CHAPTER 4

DISCUSSION

Although immunization experiments using capsular antigen preparations of well established purity have not previously been reported, many workers have inferred that capsular antigens are important in protection. Thus, Priestley $(1936)^{(48)}$ and Yaw and Kakavas $(1957)^{(49)}$ compared the protective efficacy of vaccines or antigen preparations derived from encapsulated and non - encapsulated strains, they showed that only the former were protective. Carter and Annau $(1953)^{(50)}$ also compared capsular extracts from different variant strains and Bain $(1954)^{(8)}$ reported that freshly isolated 'phase 1' organisms were more immunogenic in bovines than frequently subcultured strains and suggested that this was due to an alteration in surface properties such as encapsulation. Bain $(1955)^{(51)}$, Dhanda $(1960)^{(41)}$, and Knox and Bain $(1960)^{(52)}$ described the preparation of fractions containing capsular antigens, which exhibited protective activity associated with protein components.

Preliminary investigations of the immunogenicity of the type B capsular antigen showed that it was poorly immunogenic in rabbits when administered with aluminium hydroxide gel. There appeared to be a slight serological response in some rabbits as shown by the mouse passive protection test, but the rabbits themselves were not immune to challenge.⁽⁴¹⁾ That observation was interesting because it underlined the importance of using animals of the species for which a possible vaccine was intended during investigations of immunogenicity. Immunization of cattle with vaccine containing capsular antigens and aluminium hydroxide showed a serological response occurred since mouse protective antibodies were produced in the serum. (41)Bain $(1963)^{(41)}$ considered that cattle which had mouse - protective sera were immune to hemorrhagic septicemia.

Penn and Nagy (1976)⁽⁴¹⁾ found that fractional precipitation from aqueous solution by the addition of increasing amounts of polar organic solvents was a satisfactory method for the separation of capsular antigens and endotoxin, being technically simple and highly effective. Crude capsular antigen obtained by solvent fractionation from whole culture was purified by cetyl pyridinium chloride (CPC) precipitation.

In view of the known poor response of mice to active immunization against <u>P. multocida</u> type B the first active immunization experiment of Penn and Nagy (1974) was performed in rabbits. ⁽⁴⁰⁾ It was previously reported that a killed fowl cholera vaccine stimulated and maintained a high degree of immunity for one year in chickens raised under experimental conditions, however, the vaccine did not prevent an outbreak of acute fowl cholera. ⁽⁵³⁾

The mouse protection test in this investigation with anti - whole cell globulin (9.1 mg per mouse) approaches 100% efficiency, while the anti - capsular polysaccharide globulin (14.8 mg per mouse) showes only about 25% protection. The results was shown in table 4 page 44, and table 5 page 45. In mouse passive protection test, anti - capsular polysaccharide globulin used is not more than 29.6 mg because of the dose that excess 1.0 ml which causes necrosis in peritoneum when mice were administered subcutaneously. Because of the limited time, we did not test for minimum dose of anti - whole cell globulin that gave 100% mouse passive protection.

The results of passive protection in this trials suggest that antibodies to whole - cell and capsular antigens were formed in rabbits after vaccination. Anti - pasteurella rabbit serum has been used successfully to protect mice by Little and Lyon (1943), Robert $(1947)^{(13,14)}$, and similar test in mice has been used by Bain $(1955)^{(51)}$ for assessing immunity in cattle, but antisera from vaccinated turkeys have given equivocal results. Namioka and Murata $(1964)^{(54)}$ demonstrated that capsular antigens were important for the protective response in mice, while in chickens and turkeys the O antigens seemed more to be important.

In mouse passive protection test, an accurately determined number of organisms should be injected in order to avoid overchallenging the mice.

However, the results of these experiments suggested that the use of this, or a similar <u>P. multocida</u> vaccine, may possibly be a valuable adjunct to present methods of control and treatment of fowl cholera disease in any given flock.

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