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Propagation of Attenuated Rubella Virus Strain HPV-77 in Turkey Embryo Tissue Culture

Brief Report

By

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Because of the extensive background information and experience in man available on parenteral administration of live avian embryo vaccines, chick and duck embryo tissue cultures have been regarded as a safe medium for the growth of attenuated rubella virus for use in vaccines (1, 4, 5, 6, 10).

The present report concerns the cultivation of attenuated rubella virus in turkey embryo tissue culture.

Cell cultures were prepared from 14-day-old embryonated turkey eggs and grown in plastic flasks (Falcon, 75 cm^2) to the stationary phase or in roller bottles (New Brunswick, 1225 cm^2) using medium 199 supplemented with 6 per cent fetal calf serum and 1 per cent neomycin (50 mcg per ml). HPV-77 (7, 8) strain of rubella virus, passaged one or two times in African Green Monkey Kidney (AGMK) cells, was propagated at 32° C in turkey cell cultures maintained in Eagle's medium. Infectivity end-points (11) were performed in AGMK (9).

Table 1. *Growth of High-Passage Rubella Virus (HPV-79) Strain in Turkey Embryo Tissue Culture*

Exp. No.	Number of days following infection of the cultures	3	4	5		7	10	11	12	13	14
1	With 1%	4.0[a]	3.5	3.25	3.25	3.25	3.0	3.5	3.5	3.75	1.25
2	serum	3.5	3.25	—	—	3.0	3.5	3.5	—	—	3.5
1	Without	3.5	3.0	3.75	3.75	3.5	3.5	2.5	1.75	2.0	1.25
2	serum	3.25	1.75	—	—	1.5	2.5	2.25	—	—	3.0

[a] Infectivity end-points in $\log_{10}$/0.5 ml virus suspension.

Initially the high passage rubella virus HPV-79 was compared for its ability to grow in turkey embryo tissue culture maintained in medium with and without fetal calf serum (Table 1). Optimum growth was obtained after 3 days and was maintained during two weeks, that is until termination of experiments. In some

instances, this study was performed by changing the complete medium daily and replacing it with fresh medium. Other experiments were conducted by harvesting the complete medium alternately every one to three days. The results obtained in the infected cultures maintained without fetal calf serum were as good as those obtained in medium supplemented with serum. However, virus yield appeared to be more consistent in daily harvests when serum was used in the maintenance medium. On other hand, when grown in the absence of serum, virus yields appeared higher when harvested after a 3 day-interval.

Serial passages of rubella virus HPV-78 in turkey embryo tissue culture were then performed at 7 and 14-day-intervals. Optimum virus yield was obtained in serial passages performed either at 7 or 14-day-intervals. In general, the infected cultures maintained without fetal calf serum were as good as those maintained in the presence of 1 per cent serum.

The high passage level rubella viruses grown in monkey kidney cells and in turkey embryo tissue cultures were tested for their ability to propagate in RK-13 cells and behaved like attenuated strains, whereas the wild strain did not.

The two major considerations at the present time for rubella vaccines are the possible transmission of the vaccine virus to the pregnant woman or foetus, and the safety of the tissue system used to prepare the vaccine. Because of the latter concern, avian tissue cultures have been studied for the propagation of the virus. Chick embryo tissue culture has been shown to be unsatisfactory for rubella vaccine (6, 10) because it necessitated the overattenuation of the rubella virus strain in order to propagate well in this tissue system. Duck embryo tissue cultures allow the propagation of attenuated rubella virus (1) and are used at the present time as a safe tissue system for rubella virus vaccine.

The present data show that rubella virus strain HPV-77 propagates well in turkey embryo tissue culture. Harvesting the virus daily appears to be profitable during the first 10 days of culture when the infected cells are maintained in medium without fetal calf serum.

The persistence of the virus at 32° C in tissue culture media during 14 days proves that rubella virus is truly replicating in turkey embryo tissue culture. Further evidence for the replication of rubella in turkey embryo tissue cultures is obtained from analysis of inputs and yields on serial harvests. For example, in one study, on first passage in turkey embryo tissue culture the input was 10^4 per 0.5 ml. After an adsorption period of 2 hours at 32° C, $10^{0.5}$ per 0.5 ml was adsorbed and daily harvests yielded more than $10^{3.25}$ viral particles per 0.5 ml. On subsequent passages with a similar viral input, $10^{2.25}$ to $10^{3.5}$ particles were produced in multiple harvests.

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