

**SEROLOGICAL STUDIES IN CHOLERA WITH SPECIAL REFERENCE
TO IgG AND IgM ANTIBODIES AS DETERMINED BY
2-MER CAPTOETHANOL FRACTIONATION**

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To know the nature of antibodies in cholera with reference to IgG (7S) and IgM (19S), two different sets of sera have been selected for fractionation and subsequent titration with a vibriocidal test. They are: (i) paired, acute and convalescent sera from vibrio positive patients admitted to the Pakistan-SEATO Cholera Research Laboratory. (ii) Maternal and cord sera taken within 24 hours of delivery (for the study of transplacental transfer of antibodies).

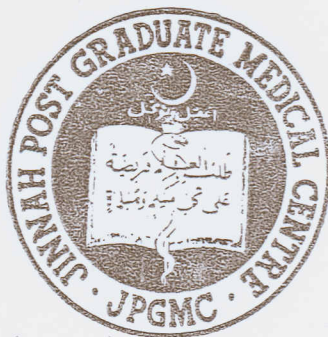
The method of fractionation is to reduce the Serum by mixing it undiluted with equal parts of 0.2M 2-mercaptoethanol, keeping it at room temperature for 1½ hours, then adding iodoacetamide in 10% molar excess to that of 2-mercaptoethanol, and lastly dialyzing the whole against a large volume of phosphate buffer saline (pH 7.2). Each sample has a control, without the addition of 2 mercaptoethanol, undergoing all of the process.

The vibriocidal test was specially designed in our laboratory using a micro-titer technique. Serially diluted serum is mixed with the Ogawa and Inaba Strains of *Vibrio Cholerae* (standardised by U.S. opacity units). It is incubated for 1 hour at 37°C for bacteriolysis to take place, then further incubated after adding heart infusion broth which simultaneously stops the activity of the complement and allows to the bacteria that have escaped lysis to grow. The cups showing no bacterial growth contains vibriolysin from the serum.

Preliminary examination of the results shows that: (i) The sera in the acute phase of disease contains low-titer vibriolysin antibody that is mostly or wholly degradable with 2 mercaptoethanol, *i.e.*, it is probably IgM. The convalescent sera possesses high titer antibodies that are partially degradable, *i.e.*, they are a combination of both IgM and IgG. (ii) Using a more sensitive vibriocidal test, the maternal sera obtained at the time of delivery shows moderate or high titer antibodies that are partially degradable, *i.e.*, combination of both IgM and IgG, but the sera of the babies are very little or not at all affected by 2 mercaptoethanol, *i.e.*, they are mostly IgG.

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