

ABSTRACT

Various experiments have been carried out in the past which attempted to isolate and purify cell fractions of Vibrio cholerae either of the classical or El Tor biotype of either Inaba or Ogawa serotype for the purpose of finding out if such antigen could be utilized in developing a potent vaccine. In this study the isolation and purification of the soluble haemagglutinin from classical Vibrio cholerae 569B, Inaba serotype has been carried out.

The soluble haemagglutinin was produced in casein-peptone broth culture medium inoculated with the organism and constantly aerated at 37° C for a period of 18 hours. The haemagglutinating activity has been consistently detected from such culture media ; from the supernatant fluid after removal of the bacterial cells by centrifugation and membrane filtration ; and also after precipitation with saturated ammonium sulphate (crude soluble haemagglutinin was obtained).

Subsequent fractionation of the crude soluble haemagglutinin (CSH) by Sephadex G₂₀₀ has revealed the presence of three protein peaks. The first peak exhibited haemagglutinating activity while the second and third peaks did not. The first peak was subjected to repeated ultracentrifugations and the deposits were pooled (P₁SH).

Following agarose gel electrophoresis of P₁SH, three protein bands were detected and were designated F, M and S. From passive haemagglutinating-inhibition tests performed on pools of these three protein fractions, it was shown that the S-protein contained a considerable amount of LPS. Thereafter, S-fraction from several agarose plates were collected, pooled and treated with ether to extract the LPS contaminant and thus obtained the pure soluble haemagglutinin fraction (extracted S) after another agarose electrophoresis. Each fraction F, M and extracted S was pooled separately and a significant haemagglutinating activity was detected from each pool.

The rabbit antisera raised against these protein fractions F, M and extracted S exhibited significant amounts of vibriocidal and neutralizing antibodies.

The protective capacities of these various antisera to soluble haemagglutinin preparations were tested using the infant mouse model in vivo system. The PD₅₀ titre was quite low but definite protection was observed against the oral homologous challenge.

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