

## Stone Formation by *Ureaplasma urealyticum* in Human Urine and Its Prevention by Urease Inhibitors

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When *Ureaplasma urealyticum* T-960 was inoculated into normal human urine ( $10^8$  viable cells per ml of urine), a white precipitate formed, with an increase in pH of the infected urine. This precipitate was identified as a mixture of struvite and whitlockite by analysis of the infrared spectrum. Its formation was completely prevented by the addition of  $10 \mu\text{M}$  *N*-benzoylphosphotriamide,  $20 \mu\text{M}$  *N*-isopentenoylphosphotriamide, or  $0.5 \text{ mM}$  caprylohydroxamic acid without the alkalization of the urine, and the *Ureaplasma* color change units were also decreased markedly by these compounds. The apparent concentrations for 50% inhibition by *N*-benzoylphosphotriamide, *N*-isopentenoylphosphotriamide, and caprylohydroxamic acid against *Ureaplasma* urease were  $7 \text{ nM}$ ,  $2 \text{ nM}$ , and  $2.2 \mu\text{M}$ , respectively. From these results, it seems that stone formation by *U. urealyticum* is prevented with these compounds, that prevention being directly attributable to the inhibition of urease activity, which causes the death of the cells.

Formation of urinary struvite stones are commonly associated with urinary infection by urea-splitting bacteria such as *Proteus* species. The bacterial urease hydrolyzes urea, leading to hyperammonuria and the alkalization of urine, which causes supersaturation with respect to struvite and calcium phosphate, with consequent crystalization of struvite and apatite. This theory was proved by the fact that this type of urinary stone was formed with infection by *Proteus mirabilis* in vitro (6, 25) in human urine and in vivo (5; M. Satoh, K. Munakata, K. Kitoh, H. Takeuchi, and O. Yoshida, submitted for publication.) in the bladders of rats and that the high urease activities (27) were detected in urine of the patients with urinary stones caused by infection. The formation of such urinary stones was prevented by treatment with urease inhibitors such as hydroxamate (HXA) (1, 5, 23, 26), which has been established to be a potent and specific inhibitor (12). Recently we reported that 2,2'-dimethylpropionylglycino-HXA is a therapeutic agent for such a type of urolithiasis and had a potent inhibitory power (16), high renal clearance (16), and no mutagenicity (17). Based on these findings, Takeuchi et al. (24) reported that this compound markedly prevented the formation of bladder stones in rats infected by *P. mirabilis*.

Phosphodiamidate (2) and *N*-acylphosphotriamide (A. V. Bayless and O. E. Millner, Jr., Chem. Abstr. 92:146458b, 1980; K. Kobashi, K. Sakaguchi, S. Takebe, J. Hase, and M. Satoh, Abstr. Int. Congr. Biochem., 12th, p. 305, 1982) were recently reported as new and more potent urease inhibitors. Millner et al. (15) showed that *N*-(diaminophosphinyl)-4-fluorobenzamide prevented stone formation in rats infected by *P. mirabilis*.

*Ureaplasma* species, frequently detected in the urogenital tracts of mammals, is a unique *Mycoplasmataceae* which produces urease (21). Friedlander and Braude (4) were successful in an attempt to form numerous stones in the bladder of rats by infection with *Ureaplasma* cells. Lann et al. (13) showed that this stone formation was prevented by the oral administration of aceto-HXA.

In this report, we show that urinary stones are formed in human urine inoculated with *Ureaplasma urealyticum* T-960 and that urease inhibitors such as HXA and *N*-acylphosphotriamide prevent stone formation.

### MATERIALS AND METHODS

**Culture of *U. urealyticum* T-960.** *U. urealyticum* T-960, which was kindly provided by Nakamura, Kurume Medical University, Kurume, Japan, was grown aerobically at  $37^\circ\text{C}$  for 15 h in Taylor-Robinson medium (TR medium) (28) modified to contain 10% horse serum.

**Formation of urinary stones.** Fresh human urine samples were obtained from volunteers without any history of urinary stone or urogenital infection diseases, made certain to be *Ureaplasma* negative, and sterilized by Seitz filtration (membrane, Toyo 85-SB, Tokyo). A volume of 0.5 ml of broth culture (described above) was inoculated into 4.5 ml of the sterile urine, followed by aerobic incubation at  $37^\circ\text{C}$  for 20 to 24 h.

**Preparation of cell suspension and cell-free fraction of *U. urealyticum*.** *Ureaplasma* cells (0.025 ml) precultured in modified TR medium were inoculated into medium (500 ml) containing 10% horse serum, 2.5% fresh yeast extract, 1.47% Bacto-PPLO without crystal violet (catalogue no 0554-02-08, Difco Laboratories, Detroit Mich.), 0.04% urea, 0.002% phenol red, and  $10^3 \text{ U}$  of penicillin G per ml (pH 6.3; adjusted with 1 N hydrochloric acid) and grown aerobically at  $37^\circ\text{C}$  for 21 h (urease activity; 36 mIU/ml of serum). Cells harvested by centrifugation at  $20,000 \times g$  for 20 min were washed twice with 10 ml of a cold physiological saline. A part of the cells obtained was suspended in 5 ml of 10 mM phosphated buffer (pH 6.3) containing 0.15 M sodium chloride (cell suspension). The others were suspended in 5 ml of 0.1 M phosphate buffer (pH 6.3), disrupted three times by ultrasonic vibration (Sonicator model W-200F; Heat Systems-Ultrasonics, Inc., Plainview, N. Y.) for 30 s at intervals of 1 min in an ice bath, and centrifuged at  $20,000 \times g$  for 20 min to remove the debris. The cell suspension and the cell-free fraction were used for the measurement of the urease activity.

**Urease inhibitors and antibiotics.** Nicotino- and caprylo-HXA were synthesized in our laboratory. *N*-Benzoylphos-

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photriamide (BPA) and *N*-isopentenoylphosphotriamide (IPA) were kindly provided by Munakata, Eisai Co., Tokyo, Japan. Tetracycline hydrochloride (Sigma Chemical Co., St. Louis, Mo.) erythromycin lactobinate (Abbott Laboratories, North Chicago, Ill.), and lincomycin hydrochloride (Sigma) were used. Every compound except caprylo-HXA was dissolved in distilled water at a concentration of 10 mM for urease inhibition and 1 mg/ml for antibiotic activity. Caprylo-HXA was dissolved in 10% ethyl alcohol at 10 mM.

**Measurement of growth of *Ureaplasma* cells.** Color change units (CCU) and CFU were measured according to the method described by Nakamura et al. (18). For the determination of CCU, a 0.2-ml sample was inoculated into 1.8 ml of modified TR medium and was serially diluted  $10^2$ - to  $10^{10}$ -fold with the same medium. These diluted samples, consisting of 10 tubes, were aerobically incubated at 37°C for 48 h. CCU represented the logarithm of the highest dilution showing the development of the alkalization of the medium, which indicated the growth of *Ureaplasma* cells. CFU represented the number of colonies formed on *Ureaplasma* differential agar (catalogue no. M52650; GIBCO Diagnostics, Madison, Wis.) plates, containing 20% horse serum, 0.25% fresh yeast extract, 0.1% urea, 0.01% cysteine hydrochloride, and  $10^3$  U of penicillin G per ml, on which the samples were inoculated and incubated in an anaerobic jar with a GasPak hydrogen and carbon dioxide generator envelope (BBL Microbiology Systems, Cockeysville, Md.) at 37°C for 4 days.

**Measurement of urease activity.** The cell suspension and cell-free fraction were diluted with 10 mM phosphate buffer (pH 6.3). The diluted solution (0.2 ml) was mixed with 0.55 ml of 0.45 M urea which was dissolved in 10 mM phosphate buffer (pH 7.3), followed by incubation for 10 min. The buffer used for the dilution and urease activity assay of the cell suspension contained 0.15 M sodium chloride. An amount of 0.2 ml of 1 N sulfuric acid was added to the reaction mixture, and 0.5 ml of the mixture was analyzed for the liberated ammonia by the indophenol reagents of Okuda and Fujii (19). Urease activity (international units) was measured as the micromoles of urea hydrolyzed per minute at 37°C.

To measure the inhibitory powers of the test compounds, 0.1 ml each of the cell suspension or the cell-free fraction and the compound solution diluted in the same buffer as described above was mixed, preincubated at 37°C for 30 min, and incubated with urea under the same assay conditions. Another assay method with radioactive urea was employed for the compounds which interfere with the indophenol reaction of ammonia. An amount of 0.1 ml each of enzyme, a test compound, and [ $^{14}$ C]urea (120 dpm/nmol; Daiichi Co., Tokyo, Japan) solution was mixed and incubated at 37°C for 30 min, followed by the addition of 0.25 ml of 2 N sulfuric acid to stop the reaction. Liberated  $^{14}$ CO<sub>2</sub> was immediately trapped into 0.2 ml of ethanolamine by the apparatus of

Ichiyama (9), and the radioactivity was measured by a Packard 300C liquid scintillation counter.

**Analysis of components of urinary stones.** Crystallographic analysis of precipitates was performed with an infrared spectrometer (Jasco A-102) by using a potassium bromide tablet. The amounts of calcium, magnesium, P<sub>i</sub>, and ammonia were measured by the calcium C-test, the magnesium B-test, the P-test (Wako Co., Osaka, Japan), and the indophenol reagents described above, respectively.

## RESULTS

**Formation of stones by *U. urealyticum* in human urine.** Viable cells ( $10^8$ ) of *U. urealyticum* T-960, with a total urease activity of 14.3 mIU, were inoculated into human urine and incubated for 24 h. The CCU increased from 8 to 10 after the first 6 h of incubation, and the pH increased from 6.7 to 8.1 after 24 h of incubation, at which time several crystals were found. However, no sediment was detected in the urine incubated with the cell-free fraction, which had a total urease activity of 477 mIU. These results showed that *U. urealyticum* grew in the urine and produced urease during the incubation period. The precipitates formed were observed in any urine specimens with different initial pHs between 5.5 and 7.6. When the urine pH was higher than 7, a little sediment was observed even in controls after incubation for 1 day, but this sediment was amorphous and apparently different from crystals. We thus used urine having a pH lower than 7.

Table 1 shows the weights and components of urinary stones produced by Jack bean urease and *Ureaplasma* cells. The pH of urine inoculated with *Ureaplasma* cells did not increase as much as it did in urine inoculated with plant urease. The weights and the amount of ammonia in the stones formed by *U. urealyticum* were significantly lower than those in stones formed by plant urease. Though there was a difference in the ratios of components between these stones, the infrared spectra resembled each other. Their spectra showed two deep absorbances at 1,050 and 3,400 cm<sup>-1</sup> and two shallow ones at 1,430 and 1,650 cm<sup>-1</sup>, and the absorbances were intermediary between the spectra of NH<sub>4</sub>MgPO<sub>4</sub> and Ca<sub>3</sub>(PO<sub>4</sub>)<sub>2</sub> (Fig. 1). These results suggest that the stones obtained from the *Ureaplasma*-infected urine were composed of struvite and whitelokite.

**Inhibition of *Ureaplasma* urease by HXA and *N*-acylphosphotriamide.** The apparent concentrations for 50% inhibition (I<sub>50</sub>) by caprylo- and nicotino-HXA were 2.9 and 4.8 μM, respectively, against the urease activity of *Ureaplasma* cells and 2.2 and 2.7 μM, respectively, against that of the cell-free fraction. They had a similar inhibitory potency regardless of the presence of the cell membrane. Their I<sub>50</sub> values were almost equal to those against bacterial ureases (7, 22). The I<sub>50</sub> values of BPA and IPA were 0.14 and 1.1 μM, respectively, against the urease activity of cells and 7.0 and 2.0 nM,

TABLE 1. Components of urinary stones<sup>a</sup>

Addition	Final pH	Wt. of stone <sup>c</sup> (mg)	μmol of:			
			Ca	Mg	P	NH <sub>3</sub>
Jack bean urease <sup>b</sup>	8.63 ± 0.10	6.23 ± 0.224	32.0 ± 2.00	5.97 ± 0.227	19.7 ± 0.763	1.04 ± 0.344
<i>U. urealyticum</i>	7.49 ± 0.048	5.19 ± 0.234	26.5 ± 3.18	4.90 ± 0.478	15.6 ± 0.770	0.156 ± 0.0409

<sup>a</sup> All the values represent the average ± standard deviation of four experiments.

<sup>b</sup> The urease activity was 10 IU.

<sup>c</sup> The stones were formed in 8.8 ml of urine.

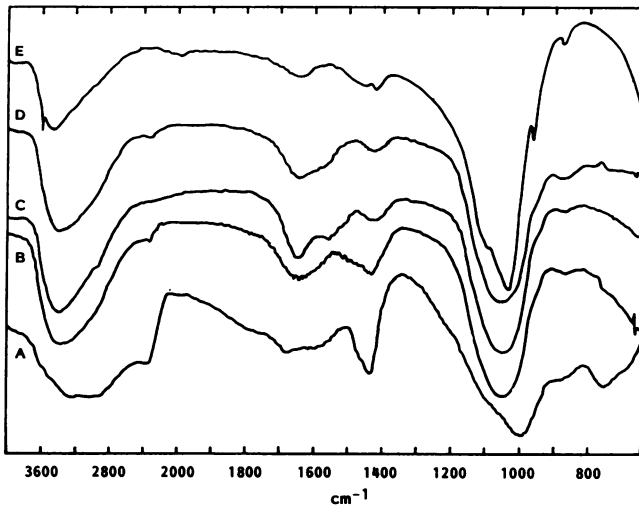


FIG. 1. Infrared spectra of urinary stones. The infrared spectra of urinary stones formed by Jack bean urease (B) and *U. urealyticum* (C and D) are shown. Stone C was obtained from urine with a final pH of 8.5, and stone D was from urine with a final pH of 7.5. The infrared spectra of NH₄MgPO₄ (A) and Ca₃(PO₄)₂ (E) are shown as authentic spectra.

respectively, against that of the cell-free fraction. The difference in I<sub>50</sub> values between viable cells and cell-free enzyme was probably due to the low permeability of phosphotriamide across the *Ureaplasma* cell membrane. The longer the preincubation period was, the stronger the BPA and IPA inhibition of the intracellular urease was. BPA and IPA were found to be markedly potent inhibitors, and their I<sub>50</sub> values were similar against Jack bean and *Proteus* ureases (Kobashi et al., 12th Int. Congr. Biochem., p. 305).

Erythromycin, tetracycline, and lincomycin did not inhibit the urease activity at all at the preincubation concentration of 33 µg/ml.

**Prevention of urinary stone formation by urease inhibitors.** Figure 2 shows urinary stone formation in human urine infected by *U. urealyticum* and its prevention by BPA. No sediment was detected in the presence of 20 µM BPA. Only a little amorphous sediment appeared at 5 µM BPA, but it was not identified as an inorganic salt because of the very small amount. At concentrations less than 5 µM BPA, as much urinary precipitate was formed as in the control without the urease inhibitor. These findings were supported by the analytical data for pH, ammonia, and CCU (Fig. 3). With the addition of 20 µM BPA, no change occurred in the

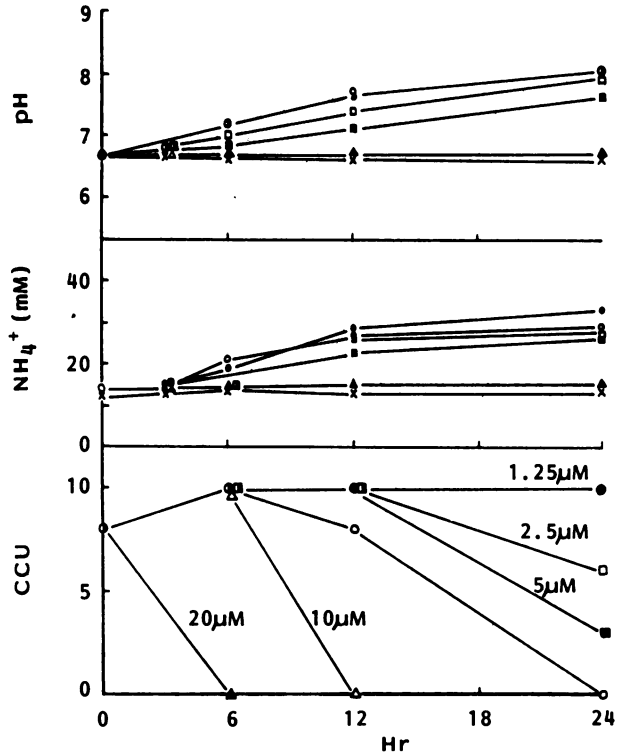


FIG. 3. Effect of BPA on alkalinization of urine and growth of *Ureaplasma* cells. Open circles and crosses represent urine infected with *Ureaplasma* cells or without *Ureaplasma* cells, respectively. Other symbols represent treatment with BPA at various concentrations; 1.25 (●), 2.5 (□), 5 (■), 10 (△), and 20 (▲) µM.

pH and ammonia concentration of the infected urine, the CCU decreased from 8 to 0, and the CFU decreased from  $5 \times 10^8$  to  $8 \times 10^6$  (data not shown) after the first 6 h of incubation. After incubation for 21 h, the CFU decreased more markedly, to less than  $2 \times 10^3$ . On the basis of these results, we concluded that stone formation was depressed by both the urease inhibition and the cytotoxic effect of BPA. By the addition of 10 µBPA, *Ureaplasma* cells grew during the first 6 h of incubation, but the growth and production of urease were not enough to increase the pH and ammonia concentrations in the urine, resulting in no crystal formation. At concentrations of 5 µM or less, the increases in pH were inhibited by BPA, depending upon the concentration, but these inhibitions were not enough to prevent urinary stone formation. Consequently, some precipitates were formed

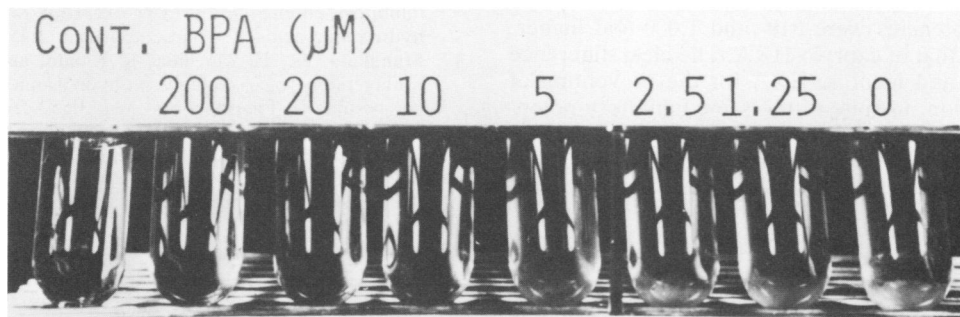


FIG. 2. Prevention of urinary stone formation by BPA. The photograph was taken after incubation for 24 h.

even after the first 6 h of incubation. CCU observed at these concentrations of BPA decreased more slowly than those of the control during the whole incubation period. These facts suggested that the addition of low concentrations of BPA reduced the rate of alkalization of urine, resulting in the delay of death of *Ureaplasma* cells. When IPA was added to an infected urine sample, the same results were obtained, except that 20  $\mu$ M IPA was necessary for the complete prevention of stone formation.

When 0.5 mM caprylo-HXA was added to the infected urine under the same conditions, the pH and ammonia concentration of the urine did not increase, the CCU decreased from 10 to 5, and the CFU decreased from  $2.8 \times 10^9$  to  $3.4 \times 10^5$  after 24 h of incubation. However, 1 mM nicotino-HXA did not prevent stone formation, although the latter was as potentially inhibitory as caprylo-HXA and IPA were against the cell suspension. From these results, it seems that potent and irreversible inhibition of the intracellular urease (11) is necessary for the complete prevention of the urinary stone formation.

Erythromycin and tetracycline were effective for the prevention of stone formation at a concentration of 6.25  $\mu$ g/ml, but lincomycin was not effective at 100  $\mu$ g/ml.

### DISCUSSION

In the present paper, we showed that *U. urealyticum* T-960 growing in human urine formed a precipitate composed of struvite and whitelockite. The struvite ( $\text{NH}_4\text{MgPO}_4 \cdot 6\text{H}_2\text{O}$ ) in the precipitate, based on the calculation of magnesium concentration, was 23% of the total weight. The infrared spectrum of this precipitate did not show any evidence of containing other magnesium compounds, such as  $\text{Mg}_3(\text{PO}_4)_2$  and  $\text{MgHPO}_4$ . The mechanism of the precipitate formation in vitro was similar to that reported previously in *P. mirabilis*-infected urine (6, 25). Accompanied by the growth of cells, the pHs and the ammonia concentrations in the urine increased, and consequently the precipitate appeared. Extensive growth of cells was necessary for precipitate formation, as supported by the following facts; (i) increase of the CFU from  $4.8 \times 10^8$  to  $3.3 \times 10^9$  during the early period of incubation, (ii) requirement of more than 1 IU of Jack bean urease per ml for precipitate formation (data not shown), and (iii) no precipitate formation as a result of treatment with antibiotics such as erythromycin and tetracycline, which were reported to depress the growth of *Ureaplasma* cells (10).

Ford (3) and Masover et al. (14) have already reported that sorbyl- and aceto-HXA inhibited the growth of *Ureaplasma* cells. But our data showed that the inhibition of *Ureaplasma* urease by BPA, IPA, and caprylo-HXA brought about the death of the cells and consequently completely prevented the precipitate formation. The effective concentrations of BPA and IPA were lower than that of caprylo-HXA, because their inhibitory potencies were 310- and 1,000-fold higher, respectively, than that of caprylo-HXA. The clear difference between caprylo- and nicotino-HXA for the prevention of precipitate formation, in spite of the same inhibitory potency, was probably because a long-chain aliphatic acyl-HXA such as caprylo-HXA irreversibly inhibited the enzyme (11) and had some antibacterial activity because of the acyl residue (8). Romano et al. (20) reported the decrease of ATP synthesis in *Ureaplasma* cells by the inhibition of urease activity with aceto-HXA. Their theory supported our results that BPA and IPA killed the *Ureaplasma* cells. Therefore, the compounds showing a preventive effect on precipitate formation in this experiment are expected to have more

clinical usefulness against *Ureaplasma* infection than aceto-HXA (13).

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### LITERATURE CITED

1. Andersen, J. A. 1975. Benurestat, a urease inhibitor for the therapy of infected ureolysis. *Invest. Urol.* **12**:381-386.
2. Dixon, N. E., C. Gazzola, J. J. Watters, R. L. Blakeley, and B. Zerner. 1975. Inhibition of Jack bean urease (EC 3.5, 1.5) by acetohydroxamate and by phosphoramidate. An equivalent weight for urease. *J. Am. Chem. Soc.* **97**:4130-4131.
3. Ford, D. K. 1972. Inhibition of growth of T-strain mycoplasmas by hydroxamic acids and by aurothiomalate. *Antimicrob. Agents Chemother.* **2**:340-343.
4. Friedlander, A. M., and A. I. Braude. 1974. Production of bladder stones by human T *Mycoplasma*. *Nature (London)* **247**:67-69.
5. Griffith, D. P., and D. M. Musher. 1973. Prevention of infected urinary stones by urease inhibition. *Invest. Urol.* **11**:228-233.
6. Griffith, D. P., D. M. Musher, and J. W. Campbell. 1973. Inhibition of bacterial urease. *Invest. Urol.* **11**:234-238.
7. Hase, J., and K. Kobashi. 1967. Inhibition of *Proteus vulgaris* urease by hydroxamic acids. *J. Biochem.* **62**:293-299.
8. Hase, J., K. Kobashi, N. Kawaguchi, and K. Sakamoto. 1971. Antimicrobial activity of hydroxamic acids. *Chem. Pharm. Bull. (Tokyo)* **19**:363-368.
9. Ichiyama, A. 1972. Tryptophan 5-monooxygenase. *Seikagaku* **44**:825-848. (In Japanese.)
10. Kishima, M., and M. Hashimoto. 1979. *In vitro* sensitivities to antimicrobial drugs of *Ureaplasma* isolated from the bovine respiratory tract, genital tract and eye. *Res. Vet. Sci.* **27**:218-222.
11. Kobashi, K. 1972. Inhibition of urease activity by hydroxamic acid and its practical application. *Seikagaku* **44**:187-204. (In Japanese.)
12. Kobashi, K., J. Hase, and K. Uehara. 1962. Specific inhibition of urease by hydroxamic acids. *Biochem. Biophys. Acta* **65**:380-383.
13. Lann, D. L., S. A. Johnson, A. M. Friedlander, and R. F. Gittes. 1977. Medical therapy of experimental infection stones. *Urology* **10**:418-420.
14. Masover, G. K., J. R. Benson, and L. K. Hayflick. 1974. Growth of T-strain mycoplasmas in medium without added urea: effect of trace amounts of urea and of a urease inhibitor. *J. Bacteriol.* **117**:765-774.
15. Millner, O. E., Jr., J. A. Andersen, M. E. Appler, C. E. Benjamin, J. G. Edwards, D. T. Humphrey, and E. M. Shearer. 1982. Fluorofamide: a potent inhibitor of bacterial urease with potential chemical utility in the treatment of infection induced urinary stones. *J. Urol.* **127**:346-350.
16. Munakata, K., K. Kobashi, S. Takebe, and J. Hase. 1980. Therapy for urolithiasis by hydroxamic acids. III. Urease inhibitory potency and urinary excretion rate of *N*-acylglycinohydroxamic acids. *J. Pharmacobio-Dyn.* **3**:451-456.
17. Munakata, K., H. Mochida, S. Kondo, and Y. Suzuki. 1980. Mutagenicity of *N*-acylglycinohydroxamic acids and related compounds. *J. Pharmacobio-Dyn.* **3**:557-561.
18. Nakamura, M., T. Itoh, and H. Ichimaru. 1982. Isolation and identification of human *Mycoplasma*, p. 1-39. *In Japan Bacteriology Education Committee (ed.), Procedure for isolation and identification of human, animal and plant Mycoplasma*. Saikon Press, Tokyo.
19. Okuda, T., and S. Fujii. 1966. The determination of ammonia in blood. *Saishin Igaku* **21**:622-627. (In Japanese.)
20. Romano, N., G. Tolone, F. Ajello, and R. La Licata. 1980. Adenosine-5'-triphosphate synthesis induced by urea hydrolysis in *Ureaplasma urealyticum*. *J. Bacteriol.* **144**:830-832.

21. Shepard, M. C., and C. D. Lunceford. 1967. Occurrence of urease in T strains of *Mycoplasma*. *J. Bacteriol.* **93**:1513-1520.
22. Shinbayashi, K., Y. Obata, and T. Yonemura. 1973. Effect of caprylohydroxamic acid on ruminal urease. *Jpn. J. Vet. Sci.* **35**:327-334.
23. Takeuchi, H., K. Kobashi, and O. Yoshida. 1980. Prevention of infected urinary stones in rats by urease inhibitor, a new hydroxamic acid derivative. *Invest. Urol.* **18**:102-105.
24. Takeuchi, H., Y. Okuda, K. Kobashi, and O. Yoshida. 1982. Treatment of infected urinary stones in rats by a new hydroxamic acid, "N-(pivaroyl) glycinohydroxamic acid." *Urol. Res.* **10**:217-219.
25. Takeuchi, H., Y. Takahashi, O. Yoshida, and K. Kobashi. 1976. Prevention of infected urinary stones by urease inhibitor. I. Infected urine and urease inhibitor. *Acta Urol. Jpn.* **22**:115-120. (In Japanese.)
26. Takeuchi, H., O. Yoshida, K. Kobashi, S. Takebe, and J. Hase. 1973. Prevention of infected urinary stones by urease inhibitor. II. Urinary excretion of hydroxamic acids and prevention of infected bladder stones in rats. *Acta Urol. Jpn.* **23**:113-118. (In Japanese.)
27. Takeuchi, H., O. Yoshida, S. Takebe, K. Kobashi, and J. Hase. 1977. Urease activity in infected urine. Relation of stone formation. *Acta Urol. Jpn.* **23**:647-651. (In Japanese.)
28. Taylor-Robinson, D., and R. J. Manchee. 1967. Adherence of mycoplasmas to glass and plastic. *J. Bacteriol.* **94**:1781-1782.