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Effects of Manganese on the Growth and Morphology of *Ureaplasma urealyticum*

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All of the 14 serotype standard strains of *Ureaplasma urealyticum* were inhibited to varying degrees by manganese. A 1 mM concentration of this cation either stopped growth or reduced the rate of growth in liquid medium. The presence of manganese also altered colonial morphology and cellular ultrastructure. Inhibition was dose dependent and strain specific. This differential response allowed the serotype strains to be divided into two broad biotypes. For the first biotype (serotypes 1, 3, 6, and 14), inhibition of growth in broth was temporary. For the second biotype (serotypes 2, 4, 5, 7, 8, 9, 10, 11, and 12), inhibition was permanent. Serotype 13 gave an intermediate response and was not classified. The effect of manganese could be at least partially blocked by magnesium but not by calcium, cobalt, copper, iron, potassium, sodium, or zinc. The concentration of magnesium yielding the maximum blocking effect was directly related to manganese sensitivity. Wild-type isolates of ureaplasma and *Mycoplasma hominis* also showed a differential response to manganese. Laboratory-adapted strains representing species of the genus *Mycoplasma* (*M. hominis*, *M. fermentans*, and *M. pneumoniae*) were inhibited by 5 but not by 1 mM manganese. The latter concentration inhibited the growth of *Acholeplasma laidlawii* and *Staphylococcus aureus*, and 5 mM manganese had no effect on *Escherichia coli*.

Strains of *Ureaplasma urealyticum* isolated from humans demonstrate at least 14 serotype specificities (13). Apart from the colonies of strain 27 (serotype standard 3), which hemadsorb guinea pig erythrocytes (2), and the cells of strain Vancouver (serotype standard 9), which are resistant to high concentrations of tetracycline (5, 11), few clear markers of strain diversity have been reported.

U. urealyticum produces a cytoplasmic urease (4, 6, 18, 24). Shepard and Lunceford have shown that the ammonia liberated in urea degradation reacts with endogenous manganese in agar medium to form a brown manganese dioxide reaction product (19). Shepard and Howard found that the addition of a solution of urea and manganous salts to growth on agar enhances this effect, allowing colonies of ureaplasmas to be differentiated from those of other mycoplasmas (17). This urease spot test is widely used by clinical laboratories. For convenience, the reagents have been incorporated into the agar medium used for primary isolation of ureaplasmas. Initially, 0.03% (wt/vol) $MnSO_4$ was used (15); later, this was reduced to half that concentration (0.015% [wt/vol]) or ca. 0.9 mM (20). To accentuate the appearance of ureaplasma colonies and thereby facilitate their identification and enumeration, we included 1 mM $MnSO_4$ in the agar used in our laboratory. The response of both laboratory-adapted serotype strains and wild-type isolates of ureaplasma to $MnSO_4$ is the subject of the following report.

(A preliminary report of this work was presented at the 78th Annual Meeting of the American Society for Microbiology, Las Vegas, Nev., 14 to 19 May 1978 [J. A. Robertson, Abstr. Annu. Meet. Am. Soc. Microbiol. 1978, G17, p. 74].)

MATERIALS AND METHODS

Organisms. The identity of the laboratory-adapted strains of *U. urealyticum* used for the initial studies has been described previously (12). The strain described therein as type 2 has since been determined to be antigenically distinct and has been designated as type 10 (13). For final studies, the standards used for serotypes 2, 5, 8, and 11 to 14 were those adopted for the expanded serotyping scheme (see Table 3) (13). Wild-type strains of ureaplasma were isolated by the Mycoplasma Laboratory, Department of Medical Microbiology, University of Alberta, using a standardized protocol (10). All ureaplasma strains demonstrated characteristic growth on agar medium and gave a positive reaction in the urease spot test (15, 17). The other mycoplasma species were obtained as follows: *Acholeplasma laidlawii* B from R. N. McElhaney, Department of Biochemistry, University of Alberta, and *Mycoplasma pneumoniae* 15331, *Mycoplasma fermentans* 19989, and *Mycoplasma hominis* 14027 from the American Type Culture Collection, Rockville, Md. *Escherichia coli* NCTC 10418 and *Staphylococcus aureus* NCTC 8530 were in the collection of this department.

Media. *U. urealyticum* and *M. hominis* were cultivated in bromothymol blue (B) broth and on genital mycoplasma (GM) agar (10) which contained a 10% (vol/vol) horse serum supplement. Horse serum contains Mg at a maximum concentration of 1.2 M (1). Both media had a pH of 6.0 (±0.1). The other mycoplasma species were grown in standard broth consisting of: PPLO (pleuropneumonia-like organism) broth without crystal violet (Difco Laboratories, Detroit, Mich.), 2.1 g; yeast extract (Difco), 0.1 g; phenol red, 1.0 ml of a 0.2% (wt/vol) solution; and water, 80 ml. After sterilization, this basal medium was supplemented with 20 ml of pooled normal horse serum (GIBCO Laboratories, Grand Island, N.Y.) and glucose and ampicillin sodium (Ayerst Laboratories, Montreal, Quebec) at final concentrations of 0.1% (wt/vol) and 1 mg/ml, respectively. The pH was adjusted to 7.4. Aqueous solutions of manganese [$MnSO_4 \cdot H_2O$, $MnCl_2 \cdot 4H_2O$, $Mn(C_2H_3O_2)_2 \cdot 4H_2O$], magnesium ($MgSO_4$, $MgCl_2 \cdot 6H_2O$), and other salts (NaCl, $C_2H_3NaO_2$, KCl, $CaCl_2$, $FeCl_3$, $CoCl_2$, $CuCl_2$, and $ZnCl_2$) were filter sterilized. All were prepared from reagent grade chemicals; for

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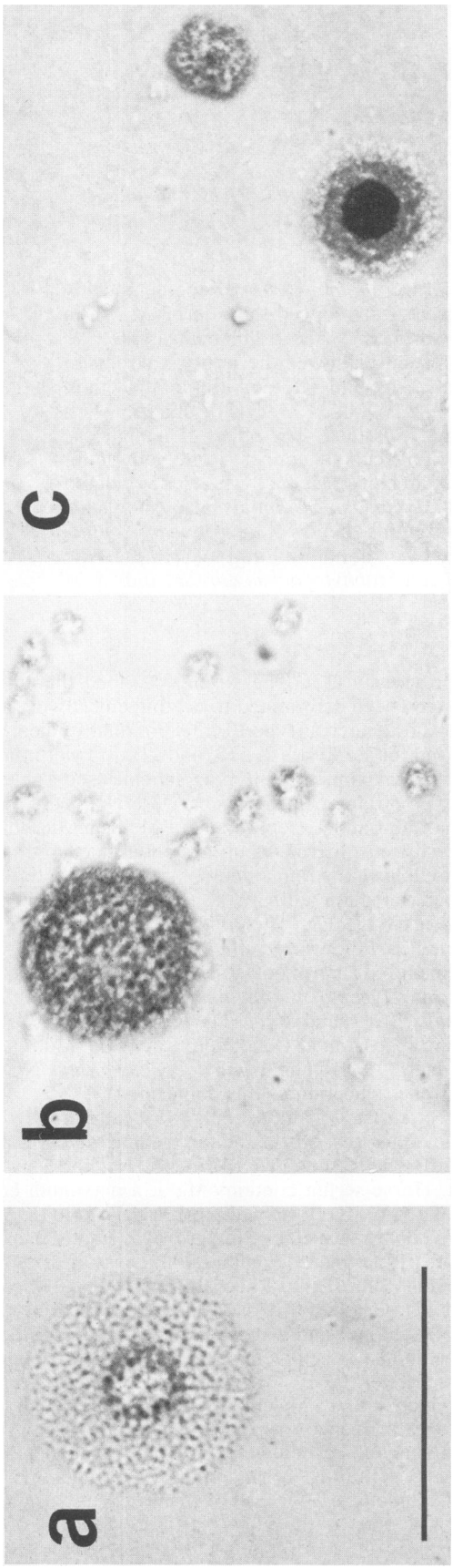

FIG. 1. The effect of $MnSO_4$ on the morphology of colonies of *U. urealyticum* 7 (serotype 1) on plain GM agar (a) GM agar with 0.5 mM $MnSO_4$ (b), and GM agar with 1.0 mM $MnSO_4$ (c). The bar represents 100 μm.

$MnSO_4 \cdot H_2O$ (Fisher Scientific Co, Fair Lawn, N.J.), the heavy metal (as lead) concentration was 0.0005%. Appropriate volumes of these solutions were added as indicated to B broth, standard broth, and GM agar. Media containing a final concentration of 1 mM $MnSO_4$ were designated as B-Mn or GM-Mn. The hydrogen ion concentrations of all media were verified and, if necessary, readjusted. For one experiment, B broth was modified for bacterial growth by omitting ampicillin sodium and adjusting the pH to 7.4. Comparative studies always used broth or agar from the same batch lot.

Qualitative studies. Dilutions of a broth culture of *U. urealyticum* T960 were spread onto GM agar to give ca. 300 colonies per 60-mm plate. After 3 days of incubation, half of the plates were left at 36°C, and the rest were placed at room temperature (RT). Every day thereafter for 11 days, one plate was taken from 36°C and another from RT. The urease spot test was performed, and a sterile scalpel was used to excise blocks of agar from both inside and outside the area used for the test. Each block was placed into B broth which was incubated for 1 day and then subcultured onto GM and blood agar media. After 2 days of incubation, GM agar was examined for the presence of ureaplasma colonies and blood agar for the absence of bacterial contaminants. B broth cultures which turned from yellow to green always gave rise to ureaplasma colonies on GM agar; no bacterial contaminants were detected.

Quantitative studies. All inocula were cultures in the logarithmic phase of growth which had been sonicated for 10 s to disperse cell clumps (10). Depending on the experiment, population estimates were based on CFU on agar or color change units (CCU) or color change units that would establish a dilution of culture that would inoculate 50% of the test wells (CCU_{50}) determinations in broth. The methodologies for these have been described previously (10, 22). Colonies on agar were examined at ×100 magnification with an inverted microscope (Diavert model; Leitz, Wetzlar, Germany) equipped with a measuring eyepiece which had been calibrated against a micrometer slide. Photomicrographs were made on Kodak Panatomic-X film under ×100 magnification in an American Optical Spencer microscope. For growth curves of *U. urealyticum*, 1 ml of inoculum culture was diluted 1/100 in B broth, and a 4-ml volume of this suspension was introduced into 36 ml of test broth. Growth was monitored by changes in pH due to alkalization from substrate (urea) degradation and also by CCU_{50} determinations.

Electron microscopy. Logarithmic-phase culture (0.2 ml) was added to 200 ml of B and B-Mn broths (pH 6.0). When the pH reached 6.8 (which represents a titer of about 10^7 in B broth), the cells were fixed in situ, collected by centrifuga-

TABLE 1. Effect of $MnSO_4$ on growth of *U. urealyticum* 27 (serotype 3) on agar

Dilution plated[a]	No. of colonies detected on GM agar with the following $MnSO_4$ concentration (mM)[b]:		
	0	0.5	1.0
10^{-2}	>300	>300	>300
10^{-3}	163[c]	238[c]	0
10^{-4}	9	0	20

[a] The volume of each sample plated was 0.025 ml.
[b] Counts shown are the mean of duplicate determinations.
[c] 6.5×10^6 and 9.5×10^6 CFU/ml for 163 and 238 colonies, respectively. The standard deviation has been shown to be ±0.5 (22).

tion, and prepared for electron microscopy as described earlier (9).

RESULTS

While conducting growth studies of *U. urealyticum*, we obtained unusual results for CFU determinations. On GM-Mn agar plates which had been inoculated with low dilutions of culture, the colonies were too numerous to count. However, on plates which had received higher dilutions of the sample and were expected to have counts of between 30 and 300 CFU/ml, no growth was detected, even when the surface of the agar was examined at ×100 magnification. Since 1 mM $MnSO_4$ had recently been added to the medium formulation as part of a species-specific indicator system, its role in this phenomenon was investigated.

Samples of dilutions of an exponential-phase culture of the same strain, 27, were plated onto GM agar containing 0, 0.5, and 1.0 mM $MnSO_4$ (GM-Mn). The effect of 1.0 mM $MnSO_4$ on the CFU of the 10^{-3} dilution (Table 1) confirmed the initially observed inhibition of strain 27 by Mn. However, Mn at half that concentration was stimulatory, a response we have reproduced in a number of subsequent experiments. Because of the variation in small numbers, we excluded counts below 30 for our calculations of CFU per milliliter. The pattern of counts in Table 1 suggests that the response to Mn was related not only to Mn concentration but also to the number of cells in the sample.

The inhibition of growth by $MnSO_4$ was reflected in its striking effect on the morphology of the colonies. A typical, $MnSO_4$ dose-related effect is shown in Fig. 1. On plain GM agar, colonies of strain 7 displayed the "fried egg" morphology considered typical of many mycoplasmas (Fig. 1a). On GM-Mn agar, several effects were noted (Fig. 1b). There was less surface growth around the periphery of the colony. Darkening due to the $MnSO_4$ indicator system was also discernible. Colonies such as this were frequently surrounded, in almost satellitic fashion, by many smaller forms which had a "cauliflower" appearance which was emphasized by the lack of response to the indicator. Because of their small size (<25 μm) and their frequent occurrence in aggregates, these small forms were difficult to enumerate or to measure, even at ×100 magnification. They were, however, included in the estimations of CFU per milliliter. The mean diameter of colonies on this agar was significantly less than on plain GM agar. When the concentration of $MnSO_4$ was doubled (Fig. 1c), some of the colonies exhibited the dark center and lighter peripheral zone which is the characteristic of ureaplasma growth on $MnSO_4$-containing agar and allows species identification.

In judging the growth of ureaplasmas on agar, we considered the numbers of colonies to be more important than their size and paid attention to the latter only when the number of CFU per milliliter was similar. Based upon their response to $MnSO_4$, the stock strains of ureaplasma then in our collection fell into two broad groups or biotypes. The first biotype contained the serotype standards 1, 3, 6, and the Boston T strain (which is antigenically similar to serotype 3); these were more refractory to $MnSO_4$ than were the rest of the strains. They are represented in Table 2 by serotype 3 (strain 27). As shown in Table 1, 0.5 mM $MnSO_4$ was stimulatory. At 1.0 mM $MnSO_4$ the counts were fewer but still exceeded those on GM agar. At this concentration of $MnSO_4$ the colonies were too small for measurement. However, all distinct entities showing the refractility characteristic of the larger ureaplasma colonies were enumerated. The second biotype contained the serotype standards 4, 5, 7, 8, 9, and 10. The presence of $MnSO_4$ caused a decrease in both numbers and size of colonies. Serotype 8 (strain T960) was moderately susceptible, and its response to $MnSO_4$ was clearly dose related. So markedly susceptible was serotype 5 (strain 354) that even 0.5 mM $MnSO_4$ reduced the number of CFU per milliliter by at least 500-fold, there being no growth on the lowest dilution of the culture that we plated. In the presence of 1 mM $MnSO_4$, halving, doubling, or quadrupling the usual 0.025% (wt/vol) urea concentration in the agar had no significant effect upon either serotype 3 or 8.

Since most laboratories use manganese-containing agar for the isolation of ureaplasmas, we performed quantitative cultures on GM agar with and without the indicator. Of 61 consecutive specimens of urethral swabs from males which yielded ureaplasmas, all grew in B broth and on GM agar, whereas only 58 of them were detected on Mn indicator-containing agar. The three specimens which failed to produce colonies on GM-Mn had low titers on plain GM agar. Twenty-six of the positive specimens had between 30 and 400 colonies on at least one of the two types of agar. Except for 1 specimen with a GM:GM-Mn ratio of 0.8, an equal or greater count was obtained consistently from GM agar; for these 25 specimens, the GM:GM-Mn ratio ranged from 1.0 to 3.5. For 11 of these specimens, the ratio of CFU per milliliter of ureaplasmas on GM:GM-Mn was within 25%, about the expected variance. However, for 9 of these 25 specimens, the ratio was between 1.25 and 2, and for 5 specimens, the ratio was over 2. Thus, heterogeneity in the response of ureaplasmas to manganese was seen with both laboratory-adapted strains and wild-type isolates.

Because a number of strains of *U. urealyticum* had failed to grow in the presence of $MnSO_4$ and certain of those which

TABLE 2. Differential effect of $MnSO_4$ on the growth of representative *U. urealyticum* strains on GM agar

Strain	CFU/ml[a]			Diameter[b] (μm)		
	Without $MnSO_4$	0.5 mM $MnSO_4$	1.0 mM $MnSO_4$	Without $MnSO_4$	0.5 mM $MnSO_4$	1.0 mM $MnSO_4$
27 (serotype 3)	6.7×10^6	9.5×10^6	7.8×10^6[c]	63 (±9)	35 (±3)	NA[d]
T960 (serotype 8)	3.8×10^7	2.6×10^7	1.7×10^7	32 (±6)	28 (±6)	15 (±3)
354 (serotype 5)	1.9×10^6	$<4 \times 10^3$	$<4 \times 10^3$	ND[e]	NA	NA

[a] CFU per milliliter are based upon the mean of duplicate counts for which the standard deviation has been shown to be ±0.5 (22).
[b] Diameters shown are the mean measurement of 20 colonies on samples containing between 30 and 300 colonies. The standard deviation for each test is given in parentheses.
[c] The count is an estimate because the small size of colonies made accurate enumeration difficult.
[d] NA, Not available because the colonies were too small to measure or were absent.
[e] ND, Not done because there were no colonies on Mn-containing agar to use as a comparison.

TABLE 3. Biotypes of *U. urealyticum* based on the response of the serotype standard strains to 1 mM manganese

Strain[a]		Growth on agar (GM:GM-Mn ratio[b])	Growth in broth (B:B-Mn titers[c])	Biotype
Designation	Serotype			
7	1	1	≦1	1
T23	2	>5	≧3	2
27	3	1	≦1	1
58	4	>5	≧3	2
354(NIH)	5	>5	≦1	2
Pi	6	1	≧3	1
Co	7	>5	≧3	2
T960(CX8)	8	>5	≧3	2
Vancouver	9	>5	≧3	2
Western	10	>5	≧3	2
K2	11	>5	≧3	2
U24	12	>5	≧3	2
U38	13	2	1.5	?
U26	14	1	≦1	1

[a] The sources of these strains have been described previously (13).

[b] CFU per milliliter on GM agar:CFU per milliliter on GM-Mn agar (±0.25), based on duplicate determinations.

[c] After 5 days of incubation, the titer in B broth was divided by the titer in B-Mn broth. The titers in B broth ranged from 6 to 9, whereas those in B-Mn broth ranged from 1 to 8. The titers were based on duplicate determinations. For a given strain in either media, the titer never differed by more than one dilution tube (i.e., factor of 10).

did often showed both qualitative and quantitative differences related to variation between agar lots (e.g., serotype 3 in Table 1 versus Tables 2 and 3), subsequent studies were based largely on the more reproducible growth of broth cultures. To identify the inhibitory component of the $MnSO_4$ indicator, growth of the three representative strains (Table 2) in B broth was compared with that in the broth with various additives. Because pH changes obtained during lag and logarithmic phases reflected CCU_{50} determinations (Fig. 2, legend), the former was used to follow growth. The response of the type strain, T960, is shown in Fig. 2. Growth in B broth proceeded as expected, with the pH readings increasing until the end of exponential growth. In broth containing 1 mM $MnSO_4$, $MnCl_2$, or $Mn(C_2H_3OH)_2$, the pH showed no significant increase. However, in broth with equimolar concentrations of chloride or acetate (provided as sodium salts), growth conformed to the pattern demonstrated by normal growth in B broth. The response of the other two strains was basically the same, with normal growth occurring only in the absence of manganese. There was, of course, the anticipated difference in the degree of the response to Mn; strain 27 was less susceptible and strain 354 was more susceptible than strain T960.

In addition to the adverse effect of Mn on growth and colony morphology, cellular ultrastructure was also altered. Strain 27, of biotype 1, was examined by electron microscopy. Thin sections of cells taken from exponential growth (pH 6.8) in B broth (Fig. 3a) showed a relatively uniform appearance. Most of the oval, elongated, and dumbbell-shaped cells were packed with ribosomes, as one would expect of cells in active growth. Although similar forms were also present in the companion culture in B-Mn broth harvested some hours later, when that culture also had reached pH 6.8, many abnormal forms were seen (Fig. 3b). The more susceptible cells of biotype 2 did not grow in B-Mn broth and, therefore, were not examined.

Because of the usefulness of the manganese indicator system in the clinical laboratory, we sought a means of reversing its inhibitory effect. Our media contained half of the usual 20% (vol/vol) serum supplement of mycoplasma and ureaplasma media. We gained no benefit from using the higher serum concentration. We then tested the effect of eight cations of biological importance (calcium, cobalt, copper, iron, magnesium, potassium, sodium, and zinc) supplied as chloride salts at final concentrations of 1, 10, and 30 mM. Of these, only Mg reduced inhibition, and it did so for all of the strains tested. The concentration of Mg which showed the greatest sparing effect was strain dependent and varied with the degree of susceptibility to Mn (Fig. 4).

Our inability to block completely the inhibitory effects of manganese on ureaplasma growth led us to compare the practical considerations of having the indicator incorporated into the agar (Fig. 5a) as opposed to applying the reagents to the colonies after incubation (Fig. 5b). We found the latter to be a more effective indicator (Fig. 5b). The time period over which a positive urease spot test could be obtained was determined by using strain T960 on GM agar. After a 3-day incubation period, half of the cultures were left in the incubator and the rest were placed at RT. For cultures at 36°C, the spot test response was strong for 7 days but detectable for 11 days. For the companion cultures at RT, the response was strong for 10 days but detectable throughout the 14-day experiment. Successful subcultures were made from areas of growth which had not come into contact with the test reagent (for 6 days at 36°C versus 14 days at RT). Attempts to subculture the organism from those areas of the agar which had been exposed to the reagent failed in every instance.

Each of the standard strains used in our expanded serotyp-

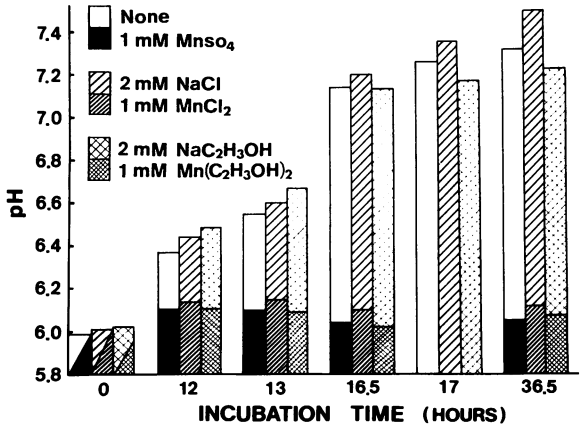

FIG. 2. The effect of manganese on the growth of *U. urealyticum* T960 (serotype 8) in broth cultures. The legend for the additions made to B broth is shown on the figure. Growth was followed by pH increases indicative of urea degradation. The Mn-containing broths were not measured at 17 h. CCU_{50} determinations were made from all cultures at least twice during incubation to verify the relationship between pH and viable cell counts during the positive growth phases. For instance, in B broth the number of CCU_{50} per milliliter increased from 3.5×10^4 at inoculation to 1.3×10^6 at 12.5 h and 5.5×10^7 at 16.5 h and had fallen to 1.3×10^2 by 36.5 h. In B-Mn broth, the number of CCU_{50} per milliliter remained stable at 2.9×10^4 until 12.5 h but then fell to 1.3×10^1 by 36.5 h.

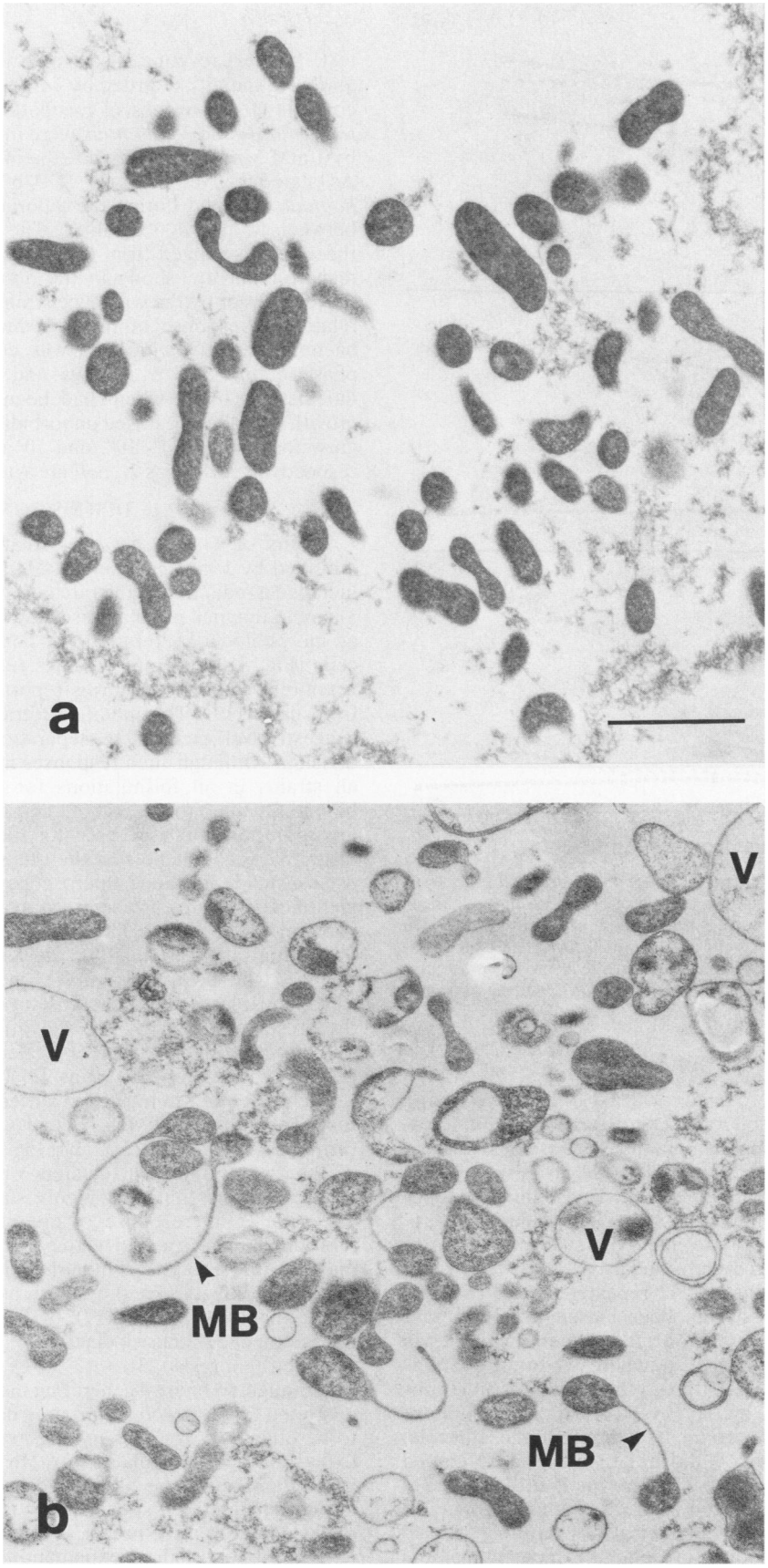

FIG. 3. The effect of manganese on the cellular morphology of *U. urealyticum* 27 (serotype 3). Cells from cultures in B broth (a) were typical of many mycoplasma species in logarithmic growth. In B-Mn (b), aberrant forms were also present. These were bilobed cells connected by a long, membranous bridge (MB) and apparently empty vesicles (V). The bar represents 1 μm. Magnification, ×19,500.

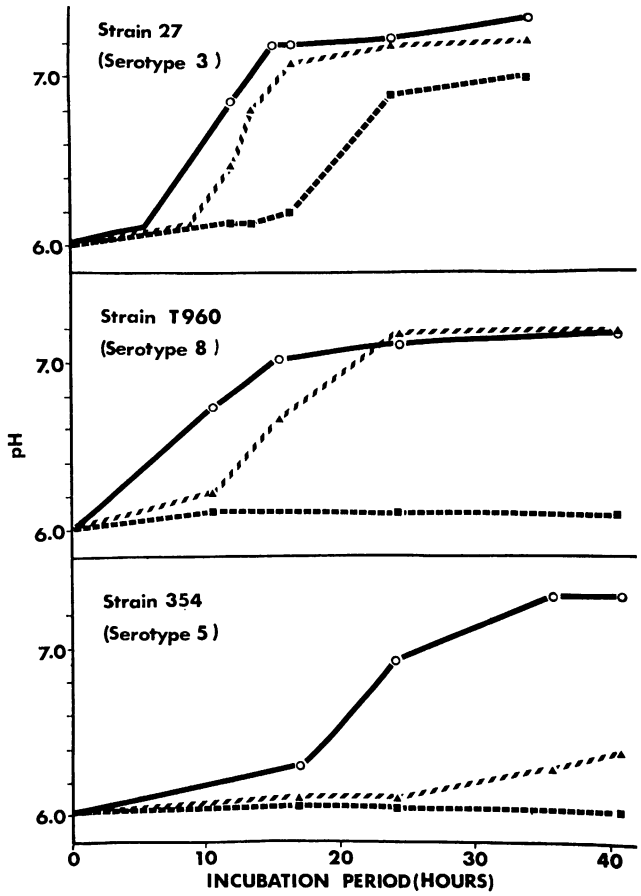

FIG. 4. Heterogeneity in the response of strains of *U. urealyticum* to manganese and in the sparing effect of magnesium. Changes in the pH of cultures of representative strains in B broth (○), B-Mn broth (■), and B-Mn broth with added $MgCl_2$ (▲). The concentration of $MgCl_2$ that gave the best sparing effect for the particular strain is shown: for strain 27, 10 mM; for strain T960, 30 mM; and for strain 354, 60 mM.

ing scheme had been cloned at least three times, and examination by immunofluorescence by use of epifluorescence revealed no heterogeneity in the response of any of the 14 antigens (12). The response of these strains to 1 mM $MnSO_4$ was then determined (Table 3). The slightly susceptible strains of biotype 1 were the serotype standards 1, 3, 6, and 14, and the moderately to markedly susceptible strains of biotype 2 were the serotype standards 2, 4, 5, 7, 8, 9, 10, 11, and 12. (Serotype standard 13 repeatedly gave an intermediate response and has not been classified.) Biotype 1 strains showed similar growth on agar with and without Mn (i.e., a GM:GM-Mn ratio near 1.0, whereas biotype 2 strains had ratios greater than 5). Although the growth of all strains was inhibited in B-Mn, by day 5 of incubation the titers of the biotype 1 strains approximated those of the controls, whereas those of biotype 2 did not then or on continued incubation. In B-Mn, the titers of biotype 2 strains were at least 1:1,000 of those obtained in the B broth controls.

Because of the deleterious effect of Mn on ureaplasmas, we examined its effect on representative members of the family *Mycoplasmataceae*. Based on CCU determinations of laboratory-adapted strains with titers of 10^7 to 10^8 (similar to those of the ureaplasmas tested), broth cultures of the four test species responded as follows. The growth of *A. laidlawii*, initially retarded by 1 mM Mn, reached that of the controls after 2 weeks of incubation. *M. pneumoniae*, *M. fermentans*, and *M. hominis* were inhibited by 5 mM but not by 1 mM Mn; for *M. hominis*, the inhibition was temporary. As stated above, colonies of 9 of the 19 isolates of *M. hominis* obtained during the laboratory trial had counts of between 30 and 400 colonies. The GM:GM-Mn ratios for these strains ranged from 0.5 to 27, an even more variable response than that shown by the laboratory-adapted strain of that species or by the wild-type strains of *U. urealyticum*. To relate the response of mycoplasmas to Mn with that of bacteria, the following trial was conducted. Logarithmic-phase cultures of *S. aureus* and *E. coli* were used to inoculate B broth which had been modified for bacterial growth (see above). Based on turbidity, the *S. aureus* culture grew to titers of 10^8, 10^6, and 10^3 in 0, 1, and 5 mM Mn, respectively, whereas *E. coli* grew to 10^8 in all three media.

DISCUSSION

Strains of *U. urealyticum* isolated from humans were inhibited by 1 mM manganese. Manifestations of this effect included a reduction in the rate of growth (Fig. 2 and 4; Table 3) and in the final populations achieved (Fig. 2 and 4) as well as morphological alterations of both colonies (Fig. 1) and cells (Fig. 3). Aberrant colonies (e.g., Fig. 1b) sometimes resembled the unusual forms reported on primary isolation from urine (19). The modified ultrastructure (Fig. 3b) was suggestive of incomplete separation of sister cells after division. Although such responses may not be expressed by all strains in all formulations for ureaplasma media, the potentially inhibitory effect of manganese should preclude its incorporation into agar used for the isolation of this organism. We recommend that the urease spot test (Fig. 5b) be used instead. If a good microscope is not available for the identification of ureaplasmas on agar, an internal indicator may be required. An alternative now exists. In his new differential agar formulation, designated A8, Shepard has replaced the 0.88 mM $MnSO_4$ indicator with equimolar $CaCl_2$ and gained a 10% increase in colony numbers (16). In the present study (data not shown), the addition of 1 mM $CaCl_2$ to B broth had no adverse effect upon either the rate of growth or final titers of strains 27, T960-CX8, or 354(NIH).

The degree of Mn inhibition varied with cation concentration (Tables 1 and 2; Fig. 1) and was strain specific (e.g., serotype 3 in Tables 1, 2, and 3; Fig. 4), allowing subdivision of the strains into two clusters or biotypes, one slightly susceptible and the other highly susceptible to 1 mM Mn. The differential response of ureaplasma strains has been reproducible on repeated testing over a number of years. For most of the serotype standards, the validity of these two biotypes is substantiated by patterns obtained by polyacrylamide gel electrophoresis (7), by DNA hybridization (3), by restriction endonuclease digests (8), and by two-dimensional gel electrophoresis (23).

Although no energy-generating mechanism has been demonstrated for *U. urealyticum*, urea degradation is considered to be obligatory for growth (e.g., reference 21). The initial explanation we postulated for Mn inhibition was that its precipitation onto the colonies restricted further growth. We thought that strain specificity in response to manganese might reflect relative urease activity. We have provided no data to support such an explanation. In 1979, Romano et al. (14) reported that crude enzyme preparations from one ureaplasma strain (P 108) were completely inactivated by 0.5 mM concentrations of heavy metal cations (Hg^{2+}, Ca^{2+},

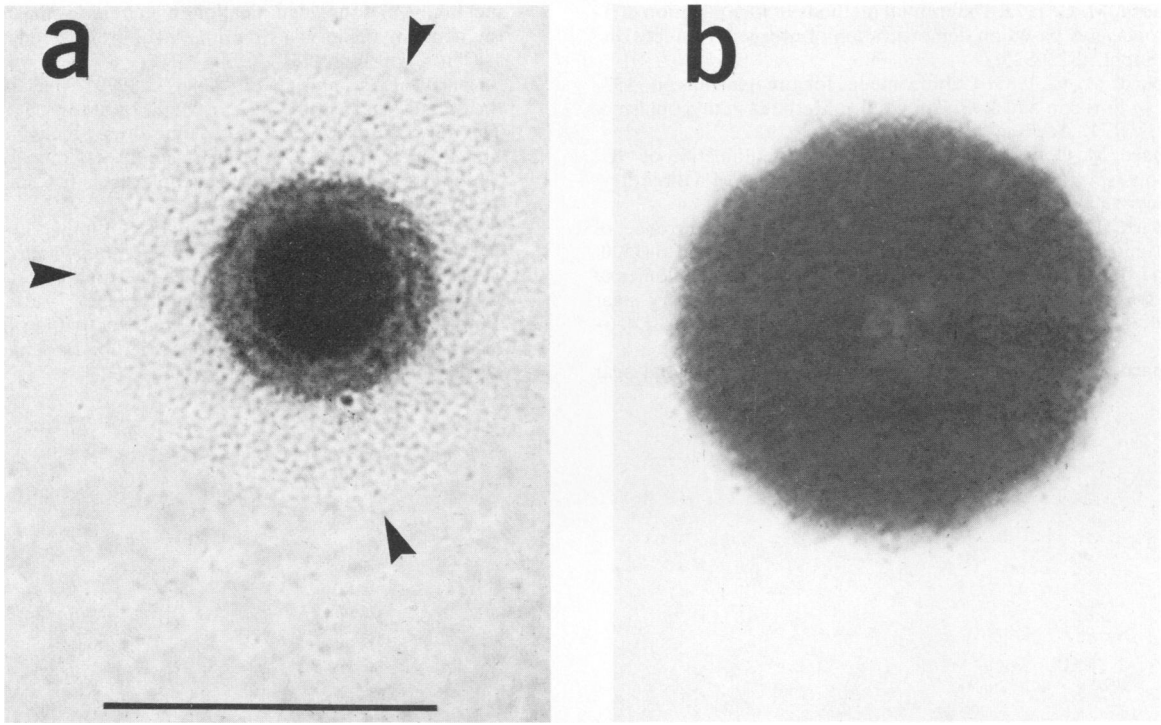

FIG. 5. The manganese indicator system used for *U. urealyticum* 27 (serotype 3). The center of the colony on A7 agar was darkened by the manganese reaction product (a). When a colony of similar size on GM agar was exposed to the urease spot test reagent, the response was more obvious (b). The bar represents 100 μm.

Fe^{2+}) but that the effects of other cations (Na^+, K^+, Ca^{2+}, Mg^{2+}, and Mn^{2+}) were negligible. We have not been able to obtain this strain to test its growth response to Mn.

Mn has a multiplicity of functions in biological systems. For all of the ureaplasma strains that we tested, Mn inhibition could be blocked by Mg, suggesting that the latter was required for an essential cellular function. However, the blocking effect of Mg was only partial, an indication that competitive inhibition of Mg by Mn was not the sole effect of Mn on the ureaplasma cells. We must consider also that, in addition to being multifactorial, the mechanisms of Mn inhibition may not be the same for all strains of the species. Clear demonstration of this would provide even further support for the concept of biotypes among strains of ureaplasmas isolated from humans. Because of the opportunity it affords for the discrimination between strains, the molecular basis for Mn inhibition is under investigation in this laboratory.

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