CHARPTER IV

DISCUSSION AND CONCLUSION

Nowadays more than 90 % of patients around the world are still post-exposure to rabies treated with Semple Vaccine. Most countries in South and Latin America use suckling mouse brain Vaccine (SMBV) for pre- and post-exposure treatment (12,42). Vaccination regimens are still being the same as Pasteur era. The high cost and low availibility in some countries of the newer and more safe rabies vaccines than the nervous tissue vaccines, such as human diploid cells vaccines make Semple and Suckling mouse brain Vaccines upper priority for vaccination.

In Latin America, SMBV is widely used for treatment. Many succeeds to improve safety, potency and reduce courses of vaccination, showing good evidence of long distance usage in future (42).

Semple and suckling mouse brain vaccines are inactivated type vaccines which are widely used for human and animal vaccination. There are so many inactivation methods being employed. The physical methods such as using ultraviolet light and heat (27), at present; phenol, beta-propiolactone (BPL) and binary ethylenimine are chemicals used for the purpose.

The present finding demonstrate that rabies vaccine preparations in which the virus was inactivated with 1:4,000 BPL produced the highest potency in both normal saline (NSS) and phosphate buffer saline (PBS). potency titre of vaccines inactivated by heat were consistently lower than the potency titre inactivated by BPL, but produced higher potency titre than inactivated by phenol (Table III and IV).

Different suspension media do not effect the potency and stability of rabies vaccine (Table III to Table VIII).

The reason that vaccine inactivated with BPL is the most potent, asif, because this chemical reacts rapidly with nucleic acid, to form 7-(2- carboxyethyl) Guanine, and binding to amino acids show that both-SH-and - SS groups to form alkylsulphonate compounds (7). Phenol is not a very satisfactory inactivating agent for rabies virus, because it acts on all of the viral protein, including the envelope glycoprotein (28). Another reason is temperature, rabies virus exposes to the heat of 37°C at various interval of times, the denaturation of proteins occurs, to phenol permitted more denatured protein, although heat inactivation takes a longer time, but the level of heat appears not to destroy all the antigenicit

No significant differences in immunogenicity were reported when aliquots of a virus pool were treated with 0.25 % to 0.5 % phenol or with 1:4,000 BPL in previous study (27). This result is contradicted to our present studies.

The use of lyophilization for preservation of the biological materials is well established, and a variety of protective materials had been employed to enhance survival during the freeze-drying procedures. Much infectivity is lost during the procedures. Many reports stated the effect of protective materials appear to be involved on the retention of infectivity of herpes virus (46), pancreatic necrosis virus (58), enteroviruses (5) and rickettsiae (6) . Dextran, the cryoprotective agent 2 or 5 %, sugars included glucose, sucrose, and lactose. Sucrose or lactose protected best against the adverse effects of drying (5, 6). Glucose, lactose proved unfavorable rickeltsiae which was kept at room temperature (5), but sucrose, was as favorable under certain conditions of

$34^{o}C$ 4 hours (5). This also resulted similarly to our experiments on lyophilized rabies virus vaccines.

The result from SMB vaccine showed a very low antigenic titre, this may because the virus titre was greatly reduced during process due to the long course of vaccine preparation time.

Semple and suckling mouse brain rabies vaccine are still widely used in developing countries. In the production of these vaccines, the potency of a killed virus depends upon inactivation methods, the stability of freeze drying products depends upon stabilizer adding. BPL inactivated vaccines showed the greatest potency both in NSS and PBS and also in lyophilized-BPL treated rabies vaccine with various stabilizers. Much higher retaintion of rabies virus antigenicity was obtained with both sucrose and lactose saline, the stability of vaccines was proved favorable in sucrose saline.

In general, vaccines potency is related to the amount of antigen, materials having an infectivity titre of less than 10^{5} LD_{50} per 0.03 ml. (for the mouse inoculated intracerebrally) will not make an inactivated vaccine of satisfactory potency (48). In our study in semple vaccines, the virus titre and the potency of inactivated vaccines were not correlated. The factors involved this event is not clear and difficult to assess in this research, the further work must be tried and done in other later related projects.