NOTES

Virulence of Ureaplasmal Urease for Mice

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Ureaplasmas killed mice within 5 min after intravenous injection. The 50% lethal dose of whole ureaplasmal organisms was 32 μg per mouse, a value also found for crystalline jackbean urease. The reaction was specific to urease, since protection was afforded by intraperitoneal injection of 200 μg of flurofamide, a potent urease inhibitor. The finding that a similar lethal effect was produced by injection of 200 μmol of NH₄⁺ indicates that the toxicity of urease is mediated by ammonium ions or free ammonia.

Ureaplasma urealyticum not only hydrolyzes urea but also requires urea for growth (8). The hydrolysis of urea by ureaplasmal urease appears to serve as a primary source of energy (15). The urease activity of ureaplasmas is high, since the specific activities of whole organism preparations were up to three times as great as those for crystalline jackbean urease (2). Jackbean urease, the first enzyme to be crystallized (in 1931), has long been known to be toxic for laboratory animals (16), and ureaplasmal urease has been implicated in the pathogenesis of ureaplasmas (3, 9, 13). That ureaplasmas have been recovered from the renal pelvis and upper urinary tracts of patients during surgery (5, 13) suggests that ureaplasmal urease could have a role in the formation of “infection” kidney stones, as do bacterial ureases.

When we sought to immunize mice with whole ureaplasmas for the production of monoclonal antibodies, we found that whole ureaplasmas killed mice rapidly. We determined that this killing effect was specific to the ureaplasmal urease and not to some other component of the ureaplasmal cell.

U. urealyticum (serovar 8, strain T-960, cloned eight times; obtained from M. Shepard) was grown in soy-peptide-fresh yeast diastase broth (6) containing 2% “agamma” horse serum, 25 mM urea, 50 mM MES [2-(N-morpholino)ethanesulfonic acid; Sigma Chemical Co., St. Louis, Mo.], and 1 mM Na₂SO₃ at pH 6.2 (8). Cultures (14 liters) were harvested by centrifugation at 8,000 × g when the pH reached 6.9 to 7.1. The pellet was washed three times in TES-saline (buffered saline containing 5 mM TES [N-tris(hydroxymethyl)methyl-2-aminoethanesulfonic acid; Sigma]), resuspended in 14 ml of TES-saline, and stored at −70°C. The yield was 0.2 to 0.5 mg of protein per liter of culture as measured by the method of Lowry et al. (10). Specific activities of ureases were determined with [¹⁴C]urea by the method of Masover et al. (11). Preparations were injected into the tails of male Swiss-Webster mice (6 to 10 weeks old and weighing 30 to 40 g) in a volume of 0.3 to 0.5 ml of buffer saline. Crystalline jackbean urease type C-3 at 1,000,000 U/g (1 U = 1 μmol of urea hydrolyzed per min at 25°C and pH 7.0) was obtained from Sigma. Labeled [¹⁴C]urea was obtained from Amersham. Flurofamide powder was a gift from Norwich-Eaton Pharmaceuticals (Norwich, N.Y.). The powder was dissolved in buffered saline at 0.2 mg/ml.

**Determination of LD₅₀.** In preliminary experiments, 200-μg doses of whole cells given intraperitoneally killed the three mice injected within 5 min, whereas with 100-μg doses, the mice were ill for 48 h but all survived. At a dose of 10 μg, no effect was seen. In contrast, 100 μg administered intraperitoneally killed all mice, whereas all survived challenge at 10 μg (Table 1). The 50% lethal dose (LD₅₀) was 32 μg (range, 16 to 64 μg [4]) for intravenous injection, as calculated by the Reed-Muench method (4, 14). The intravenous injection of 100 μg of crystalline jackbean urease was also lethal for mice, and the LD₅₀ was also 32 μg (range, 16 to 64 μg). The specific activities also were similar: 0.1 U for the ureaplasmal preparation and 0.3 U for the jackbean urease (specific activity is defined as micromoles of urea hydrolyzed per microgram of protein per minute). This particular batch of ureaplasmal urease was significantly less active than those reported previously (2).

**Time course.** A characteristic time course and set of toxic sequelae were observed in the experimental animals after inoculation. All animals became quiet and hyperventilated. Shortly thereafter or concurrently, the animals showed a progressive ataxia or staggering of gait. A neuromuscular twitching and hyperreactivity were noted until the animals became comatose, almost always within 5 min. Finally, a sudden rigid convolution resulted in death, often precipitated by the slightest stimulation. These events suggested acute neurotoxicity, such as is consistent with ammonia intoxication. When animals surviving lower challenge doses of urease were challenged with 100 μg of whole cells 4 weeks after the first challenge, their deaths followed the typical course.

**Protection by flurofamide.** Flurofamide (12) is 1,000 times as active as acetohydroxamic acid as an inhibitor of urease, and it acts as an antimicrobial agent for ureaplasmas (7). When flurofamide (200 μg, approximately 6 μg/g, or three times the MIC [7]) was given intraperitoneally 2 h prior to intravenous challenge with a known lethal dose of whole ureaplasmas (100 μg), the mice showed no signs or symptoms of illness (Table 2). Control mice not given flurofamide died within 5 min of challenge. Flurofamide protected mice from challenge with either the supernatant of sonicated cells

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or whole cells, indicating that flurofamide exerted its effect by inhibiting urease activity. Living cells were not required because the supernatant of sonicated cells was as lethal as whole cells (Table 2). Treatment of ureaplasmas at 65°C for 2 to 4 h produced a preparation without urease activity that was nontoxic to mice at a dose of 100 μg (Table 1).

**Ammonium toxicity.** In order to further define the mechanism of death, ammonium chloride was administered intravenously. Both animals receiving 200 μmol of NH₄Cl (5 μmol/g) died in the typical fashion, and one of the two animals survived a dose of 100 μmol (2.5 μmol/g). Flurofamide at 200 μg given intraperitoneally 2 h before injections to protect mice from an intravenous challenge with ammonium chloride (200 μmol). The toxic dose of ammonia (200 μmol per mouse) is close to the theoretical amount of ammonia generated by 100 μg of urease at a specific activity of 0.1 U/μg. Under optimum conditions, 100 μg of urease could generate 20 μmol of ammonia per min or 100 μmol in 5 min.

This murine model shows that ureaplasma urease is approximately as lethal as crystalline jackbean urease and that toxicity is mediated by the production of ammonia. Although this model utilizes larger amounts of organisms than would be found in natural infections, it is likely that the mechanism shown here may play a role in tissues immediately adjacent to live ureaplasmas. The pH of kidney urine in a ligated canine kidney experimentally infected with ureaplasmas increased, suggesting that urease has pathogenic potential in a dog model (9). Chronic interstitial renal inflammation was accompanied by an antibody response to ureaplasmas (9).

Since flurofamide prevents the toxic effects of urease, it likely has clinical and experimental applications. Ball and McCaughhey showed that parenterally administered flurofamide eliminated experimentally induced ureaplasmal vaginal infections of sheep (1). In our hands, flurofamide has proven useful in protecting mice from the lethal effects of ureaplasmas in immunization protocols for monoclonal antibody production. The clinical course of urease toxicity in mice is consistent with acute neurotoxicity, because the injection of 200 μmol of NH₄Cl intravenously killed the animals immediately.

**References**


