LIPOPOLYSACCHARIDES FROM *RICKETTSIACEAE*: *LIMULUS* ENDOTOXIN ASSAY AND PATHOGENETIC MEDIATORS IN RICKETTSIOSIS

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Members of *Rickettsiaceae* family possess lipopolysaccharides (LPS) with endotoxic activity (*1*). We examined the ability of LPS from some rickettsiae to gelify the *Limulus* amoebocyte lysate, a typical assay of endotoxic potency (*2*). The preparations studied were: LPS from *Rickettsia typhi*, *R. slovaca*, *Coxiella burnetii in* phase I and II (kindly supplied by Dr S. Schramek, Institute of Virology, Bratislava, Czechoslovakia) and LPS from *C. burnetii* phase I (provided by Dr. E. Ribi, Rocky Mountain Laboratories, Hamilton, Montana, U.S.A.). All LPS were suspended in isotonic saline by vortex mixing; LPS from *C. burnetii* I and II were also studied after sonication for 5 min. The assay for gelation of *Limulus* lysate was done in duplicate with Pyrotest (Difco) and LAL (Microbiological Associates) as described (*3*). 0.1 ml each of lysate and LPS were mixed and presence or absence of a firm gel was noted after an undisturbed incubation or 60 min at 37 °C. The positive control consisted of the addition of 0.1 ml of a 1 ng/ml standard solution of *Escherichia coli* 0111:B4 LPS (Difco) to the lysate; addition of endotoxin-free saline constituted a negative control.

All the LPS were highly reactive producing a firm gel in the assay. LPS from *R. typhi* and *R. slovaca* showed a *Limulus* activity equivalent to 10 ng of endotoxin per ml, LPS from *C. burnetii* I an activity ranging between 10 (Dr. Schramek's preparation) and 5 (Dr. Ribi's preparation) ng per ml, while LPS from *C. burnetii* II an activity equivalent to 1 ng of endotoxin per ml. After the investigations on the LPS form *Rochalimaea quintana* (*4*) and from *C. burnetii* I (*5*), our observations represent the first evidence of a *Limulus* endotoxin activity in some members of the genus *Rickettsia* (*R. typhi* and *R. slovaca*). Our results indicate that these organisms possess another biological property of the more than 30 biological activities attributed to endotoxins of typical Gram-negative bacteria (*6*). It is tempting to speculate that some of the clinical manifestations described in rickettsiosis may be attributed to biological activity of an endotoxin and that endotoxaemia might contribute to some of these events. It is difficult to correlate endoxin content of organisms during infection with pathological effects, but this does not mean that no correlation exists. Walker and Mattern (*7*) suggested an ,,unidentified'' circulating toxin as a possible pathogenetic mediator of acute renal failure in Rocky Mountain spotted fever. Unfortunately, without endotoxin assay (not done even in experimental rickettsiosis) this seems hardly probable.

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