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Detection of Staphylococcal Nuclease Elaborated During Lethal Infections in Mice

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Active staphylococcal nuclease was detected in tissues of lethally infected mice. Organ distribution and levels of the enzyme varied with the bacterial strain used.

Secretion of a nuclease unique in its calcium requirement, high *p*H optimum, heat stability, and low molecular weight is an important criterion of pathogenic staphylococcal strains (1) and can be specifically measured in a variety of plant and animal tissues, whether sterile or heavily contaminated with microorganisms (3). The potential significance of this enzyme in disease (D. G. Stuart, Ph.D. thesis, Univ. of New Hampshire, Durham, 1967) and the possibility that it could provide information concerning the in vivo metabolic activities of *Staphylococcus aureus* led us to study whether it was elaborated in the tissues of mice with lethal staphylococcal infections.

White mice (20 to 25 g males) were inoculated intraperitoneally with washed cells of *S. aureus* UNH570, a strain characteristic of bovine-derived strains and highly virulent for mice (4, 5), or 14609, a strain characteristic of human-derived strains highly virulent for mice (4). The animals were sacrificed either shortly before death or approximately 1 hr before the mean time of death, 5.5 hr. Tissue samples were homogenized in saline 1:1 (w/v), and plasma was diluted 1:1 (v/v) with saline. Samples so prepared were diluted in equal volumes of 0.02 M to $CaCl_2$-0.05 M Tris-hydrochloride buffer (*p*H 8.8) and heated for 10 min at 100 C. Cellulose nitrate filters (Schleicher & Schuell B-6 Selectron) carrying bound, ^{14}C-labeled *Escherichia coli* deoxyribonucleic acid (DNA) (10) were pretreated by incubation in a solution of Ficoll, polyvinylpyrrolidone, and bovine albumin to prevent rebinding of enzymatically released DNA (7). Filters to which the buffered samples had been added were incubated in scintillation vials at 37 C. Three filters were incubated in a buffer-water mixture (1:1) to serve as controls. After 30 min of incubation, the filters were washed four times with a solution of 0.45 M NaCl plus 0.04 M sodium citrate, dissolved in 5 ml of Aquasol (New England Nuclear Corp.) scintillation fluid, and assayed in a liquid scintillation spectrometer. Significance of differences in treatment groups was determined by Duncan's new multiple-range test (12).

Analysis of the tissues of infected mice, mice receiving injections of washed, heat-killed cells, and tissues from untreated mice mixed with culture broth supernatant fluid of UNH570 are shown in Table 1.

Tissues mixed with culture supernatant fluid showed spleen, kidney, liver, and carcass to possess some ability to quench the assay, 6, 9, 12, and 81%, respectively. Consequently, the activity measured in these tissues in the other experimental combinations was appropriately corrected.

Mice injected with washed, heat-killed cells showed no evidence of morbidity or mortality, and, although the tissues of these animals showed slightly greater activities than did comparable tissues from control animals, the differences were not significant.

The possibility that the activity we observed might have been due to nonspecific factors arising in the moribund animal was tested by fatally infecting mice with *Klebsiella pneumoniae* and assaying their livers. No nuclease activity was detected.

Nuclease is elaborated by *S. aureus* during lethal infection of the mouse and retains its enzymatic activity in the tissues examined (Table 1). The analysis of the 14609 infections showed that 75 to 100% of the nuclease activity became detectable in the last 25% of the course of infection. Either the pathogen produces the bulk of the enzyme in this interval or failure of the clearance ability of the host tissue allows its accumulation.

The observed distribution of the activity in spleen, liver, kidney, and lung resembles distribution patterns observed by others (2, 6, 8, 9, 11) with alpha toxin and enterotoxin in mice, monkeys, and rabbits and is not unexpected because of the detoxifying function of these tissues. However, the absolute amount of activity detected and its relative distribution vary markedly with the strain of pathogen. The animal-derived UNH570

TABLE 1. *Tissue nuclease activity in mice inoculated with Staphylococcus aureus*

Tissue[b]	Nuclease activity[a] (μg of DNA released $g^{-1}hr^{-1}$)					
	Uninoculated mice	Heat-killed cell inoculation (strain UNH570)	Live-cell inoculation (strain UNH570)[c]	Live-cell inoculation (strain 14609)		Normal tissue + UNH-570 culture supernatant[f]
				1.5 Hr before death[d]	At death[e]	
Carcass[g]	3.4	6.8	5.8	NA[h]	NA[h]	15.2
Brain	0.7	2.0	1.3	0.7	13.2	76.0
Heart	1.7	2.1	2.5	1.7	10.1	77.6
Lung	0.9	1.9	7.7	2.8	17.4	76.8
Liver	−0.9	1.4	13.4	0.1	22.2	69.6
Spleen	−0.9	0.5	3.5	0.9	23.9	74.4
Kidney	3.0	3.9	7.9	9.2	51.3	72.0
Plasma	0	0	13.6	14.9	59.2	79.2
None						79.2

[a] Activity was calculated as follows: (counts per minute of control filter − counts per minute of sample filter)/(counts per minute of control filter) × (20 μg of DNA/filter)/(grams of tissue or milliliters of plasma/hr). The kidney, liver, spleen, and carcass values are corrected for the ability of these tissues to quench the assay.

[b] Five to 10 mice were pooled per sample.

[c] A 1-cm^3 amount of sterile saline containing 3×10^9 colony-forming units was inoculated intraperitoneally. Mice had begun to die at the time the first sample was taken.

[d] A 1-cm^3 amount of a washed 8-hr culture containing 4.8×10^8 colony-forming units was inoculated intraperitoneally 200 to 260 min before samples taken. No mice died during sampling period.

[e] A 1-cm^3 amount of a washed 5-hr culture containing 2.7×10^8 colony-forming units inoculated intraperitoneally 285 to 330 min before samples were taken. Mice had begun to die at the time the first sample was taken.

[f] A 1-ml amount of culture supernatant fluid was mixed with an equal volume of normal tissue homogenate.

[g] Remainder of animal after skin and organs to be sampled were removed.

[h] NA, Tissue not assayed.

strain yields much lower levels overall, with activities highest and about equal in plasma and liver. The activities in the lung and kidneys were lower and also about equal. The human-derived 14609 strain yields 2- to 12-fold higher nuclease levels, depending on the tissue, with activities highest and about equal in plasma and kidneys. Liver and spleen showed about one-half the activity of plasma and kidney, and the lung about one-third as much. With the 14609 strain, nuclease activity is detectable in brain and heart.

The differences in tissue distribution may be due to a tissue specificity of the enzyme or bacterial cell that differs between the strains or may reflect a greater capability of the UNH570 strain to damage hepatic clearance and the 14609 strain to damage renal clearance. It has been reported in two studies (9, 11) that the kidneys are the major site for detoxification of staphylococcal exotoxins. In both of these studies, however, the bacterial products were injected without infecting the host. Our results with an infected host show that the strain of *S. aureus* influences the tissue distribution of its exoproducts.

It has been pointed out (4) that the culture characteristics associated with mouse virulence in *S. aureus* are somewhat different for populations derived from human and animal hosts. The observations reported here indicate that members of these populations express virulence differently in vivo.

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