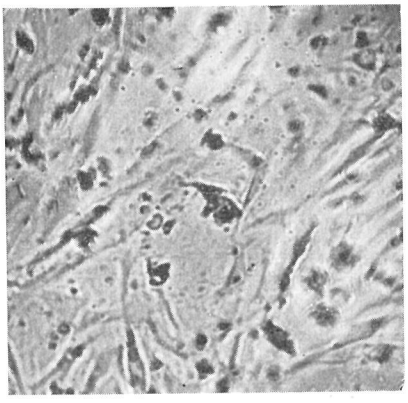
Propagation of Egg-Adapted Poliovirus (Type 1, Brunhilde Strain) in Chick Embryo Tissue Culture

As we reported previously[1], poliovirus (type 1, Brunhilde strain) has been serially cultivated in the developing chick embryo by the chorioallantoic cavity route. This passaged poliovirus was not cytopathogenic for FL or monkey kidney cells, and the proof of multiplication of passaged virus in developing chick egg rested mainly on the interference test with mumps virus.

In the present study, further convincing evidence was obtained for the serial passage of poliovirus in developing chick eggs. The 27th generation of chick embryo passaged poliovirus material, consisting of infected chorioallantoic membrane and fluid, was transferred to a chick embryo tissue culture. The chick



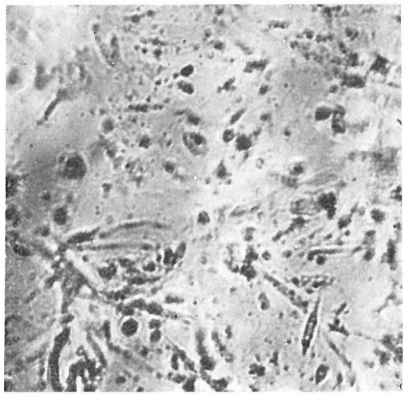


Fig. 1, 2. Cytopathic changes in chick cells 7 days after addition of 6th chick cell passage of poliovirus Cellular shrinkage and nuclear degeneration is prominent

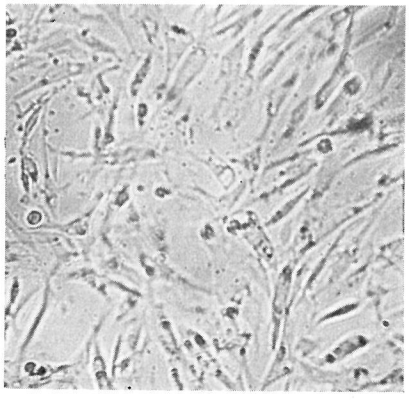
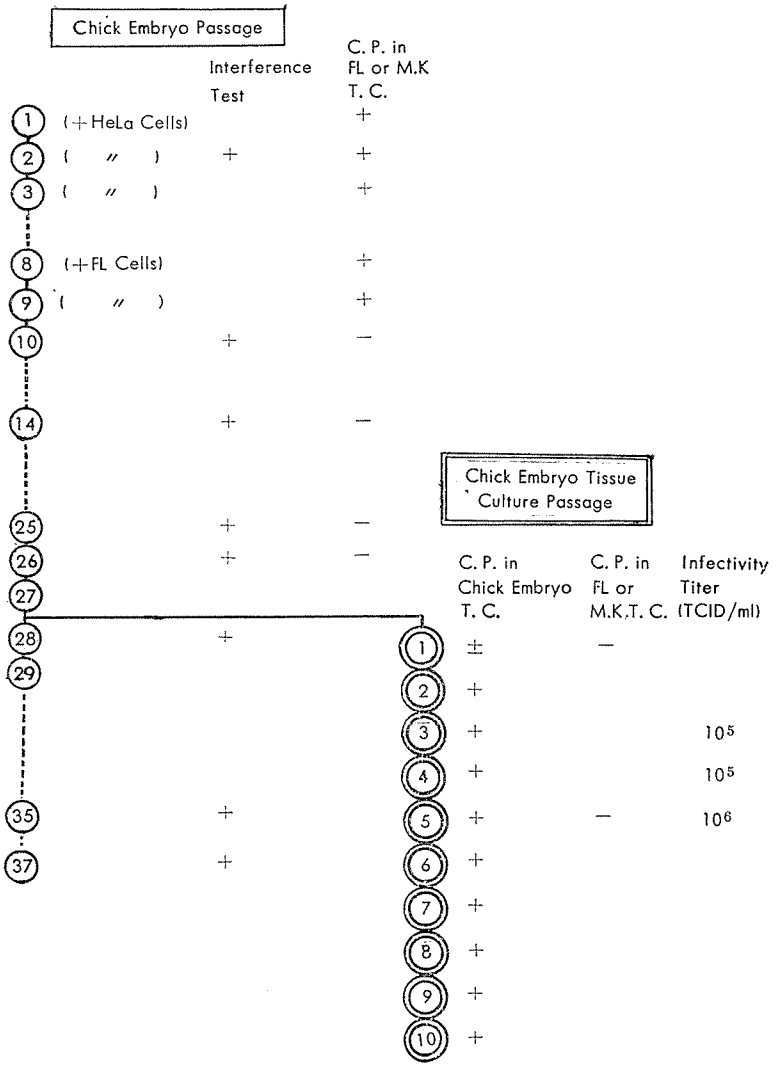


Fig. 3. Chick cells in control culture under the same conditions as above

embryo tissue culture was prepared in the following way. Seven to 10 day old chick embryo was minced, trypsinized and dispersed with LE solution (+3% calf serum). After formation of a monolayer, cells were further trypsinized and dispersed with the same medium. Two to 3 days later, when a monolayer sheet

Table 1. Serial Cultivation of Poliovirus (Type 1. Brunhilde) In Developing Chick Embryo and in Chick Embryo Tissue Culture



C.P.=Cytopathic Change
M.K.T.C.=Monkey Kidney Tissue Culture
TCID=Tissue Culture Infectious Dose

had been formed, the virus material was inoculated and 199 medium (+3% calf serum) was added as maintenance medium.

The observation period of cytopathic phenomenon of the chick cells was 14 days. In the first passage, no distinct cytopathic change was detected. Fluid was harvested at the end of the observation period and was inoculated into the newly prepared chick embryo tissue culture. Then the cellular change became apparent after about 7 days.

As shown in Figs. 1, 2, a striking shrinkage of the cytoplasm was readily detected and nuclear degeneration was prominent in the infected tissue culture, whereas no such severe alteration occurred in the control cells. These degenerated cells seemed not to fall off walls of the bottles even in the late stage, differing from FL cells infected with the wild poliovirus. The pH of the medium of the infected tissue ceased to change earlier than that in the control culture. The fluid harvested at the time of appearance of cytopathic change was successively inoculated into chick embryo tissue cultures, and a definite cellular change was noticed in every passage.

Table 2. Result of Neutralization Test with Monkey Sera and Virus of the 5 th Passage in Chick Cells

Serum	Infectivity Titer in Chick Cells	Neutralization Index
Immune Monkey Serum	$<10^1$ (TCID)	>3
Normal Monkey Serum	10^4 (TCID)	

The passaged virus was proved to be non-cytopathogenic for FL or monkey kidney cells and occasionally titrated in chick embryo tissue culture propagated in prescription bottles. Titers of 10^5 TCID/ml at 3rd and 4th generation and 10^6 TCID/ml at 5th generation were obtained. The cytopathogenic effect of passaged virus could be neutralized with specific immune monkey serum for type 1 poliovirus. The detailed immunological and biological properties of this chick embryo tissue culture passaged virus will be described later.

REFERENCE

1) Takahashi, M., Yamamura, T., Toyoshima, K., Miki, T. and Okuno, Y. (1960). Studies on attenuation of poliovirus. I. Serial cultivation of poliovirus (Type 1, Brunhilde strain) in developing chick embroys. *Biken's J.* **3,** 249-257.

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