CHAPTER 3

RESULTS

3.1 PIG KIDNEY CELLS IN BALANCED SALT SOLUTIONS

The growth of pig kidney cells in Hank's and Earle's balanced salt solutions were compared as follows :

Primary pig kidney tissue cultures were prepared from pigs aged 4-6 weeks old. Kidneys were removed, minced, trypsinized and seeded in growth medium in a concentration of 3.0×10^5 cells/ml. One pair of kidneys could produced about 2000 ml of cells suspension. The growth medium consisting of Earle's balanced salt solution and Hank's balanced salt solution, each containing 0.5% lactalbumin hydrolysate and 3% fetal bovine or calf serum. Monolayer cultures were grown in the Roux bottles, usually at 37°C. The similar confluent growth was nearly complete at four to five days from these two media but the cell culture deteriorated before completion of the 21 days. Using 5% fetal bovine or calf serum, the cells remained in good condition for sufficient time to complete the test.

3.2 YIELD OF ERA STRAIN RABIES VIRUS AND VACCINE FROM VARIOUS DIFFERENT CONDITIONS.

3.2.1 The comparison of propagation of ERA strain of rabies virus in pig kidney culture incubated at 34°C and 36°C.

To determine the optimal day of fluid harvested, when the maximal viral concentration was present, the following experiment was carried out.

Primary pig kidney tissue cultures were prepared by using LE medium containing 5% calf serum (Fig. 1 p. 59). The infected cultures with ERA strain of rabies virus titer $10^{-4.37}LD_{50}/0.03$ ml were placed in the incubator at 34°C and 36°C. Each of every two days fluids were harvested, beginning with the second day after inoculation and ending on the eighteenth. No cytopathic changes were observed in the infected cultures (Fig. 2 p. 59) but the fluids yielded the viruses. The harvested fluids were titrated intracerebrally in mice. The results of the experiments are shown in Table 17, p. 60 and Fig. 3, p. 61 and in Table 19, p. 64, Fig. 5, p. 65.

3.2.2 The comparison of propagation of ERA strain of rabies virus in primary pig kidney cultures, using Earle's balanced salt solution (LE) and Hank's balanced salt solution (LH) at 34°C.

This experiment was repeated along the same lines, as described in the comparison in propagation of Rabies Virus in Pig Kidney culture at 34°C and 36°C, except that the LH medium and LE medium were used for the growth medium incubated at 34°C. The results are shown in Table 18, p. 62 and Fig. 4, p. 63 and Table 20, p. 66, Fig. 6, p. 67

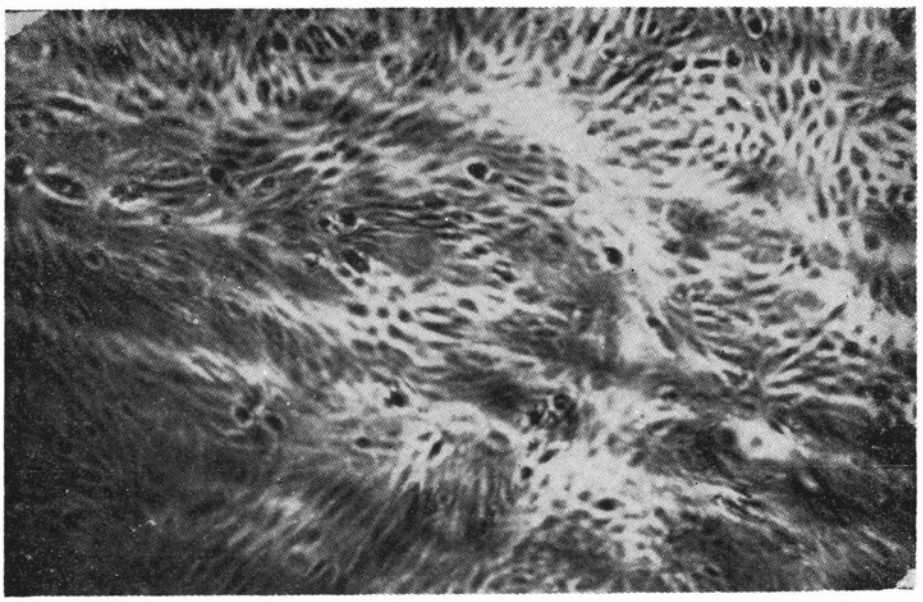

Fig. 1 Normal Pig Kidney Tissue Culture X 400

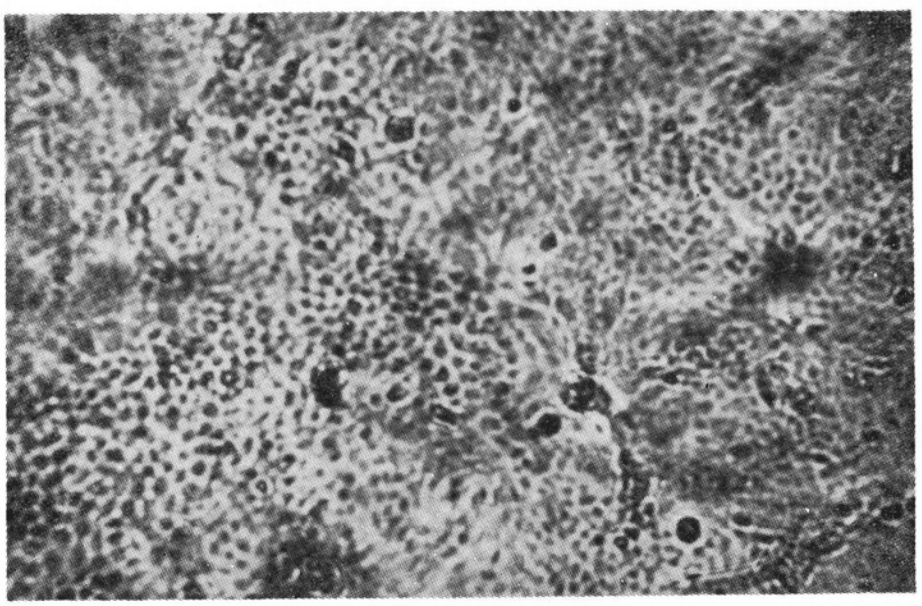

Fig. 2 Pig Kidney Tissue Culture two weeks after infection with rabies virus X 400

TABLE 17

Growth of rabies virus in pig kidney culture at 34°C and 36°C

Serial number	Time of harvested day	Titers of culture fluid (Mouse LD_{50}/0.03 ml)	
		Incubated at 34°C	Incubated at 36°C
1	2	$10^{-3.37}$	$10^{-3.25}$
2	4	$10^{-3.63}$	$10^{-3.57}$
3	6	$10^{-4.32}$	$10^{-4.32}$
4	8	$10^{-4.44}$	$10^{-4.70}$
5	10	$10^{-3.68}$	$10^{-4.25}$
6	12	$10^{-4.22}$	$10^{-4.22}$
7	14	$10^{-3.70}$	$10^{-3.84}$
8	16	$10^{-3.50}$	$10^{-3.83}$
9	18	$10^{-3.52}$	$10^{-3.00}$
10	20	$10^{-3.17}$	$10^{-3.00}$

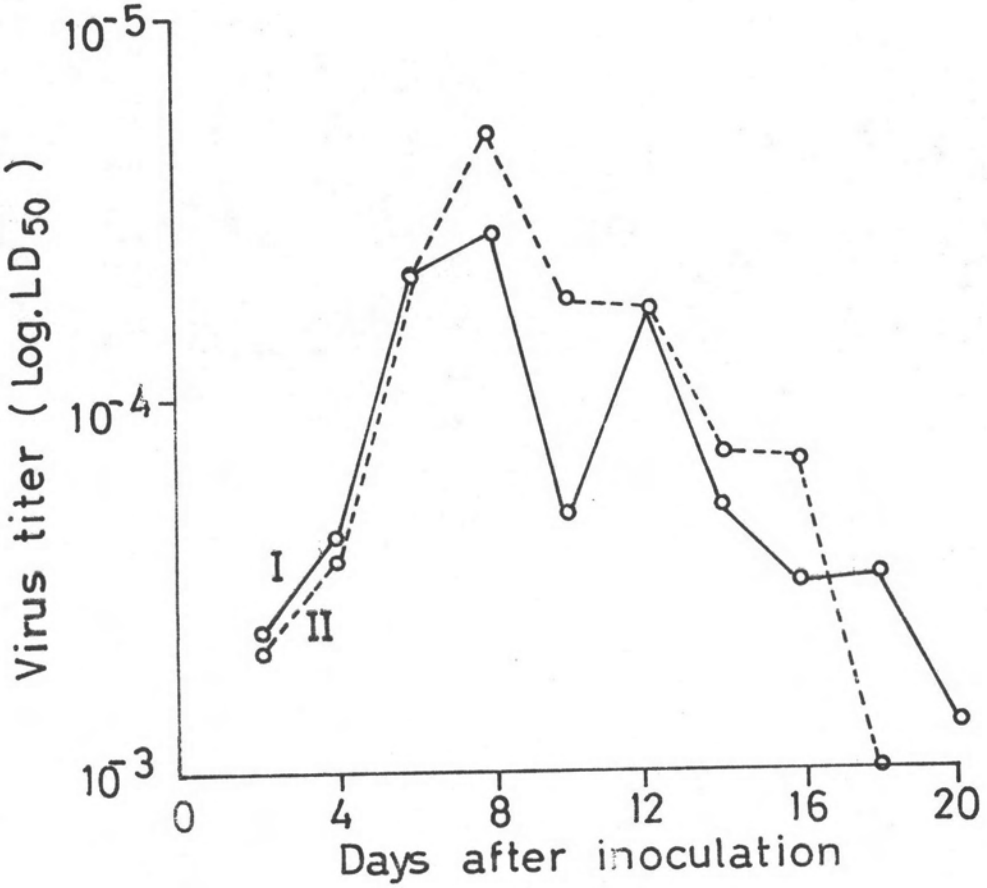

Fig. 3 The comparison of rabies virus titers in pig kidney culture at 34° C and 36° C

o——o Incubation at 34° C

o - - -o Incubation at 36° C

TABLE 18

Rabies virus titers in pig kidney cultures using LE medium and LH medium

Serial number	Time of fluid harvested days	Titers of culture fluid (Mouse LD_{50}/10.03 ml)	
		LE medium	LH medium
1	2	$10^{-3.37}$	-
2	4	$10^{-3.63}$	$10^{-3.50}$
3	6	$10^{-4.32}$	$10^{-4.40}$
4	8	$10^{-4.44}$	$10^{-4.40}$
5	10	$10^{-3.68}$	$10^{-3.46}$
6	12	$10^{-4.22}$	$10^{-4.00}$
7	14	$10^{-3.70}$	$10^{-4.00}$
8	16	$10^{-3.50}$	$10^{-4.16}$
9	18	$10^{-3.52}$	$10^{-3.68}$
10	20	$10^{-3.17}$	$10^{-4.16}$

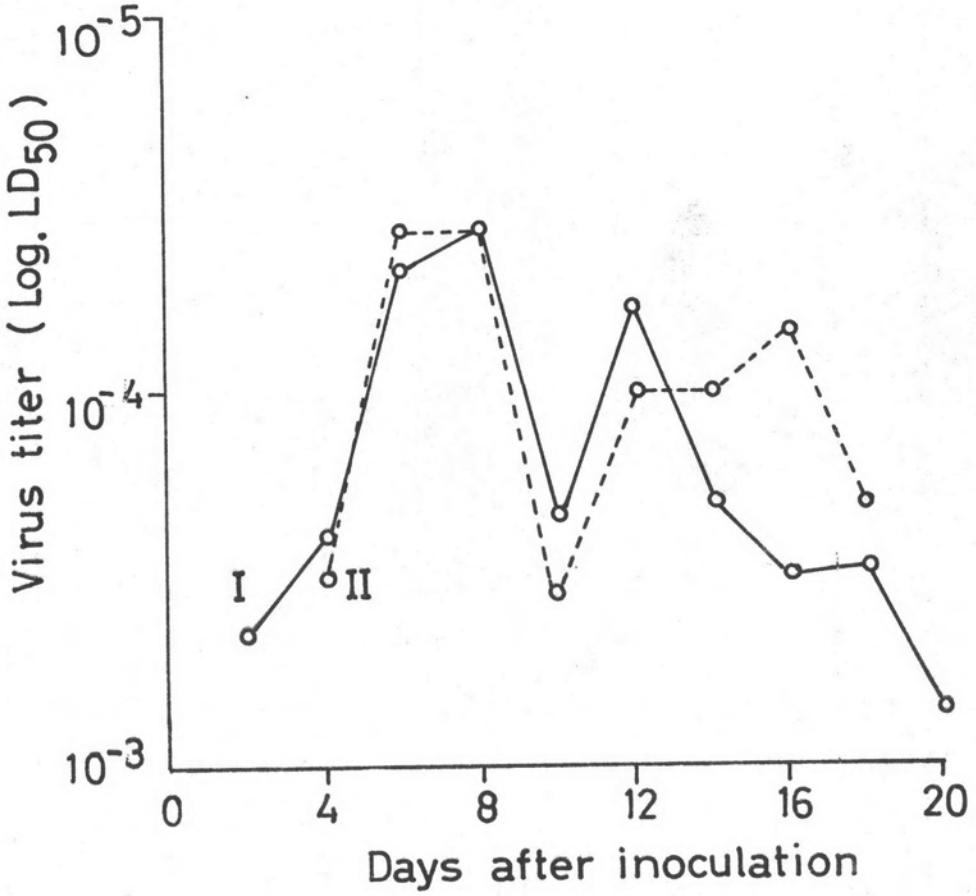

Fig. 4 The comparison of rabies virus titers in pig kidney culture using LE medium and LH medium

——o—— LE medium

- - - o - - - LH medium

TABLE 19

Growth of rabies virus in pig kidney culture at 34°C and 36°C

Serial number	Time of harvested days	Titers of culture fluid (Mouse LD_{50}/0.03 ml)	
		Incubated at 34°C	Incubated at 36°C
1	2	$10^{-3.00}$	$10^{-3.00}$
2	4	$10^{-4.32}$	$10^{-3.63}$
3	6	$10^{-4.52}$	$10^{-4.50}$
4	8	$10^{-4.83}$	$10^{-3.62}$
5	10	$10^{-4.50}$	$10^{-4.83}$
6	12	$10^{-5.00}$	$10^{-4.63}$
7	14	$10^{-5.50}$	$10^{-5.17}$
8	16	$10^{-4.50}$	$10^{-5.17}$

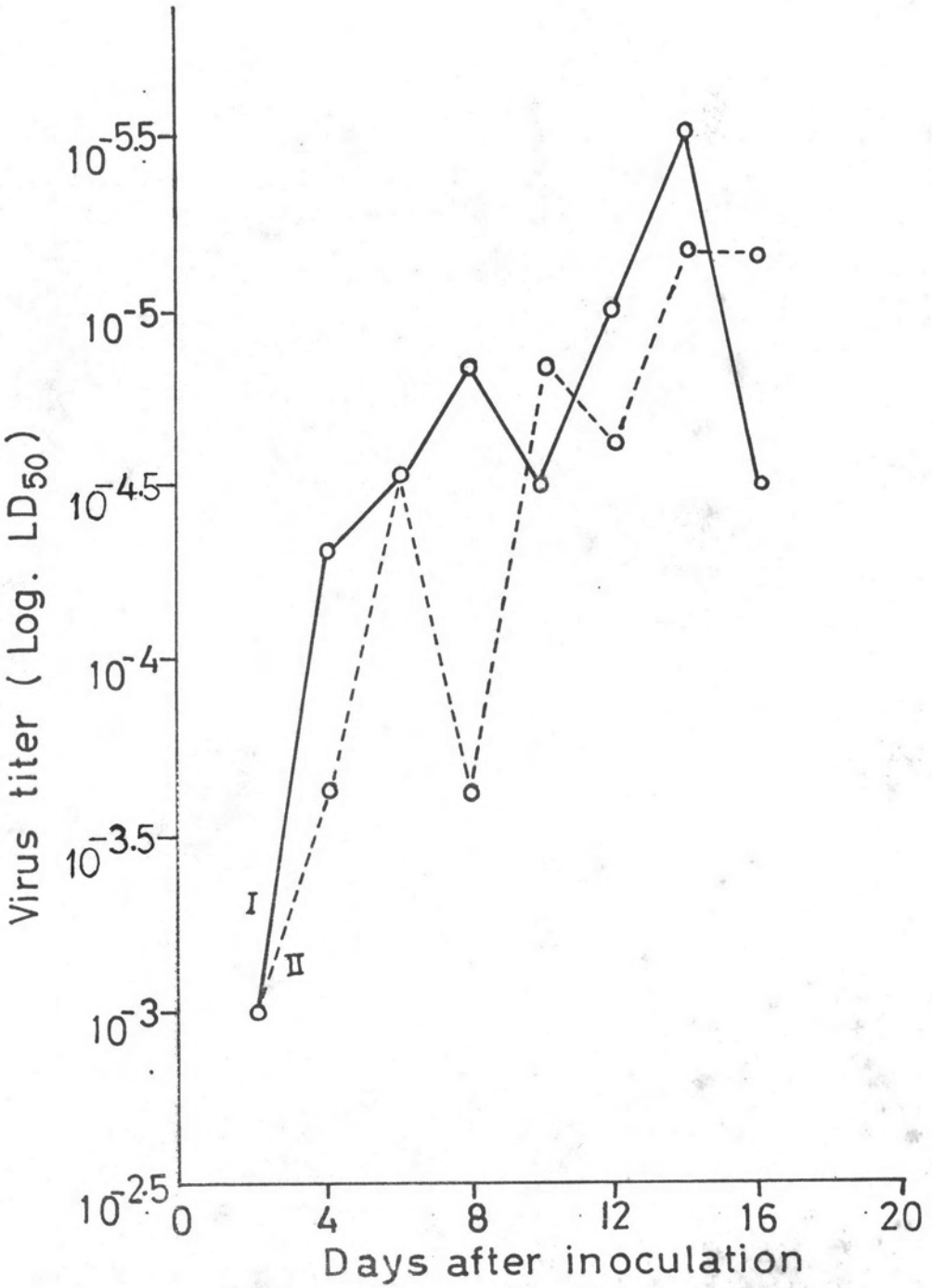

Fig. 5 The comparison of rabies virus titers in pig kidney culture at 34° C and 36° C

o——o Incubation at 34° C

o- - - -o Incubation at 36° C

TABLE 20

Rabies virus titers in pig kidney cultures using LE medium and LH medium

Serial number	Time of fluid harvested day	Titers of culture fluid (Mouse LD_{50}/0.03 ml)	
		LE medium	LH medium
1	2	$10^{-3.00}$	$10^{-2.63}$
2	4	$10^{-4.32}$	$10^{-3.75}$
3	6	$10^{-4.52}$	$10^{-4.17}$
4	8	$10^{-4.83}$	$10^{-3.88}$
5	10	$10^{-4.50}$	$10^{-4.83}$
6	12	$10^{-5.00}$	$10^{-5.00}$
7	14	$10^{-5.50}$	$10^{-4.50}$
8	16	$10^{-4.50}$	$10^{-3.50}$

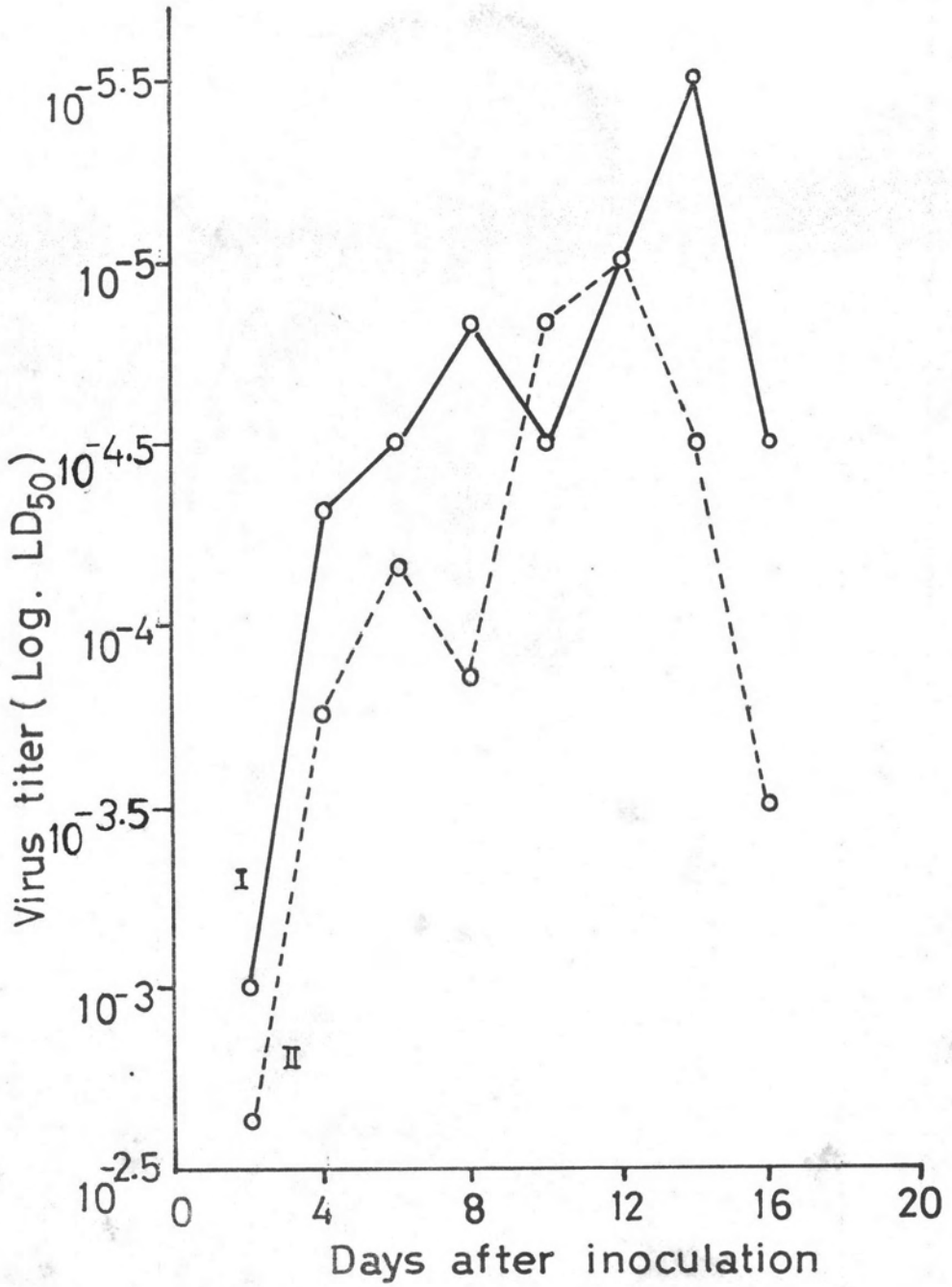

Fig. 6 The comparison of rabies virus titers in pig kidney culture using LE medium and LH medium.

o——o LE medium

o- - - -o LH medium

3.2.3 Rabies virus titers in pig kidney tissue culture from different days harvested.

From the above the opitimal days harvested occurred on the eighth days post inoculation. It is evident that the virus multiplies quite rapidly since replacement of fluid on infected cells results in a titer usually equal to that present before fluid change. This means that the fluids can be harvested at least three times from the one monolayer culture. So in this experiment the fluids were harvested at each 7 and 9 days post inoculation. After harvested, freshed LE medium with 2% calf serum was added and the cells reincubated at 34°C. The results are shown in the Table 21, p.69, Fig. 7, p. 70.

TABLE 21

Rabies virus titers in primary pig kidney tissue culture from differnet days harvested

Titers of seed virus	Infectivity titer of culture fluid (Mouse LD_{50}/0.03 ml)								
	Harvested Days								
	5th	7th	9th	12th	14th	18th	19th	21th	27th
$10^{-4.37}$		10^{-4}			$10^{-4.38}$			$10^{-4.63}$	
$10^{-4.37}$			$10^{-4.32}$			$10^{-4.5}$			$10^{-4.63}$
$10^{-3.68}$			$10^{-3.83}$			$10^{-3.63}$			$10^{-4.16}$
$10^{-4.62}$	$10^{-4.32}$			$10^{-4.73}$			$10^{-5.32}$		

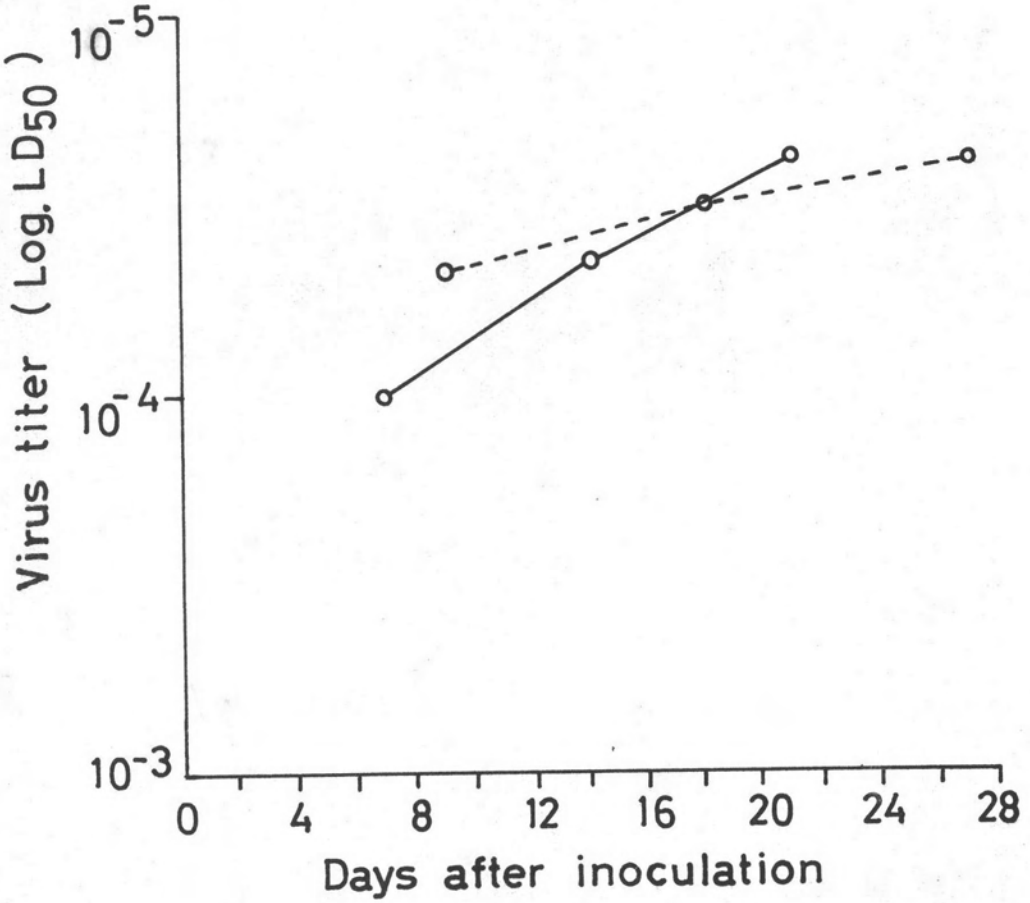

Fig.7 The comparison of rabies virus titers from harvested fluids changed every 7 days and 9 days.

o——o 7 days harvested fluid

o- - - -o 9 days harvested fluid

TABLE 22

Virus titers of fluid vaccine kept at 4°C.

Virus titers of harvested fluid	Virus * titers of harvested fluid kept at 4°C		
	3^{rd} month	6^{th} month	7^{th} month
$10^{-4.50}$	$10^{-4.38}$	$10^{-3.68}$	$10^{-3.00}$

* Virus Titers = Mouse LD_{50}/0.03 ml.

TABLE 23

Virus titers of fluid vaccine kept at room temperature

Virus titers of harvested fluid	Virus titers of harvested fluid after kept at room temp. (29-31°C.)				
	1^{st} day	2^{nd} day	3^{rd} day	6^{th} day	10^{th} day
$10^{-4.38}$	$10^{-3.50}$	$10^{-3.167}$	$10^{-1.84}$	$10^{-1.44}$	$10^{-1.00}$

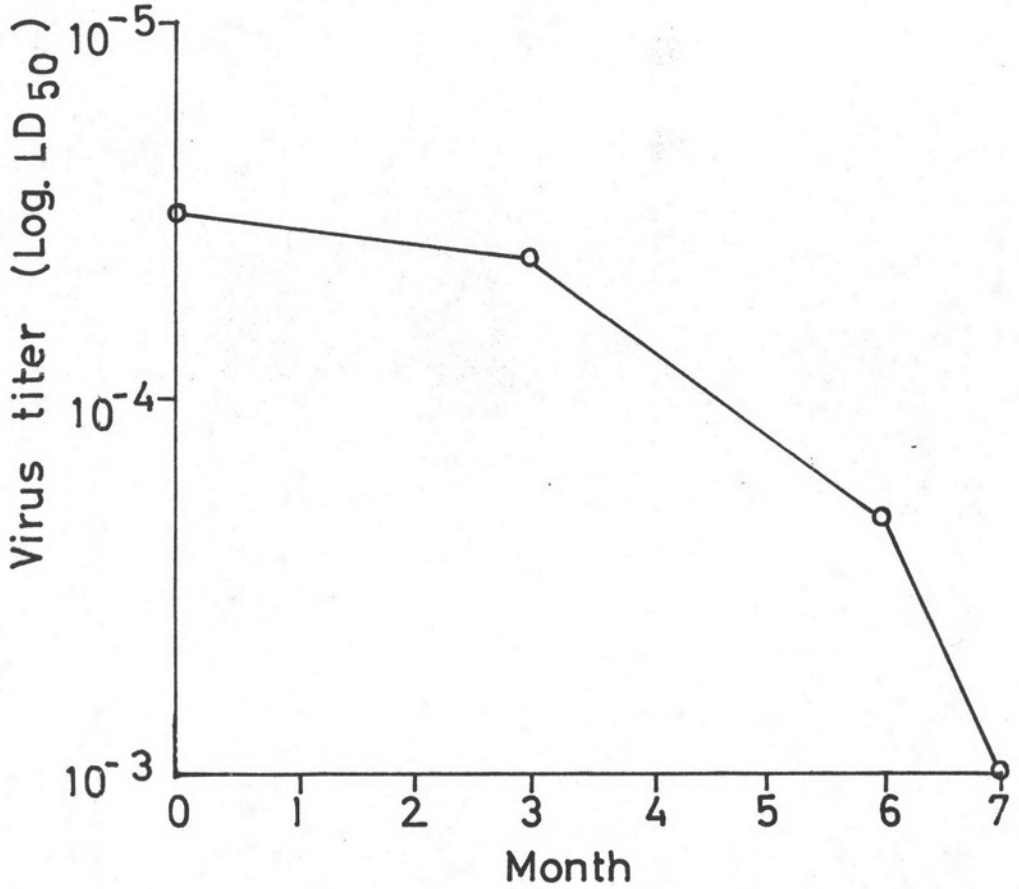

Fig. 8 Rabies virus titer of fluid vaccine kept at 4° C

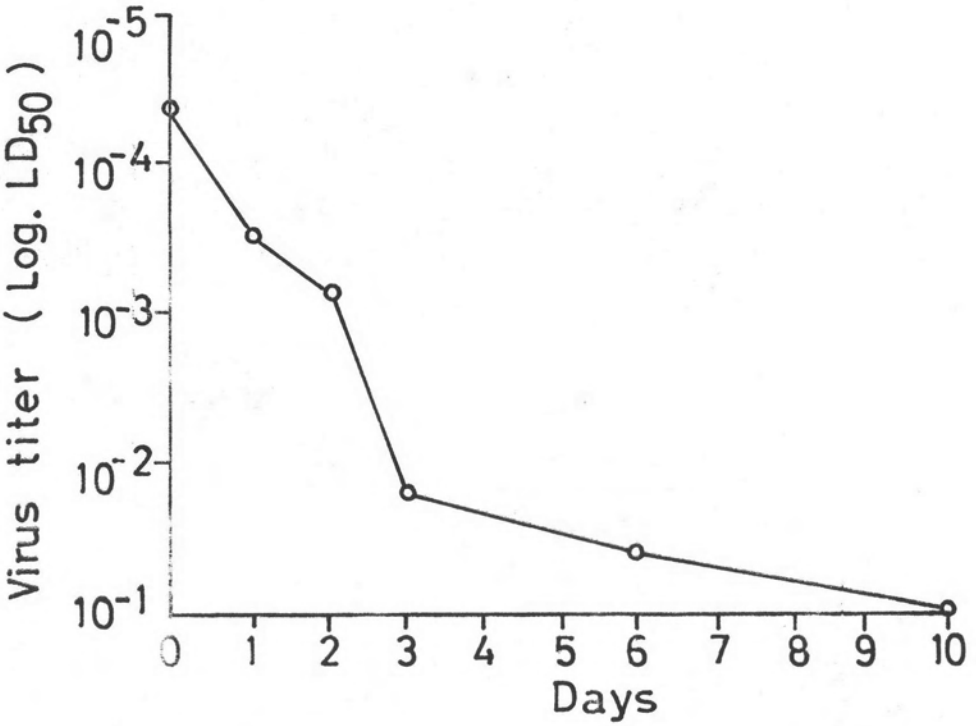

Fig.9 Rabies virus titers of fluid vaccine kept at room temperature (29-31°C)

3.3 VACCINE TESTING

It was important to examine the finished products by the following tests :

3.3.1 Titration : The results of virus titration of vaccine are shown in Table 24, p. 74.

TABLE 24

Virus titers of lyophilized MLV-ERA strain

Titers of Fluid Harvested Vaccines	* Titers of Lyophilized Vaccines
$10^{-4.38}$	$10^{-4.16}$
$10^{-4.73}$	$10^{-4.4}$

* Titers = Mouse LD_{50}/0.03 ml.

3.3.2 Sterility test

Bacterial contamination	The final product free of contamination.
Mold contamination	The final product free of contamination.

3.3.3 Safety test

All of mice remain alive and healthy during 21 days of the test.

3.3.4 Antigenicity test

The lyophilized vaccine was performed according to the procedure mentioned in antigenicity on p. 56. The results of testing are shown in Table 25, p. 76.

TABLE 25

Challenge results in guinea pig vaccinated with ERA vaccine

Vaccine Dilution	Number Vaccinated	Survived Vaccination	*Challenge			
			Vaccinates		Controls	
			** D/C	*** Antibody Titer	D/C	*** Antibody Titer
1/10	10	10	0/10	87, 51, 46	3/5	0, 0

* Challenge Dose 8,000 LD_{50}/0.25 ml.

** D/C = Death/Challenged

*** Reciprocal antibody titer against challenge dose 75 LD_{50}.