Pathogenicity of Stable L-Phase Variants of \textit{Staphylococcus aureus}: Failure to Colonize Normal and Oxamide-Induced Hydronephrotic Renal Medulla of Rats

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The direct intramedullary inoculation of stable L-phase variants of \textit{Staphylococcus aureus} failed to colonize normal and hydronephrotic rat kidneys induced by oral feeding of oxamide.

The intravenous inoculation of stable L-phase variants of different bacteria has failed to initiate renal infections in experimental animals (2, 11, 12; unpublished data). One reason for this failure was the inability of these variants to reach the kidneys. After intravenous inoculation, L-phase variants could have been eliminated by the suboptimal serum osmolality, the serum bactericidal activity, or the phagocytosis by leukocytes as well as reticuloendothelial cells. Direct inoculation of the L-phase variants into the hypertonic renal medulla that is most susceptible to infection (1, 3) would bypass these natural body defense mechanisms.

A strain of stable L-phase variants of \textit{Staphylococcus aureus} originally induced by lyso- staphin (9, 10) was inoculated into the renal medulla of male Sprague-Dawley rats weighing approximately 125 g.

\textbf{Group I.} Rats were fed Purina Rat Chow and water ad libitum. Under pentobarbital anesthesia and aseptic conditions, the kidneys were exposed by a mid-abdominal incision. A 0.1-ml amount of stable L-phase variants (10^8 to 10^7 colony-forming units) was inoculated into the medulla of each kidney. The incision was closed with 4-0 silk sutures. Groups of three to six animals were sacrificed at intervals from 10 min to 7 days. The bladder urine was aspirated and both kidneys were removed aseptically. One kidney was used for histological examination. The remaining kidney and bladder urine were cultured for L-phase variants by methods previously described (6).

At the time of sacrifice the kidneys appeared grossly normal. No microorganisms were seen on Gram stain of kidney sections, and the only abnormal finding was mild tubular dilatation in the area of the needle tract in rats sacrificed 7 days after inoculation. L-phase variants (10^2) were consistently recovered from kidneys of rats sacrificed at 10 min and were occasionally recovered at 30 min and 1 h after inoculation, but none were recovered thereafter. L-phase variants in decreasing numbers (10^6/ml at 10 min to 10^2/ml at 5 h, and none after) were recovered from the bladder urine.

\textbf{Group II.} Six rats were fed 1.2 g of a water-insoluble crystallogenic agent, oxamide (Eastman Kodak Co., Rochester, N.Y.), per 100 g of ground Purina Rat Chow and water ad libitum for 2 days. The kidneys and ureters were grossly and microscopically normal in animals sacrificed 1 to 5 days later.

Thirteen rats were fed oxamide for 4 days and sacrificed 1 to 12 days later. Mild to extremely severe bilateral hydronephrotic swollen kidneys were found in rats sacrificed at 1 to 3 days after oxamide was discontinued. Crystals of oxamide were found in the ureters of many animals (Fig. 1). The kidneys and ureters were grossly normal in animals sacrificed at day 5 and thereafter. With microscopy there were foci of tubular necrosis with occasional mild tubular epithelial cell degeneration, granular casts, polymorphonuclear neutrophil infiltration, and occasional crystals in tubules and the renal pelvis. These findings became less severe as the interval between the discontinuation of oxamide and the time of sacrifice lengthened. Normal renal histology was found in rats sacrificed at day 12.

\textbf{Group III.} Rats were fed oxamide for 4 days. One day after oxamide was discontinued, L-phase variants were inoculated into the renal medulla. Groups of six rats were sacrificed 1 to 7 days after inoculation. All rats had hydrourerets and swollen kidneys at the time of intramedullary inoculation of L-phase variants (Fig. 2). At the time of sacrifice, the degree of obstruction had decreased and occasionally the ureters and
kiıldıns looked normal grossly. There was no microorganism seen on Gram stain of kidney tissues, and the renal histopathology was similar to that of animals fed oxamide alone for 4 days.

No L-phase variants were recovered from the kidneys or the bladder urine. A mannitol-positive, coagulase-positive staphylococcus recovered from the kidney of one animal inoculated 7 days earlier with the stable L-phase variants probably represented a contaminant, but it would be impossible to rule out in vivo reversion (8).

These results indicated that direct intramedullary inoculation of stable L-phase variants of S. aureus failed to colonize normal as well as hydronephrotic rat kidneys. From this and other investigations, it seems very doubtful that stable L-phase variants are responsible for significant clinical infection.

One other important aspect of this investigation is the reproducible production of reversible bilateral hydronephrosis in rats by oral feeding of oxamide for 4 days. Although oxamide has been used in previous investigations, no hydronephrosis was produced due to the shorter duration of feeding (4, 5, 7). This model may be useful in other investigations where reversible bilateral hydronephrosis in rats is desired.

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LITERATURE CITED


