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Studies on the Prophylaxis of Measles with Attenuated Living Virus
II. Cultivation of Measles Virus Isolated by Tissue Culture in Developing Chick Egg

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SUMMARY

It was shown that both the Edmonston and Toyoshima strains of measles virus, could be serially cultivated over many generations in developing chick embryos either in the chorioallantois or in the amnion. This was confirmed by the interference test and by back-inoculation to FL cells. Chorioallantoic passaged virus of the second and later generations had no cytopathogenicity towards FL cells, while its interfering activity was retained. Amnion passaged virus, however, retained both cytopathogenicity and interfering activity.

INTRODUCTION

Cultivation of measles virus in chick embryos has been studied by Torres (1935) and numerous later investigators. Until recently the work has not made much progress. Rake *et al.* (1940-1943) reported that inoculation of chick embryo passaged measles virus in man or monkey resulted in some cases in the appearance of measles symptoms and consequent immunity against this disease. From these results, it was stated that cultivation of measles virus in chick embryos was possible. However, the results of experiments were not constant and it was noted that the virus gradually disappeared during egg passage. The cause for this unsuccessfulness can be considered to be the lack of a method for evaluating virus propagation. They (1949) discontinued the investigation with the words "Unfortunately, the reason for the failure of the vaccine to afford better protection are not evident, nor does it seem probable that they will be fully understood until better methods are available for working with the virus *in vitro* and in the laboratory."

Studies similar to those of Rake *et al.* have been conducted by the authors since 1943, and later a so-called "better method" was devised (Taniguchi *et al.*, 1956). This has been called the "interference diagnosis method" and with it, it has been possible to show that measles virus could be successfully passed for more than 10 generations in 4 of 56 cases by inoculation of measles virus material

directly in the chorioallantois of chick embryos. Even with this method, however, the rate of isolation of the virus was low and long-term serial passage and maintenance of the isolated strain was difficult.

In 1954, Enders and Peebles successfully cultivated measles virus in tissue cultures and we also succeeded in isolating the virus using FL cells (Toyoshima *et al.*, 1959). The virus isolated by this method, the Toyoshima strain, and the Edmonston strain of Dr. Enders, were inoculated in chick embryos and the previous experiments were repeated. For evaluation of virus multiplication, the interference diagnosis method as well as back-inoculation into tissue cultures and testing for cytopathogenicity, were used. It was found that both strain of measles virus could be serially cultivated in the chick embryo by both the amniotic and chorioallantoic routes.

MATERIALS AND METHODS

1. *Virus*

The Toyoshima strain of measles virus isolated and maintained in FL cells in our laboratory (Toyoshima *et al.*, 1959), and the Edmonston strain (Enders *et al.*, 1954) passed FL cells were used.

2. *Chick embryo Passage*

A. *Chorioallantoic cavity*

0.2 ml aliquots of the tissue culture fluid from the 8th FL cell passage of Edmonston virus and the 7th FL cell passage of Toyoshima virus were inoculated respectively into the chorioallantoic cavity of 7 day eggs and membrane-fluid mixtures were homogenized after 7 days incubation. They were then centrifuged at 3.000 rpm for 15 minutes and 0.2 ml of the supernatant was used as the passage virus material.

B. *Amniotic cavity*

0.2 ml aliquots of the tissue culture fluid from the 8th FL cell passage Edmonston strain was inoculated in the amniotic cavity of chick embryos on the 7th day of development. After incubation for 7 days, the amniotic membranes and fluids were harvested and homogenized, and then centrifuged as above. 0.2 ml of the supernatant was inoculated in the amniotic cavity for serial passages. The procedure for cultivation and passage of Toyoshima virus was identical.

3. *Back-inoculation into FL cells*

Egg passage virus materials were examined for cytopathogenicity to FL cells. 0.2 ml of each sample of the serially passed virus material from the chorioallantois and amnion was back-inoculated into 3 tubes of FL cells. The observation period for cytopathic effects was usually 14 days and a second or third passage in FL cells was neccessitated in cases when cytopathic changes were dubious in the first passage.

4. *Diagnosis by the interference method*

The details of this method were reported previously. (Taniguchi *et al.*, 1956). 4 days after inoculation of the measles virus material in the chorioallantoic cavity, mumps virus was inoculated as a challenging virus by the same route. The eggs were incubated for a further 3—4 days. The chorioallantoic fluid was harvested and the yield of mumps virus was measured by the hemagglutination test. 6 eggs were usually employed for each test. 3 eggs were treated as described above and the other 3 were inoculated only with challenging mumps virus.

As for the amnion passage virus material, the material was also inoculated in the

chorioallantoic cavity, where the interference was tested as described above. This was because inoculation of the challenging mumps virus directly in the amniotic cavity was presumed to be detrimental to the developing chich embryo.

RESULTS

1. *Cultivation of measles virus in chorioallantoic cavities of embryonated eggs*

Measles virus of Edmonston strain was serially cultivated in the chorioallantoic cavity for 14 generations as shown in Table 1. The interval of passage was usually one week unless specified in Table.

In the interference tests, although the hemagglutination titer of mumps virus of the control egg was not always constant probably because of insufficient adaptation of this virus to chorioallantois after passage in the amnion, considerable difference was noticed between the hemagglutination titers of the experimental and control eggs. As stated in the previous paper, these differences could be attributed to an interfering action of the measles virus which had previously propagated in the chorioallantoic cavity. The specificity of this method was established by the fact that no interfering phenomena were observed when antibody of measles virus was added. Serially cultivated virus of 14 generation showed

Table 1. Serial Cultivation of Measles Virus in Chorioallantoic Cavities of Embryonated Eggs: I.

Edmonston strain (the 9th FL cell passage)	Interference Test	
	Experimental eggs	Control eggs
①		
② ····→	0, 0, 0	2^3, 2^3, 2^2
③		
④ ····→	2^2, 0, 0	2^8, 2^8, 2^8
⑤ ····→	0, 0	2^7, 2^7
⑥ ····→	2^2, 2^2, 2^2	2^5, 2^5, 2^4
⑦		
⑧ ····→	0, 0, 0	2^5, 2^6
→ stored at–20°C for 17 days		
⑨ ····→	0, 2^8, 2^8	2^6, 2^6, 2^7
⑩ ····→	0, 0, 0	0, 0, 0
⑪ ····→	0, 0, 0	2^4, 2^6, 2^7
→ stored at–20°C for 6 days		
⑫ ····→	0, 0	2^5, 2^{10}, 2^{10}
⑬ ····→	0, 0, 0	0, 0, 0
⑭ ····→	0, 0, 0	2^5, 0, 0
FL cell C.P. (—)		

The circles indicate embryonated eggs, and the figures in the circles show the number of the passage.

Each figure expresses the hemagglutination titer of mumps virus in the respective egg.

no cytopathic effect in FL cells and no characteristic morphological change was detected in the section preparations of chorioallantoic membranes.

The Toyoshima strain was cultivated in the chorioallantoic cavity in the same manner and similar results were obtained, as demonstrated in Table 2. Each generation was back-inoculated into FL cells, and only virus material of the first generation resulted in cytopathic change toward the end of the 14 day observation period. After a second passage no virus material ever exhibited cytopathic effects in FL cells.

Table 2. Serial Cultivation of Measles Virus in Chorioallantoic Cavities of Embryonated Eggs. II.

Toyoshima strain (the 7th FL cell passage)	Interference Test	
	Experimental eggs	Control eggs
① ⟶	0, 2^3, 2^4	2^8, 2^8, 2^8
↳ FL c.p. (+)		
② ⟶	0, 0, 0	0, 0, 0
↳ FL c.p. (−)		
③ ⟶	0, 0, 0	2^7, 2^7, 0
↳ FL c.p. (−)		
④ ⟶	0, 0, 0	0, 0, 0
↳ FL c.p. (−)		
⑤ ⟶	0, 0, 0	0, 0, 0
↳ FL c.p. (−)		
⑥		
⑦ ⟶	0, 0, 0	0, 0, 0
⑧ ⟶	0, 0, 0	2^6, 2^6, 2^7

The circles indicate embryonated eggs, and the figures in the circles show the number of the passage.

Each figure expresses the hemagglutination titer of mumps virus in the respective egg.

2. *Cultivation of measles virus in the amniotic cavity of embryonated eggs*

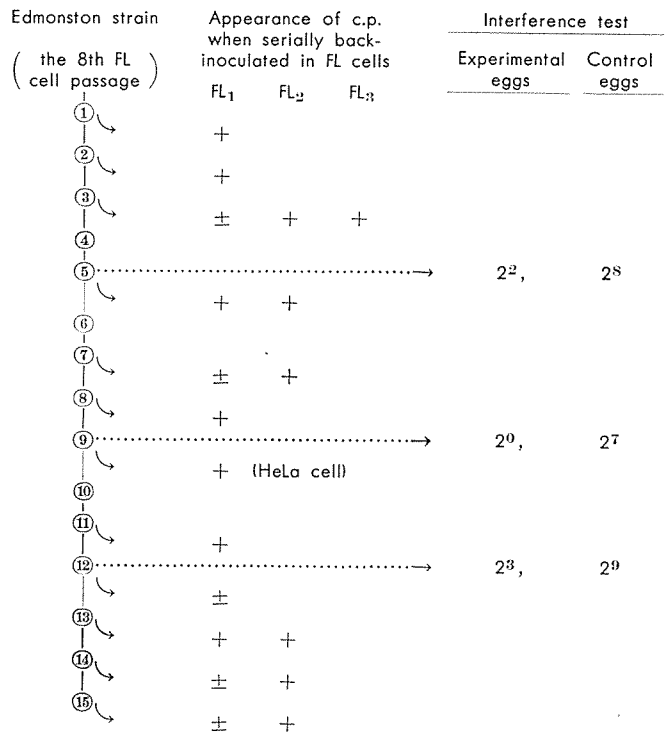
The Edmonston strain of measles virus was serially cultivated in the amniotic cavities of 7 day embryonated eggs. The first inoculum consisted of the Edmonston strain of the 8th passage in FL cells. In subsequent serial passages up to 15th generation, 0.1 ml of a suspension of the amniotic membrane and fluid was employed as inoculum. As shown in Table 3, the 5th, 9th and 12th passage material gave positive results in the interference test.

The introduction of the passage virus material of the first to the 15th into tissue culture of FL cells, resulted in typical cytopathic changes indicative of multiplication of the measles virus. When no typical cytopathic change was observed during the 14 day observation period of the first tissue culture passage, a distinct change was detected in the second or third passage of FL cells.

Table 4 shows the result of serial cultivation of the Toyoshima strain in the amniotic cavity in the same way as described above.

The results were similar to those obtained with the Edmonston strain, and demonstrated that this strain could be serially cultivated in the amniotic cavity.

Table 3. Serial Cultivation of Measles Virus in Amniotic Cavities of Embryonated Eggs. I.



The circles indicate embryonated eggs, and the figures in the circles show the number of the passage.

Each figure expresses the hemagglutination titer of mumps virus in the respective egg.

DISCUSSION

It has been reported that multiplication of a virus in chick embryos can be evaluated by the so-called interference diagnosis method (Okuno *et al.*, 1956). Our previous report that propagation of measles virus cultivated in chick embryos could be tested by the interference phenomenon with the mumps virus, has been verified in the present study.

Measles virus grown in tissue culture was used as the material for the first inoculation of the chick embryo and readily passaged. The rate of successful passage was low in the previous study, possibly due to the small quantity of virus in the material taken from the patient.

Long-term passage of measles virus in chick embryos is possible by either the amniotic or chorioallantoic routes, but the pathogenicity against FL cells differs. Amnion passage virus retains cytopathogenicity against FL cells, while this property is rapidly lost by chorioallantoic passage. In other words, the virus may undergo a change in pathogenicity when passed through the chorioallantoic route

Table 4. Serial Cultivation of Measles Virus in Amniotic Cavities of Embryonated Eggs. II.

Toyoshima strain (the 7th FL cell passage)	Appearance of c.p. when back-inoculated in FL cells
①	+
②	+
③	+
④	+
⑤	+
⑥	+
⑦	+
⑧	+
⑨	+
⑩	+
⑪	+
⑫	+
⑬	+
⑭	+
⑮	+
⑯	+
⋮	
㉕	+

The circles indicate embryonated eggs, and the figures in the circles show the number of the passage.

and it is believed that this altered virus may be useful for the production of living virus vaccine. Studies on this possibility are presented in the following paper.

Similar results have been obtained with both the Toyoshima and Edmonston strains.

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