

Inhibitory Effect of Heparin on the Limulus Test for Endotoxin¹

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Heparin can inhibit the Limulus test for endotoxin unless 0.05 M CaCl₂ and 0.154 M NaCl are added to the lysate.

Previous reports (1-3) have indicated that heparin, even at 1,000 U/ml (1, 2), does not inhibit detection of endotoxin with the Limulus test. Studies in this laboratory have found heparin to be inhibitory but this effect can be overcome by addition of 0.05 M CaCl₂ and 0.154 M NaCl to Limulus lysate.

Limulus lysate was prepared according to the method of Sullivan and Watson (4). Heparin (1,000 U/ml; sodium salt: Sigma Chemical Company, St. Louis, Mo.) dissolved in pyrogen-free distilled water (Travenol, Deerfield, Ill.) was serially diluted twofold to 1 U/ml. Each tube containing 0.1 ml of heparin solution was then spiked with 0.01 ml of *Klebsiella pneumoniae* endotoxin (10 ng/ml) furnished by the Food and Drug Administration and 0.1 ml of lysate added to each tube. The tubes were then incubated at 37 C for 60 min. A solid gel which remained intact after inverting the tube (10 by 75 mm) 180° indicated no inhibition by heparin. The effect of various salt additions to the lysate for purposes of overcoming the inhibition by heparin was done by adding to chloroform-extracted lysate the following: (i) nothing, (ii) 0.154 M NaCl, (iii) 0.05 M CaCl₂, and (iv) 0.154 M NaCl plus 0.05 M CaCl₂. Chloroform extraction was necessary to remove an inhibitor in the lysate as previously discussed (4). Controls consisted of lysate with pyrogen-free water and lysate with heparin in the absence of added endotoxin. The lysate used would clot at less than 1 ng of endotoxin per ml with or without added salts.

Heparin inhibition of the reaction of endotoxin with Limulus lysate occurred when 0.05 M CaCl₂ and 0.154 M NaCl were not added to the lysate (Table 1). Addition of either salt alone only slightly relieved the inhibition. No inhibition, however, was found with lysate containing both salts up to 1,000 U of heparin per ml. That the heparin used was free of endotoxin was shown by serial twofold dilutions from 1,000 to 1 U/ml, which all gave a negative reac-

TABLE 1. Effect of heparin on reaction of endotoxin with Limulus lysate

Addition to lysate	Inhibitory level of heparin (U/ml) ^a
None	≤1.0
0.05 M CaCl ₂	7.5
0.154 M NaCl	7.5
0.05 M CaCl ₂ + 0.154 M NaCl	>1,000.0 ^b

^a Lowest concentration of heparin (U/ml) preventing gelation of lysate in presence of 1 ng of endotoxin per ml.

^b No inhibition found at 1,000 U/ml. It is not known whether higher concentrations of heparin would be inhibitory under these conditions.

tion with lysate containing 0.05 M CaCl₂ and 0.154 M NaCl.

Results of this study show that heparin can interfere with detection of endotoxin with the Limulus test. Heparin inhibition can be prevented by using lysate to which Ca²⁺ and Na⁺ have been added. The mechanism by which heparin inhibits the Limulus test is not known. The presence of sulfate, carboxyl, or other functional groups in the polysaccharide structure of heparin may account for its inhibitory effect. Ca²⁺ and Na⁺ may block such reactive groups that might interfere with one or more steps involved in the gelation of lysate by endotoxin (4).

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