyticum* cells are larger than those of *M. hominis* (10), cultures of ureaplasmas never exceed 10^8 organisms per ml (9). Because of the low biomass, volatile or nonvolatile end products of ureaplasma could conceivably be below detection. However, the results of these studies provide no evidence that either *M. hominis* or *U. urealyticum* metabolizes any of the substrates that we examined.

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