CHAPTER 4

DISCUSSION

Successful attempts to propagate rabies virus in pig kidney tissue culture have been summerized by Dr. M.K. Abelseth (2). The latest development in vaccines called Rabies Vaccine Modified Live Virus, Porcine Tissue Culture Origin, ERA strain (8, 35).

The method of growing pig kidney tissue culture using growth medium consisted of LE medium and LH medium with 3% fetal bovine or calf serum. The monolayer cultures were made in the Roux bottles keeping at 37°C incubator. The confluent growths (Fig. 1, p. 59) were equally almost complete at four to five days but the tissue cultures deteriorated before completion of the 21 days incubation. By using 5% fetal bovine or calf serum, the tissues were found to be in good condition for completion of the test. The fluids showed the occurrence of abundant viruses without cytopathic changes observed in the infected pig kidney tissue cultures.

The patterns of the propagation of virus growth curves in pig kidney cells at 34°C from two experiments are shown to be correlated (Fig. 3, p. 61 and Fig. 5, p. 65) The virus titers in the culture fluids had increased and reached the maximum on the eighth day. Between the eighth day and the tenth day the titer fluctuated and declined from the tenth day to sixteenth day. The comparison of the above mentioned propagation at 34°C with those propagated at 36°C

showed the virus titers obtained to be not much differnet between the two curves of each experiment (Curve I and Curve II, Fig. 3, p. 61 and Fig. 5, p. 65), but the patterns of virus growth curves differed. Moreover the cell cultures grown at 36°C deteriorated rapidly. The results of these two curves from both trials indicated that the optimal day to harvest the fluids could be from the sixth to the eighth day after infection at 34°C.

The propagation of ERA strain of rabies virus in pig kidney culture using the two kinds of growth media, i.e. the LE and LH media, incubated at 34°C were also done in two trials (Table 18, p. 62, Fig. 4, p. 63 and Table 20, p. 66, Fig. 6, p. 67). The patterns of the two curves and the results between the two experiments are quite similar except the peaks of the maximum virus titers appeared to be opposite, the first peak was higher the second peak was lower in one experiment (Fig. 4, p. 63) and the first peak was lower, the second peak was higher in the other experiment (Fig. 6, p. 67). However the results of these curves indicated that the maximum titer of infected viruses from harvested fluids appeared between the sixth and eighth day when using LE medium. The viruses multiplied quite rapidly and was released continually from the cells. The pH of the medium gradually became acid resulting to the deterioration of tissue culture. The replacement of fresh medium every seventh and ninth day will help the virus multiplication and at least fluids could be harvested three times from one monolayer cultures. The virus multiplications of the replacement medium were equal in titers (Table 21, p. 69 and Fig. 7, p. 70). Thus,

it is the best time for fluid harvest. After each lot of fluid was harvested, fresh LE medium with 2% fetal bovine or calf serum was added and the cells were reincubated at 34°C incubator. The viruses obtained from fluid harvested at the ninth day, reached a maximum titer of $10^{-4.63}$ LD_{50}/0.03 ml (Table 21, p. 69).

The best storage condition of vaccine is freeze-drying, yet the fluid vaccine could be kept at 4°C for 7 months with effective potency (Table 22, p. 71 and Fig. 8, p. 72). In order to compensate with the improper conditions of transportation, which would cause dropping of the titers, it is recommended the fluid vaccine should be kept at 4°C for not longer than 3 months. At room temperature (29°C - 31°C) the fluid vaccine would be no longer effective after two days (Table 23, p. 71 and Fig. 9, p. 73).

The addition of a stabilizer to the vaccine before lyophilization greatly preserved the activity of live virus (Table 24, p. 74). The titer of vaccine after lyophilization dropped from $10^{-4.38}$ to $10^{-4.16}$ LD_{50}/0.03 ml, which was high enough to meet the Standard of Potency (not less than $10^{-2.5}$ LD_{50}/0.03 ml.) (p. 55).

The finished vaccine must be free from bacterial and mold contamination and must be tested for safety and effectiveness by proper methods (pp. 52-56)

The antibody titers among the ten vaccinated guinea pigs were 46 to 87 reciprocal numbers, which could protect 100% of the test animals and caused 60% of five control animals die within 21 days of the test. This antigenicity test is conformed to the limit of

Connaught Laboratories (the test is considered satisfactory when at least 70% of the vaccinated animals survive and 80% of the control die within 21 days period of observation)(p. 56).