Preparation of High Potency Poliovirus in FL Cell Cultures at Room Temperature.* (24961)

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It was shown in earlier studies(1) of the reproduction of poliovirus at 37°C in various cell strains of human derivation that appreciable quantities of infectious virus appear in cells about 6 hours after infection; the titer increases rapidly thereafter and reaches a peak at about 12 hours. The intracellular concentration of virus then declines quickly due to release into the culture fluid. Highly potent preparations of virus can be harvested from the infected cells by mechanical disruption in a Mickle tissue disintegrator, but the harvest must be timed carefully if a good yield is desired. In the present study it has been found that during cultivation at 32°C, virus accumulates almost as rapidly and reaches practically the same intracellular titer as at 37°C. However, release of virus at 32°C is much slower than at 37°C and as a result, the intracellular concentration of virus remains at or near the peak level for 4 to 6 hours. For purposes of production of highly potent virus by mechanical disruption of infected cells it is more convenient to operate at lower temperature, since the need for careful timing of harvest is thus obviated. Furthermore, it has been found that careful control of temperature is unnecessary, since potent virus preparations for use in immunological studies have been obtained consistently by cultivation at room temperature for approximately 18 to 20 hours, followed by extraction of virus from infected cells.

Materials and methods. Poliovirus Type 1, Mahoney strain, was used. Virus infectivity assays were made by plaque technic in rhesus monkey kidney cultures, viral antigen was assayed by complement fixation (CF) tests with human convalescent sera, and FL cells for virus propagation were grown as described elsewhere(1,2).

Results. Kinetics of virus formation and release at 37° and 32°C. Four days after seeding, 25 replicate 32 oz bottle cultures of FL cells were washed 3 times with 10 ml portions of maintenance fluid and infected by addition of 25 ml of maintenance fluid containing sufficient virus to provide a multiplicity of 28 plaque forming units (PFU)/cell. After 3 hours incubation at 37°C the maintenance fluid was removed and cultures washed 3 times with 10 ml portions of maintenance fluid. Ten cultures were incubated at 32°C and another 10 bottles were kept at 37°C. The remaining 5 cultures were used for counts of cell nuclei(3). At intervals shown in Fig. 1, samples consisting of one culture were removed from respective incubators. The fluid phase was withdrawn and assayed for infectivity. The cells were scraped off with a rubber tipped glass rod, suspended in chilled veronal buffer(1) and centrifuged at 2000 rpm for 2 minutes. The supernate was discarded. The cells were resuspended in 1 ml of veronal buffer, disrupted in Mickle Tissue Disintegrator as described previously(1), and extracts so obtained were assayed for virus infectivity. The results, expressed in terms of PFU/cell, are shown in Fig. 1.

It is evident that decrease in temperature affects release of virus much more markedly than its formation. As a result, a high intracellular concentration of virus persists for 4

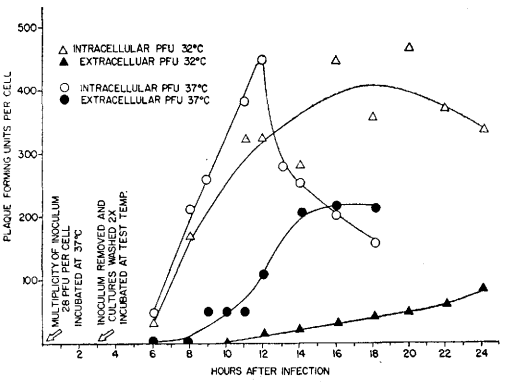

FIG. 1. Formation and release of poliovirus type 1 in FL cells incubated at 37 and 32°C.

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TABLE I. Assay of Intracellular Virus Prepared from Infected FL Cells Maintained at Room Temperature Overnight (18-20 Hr), 4 Lots.

Lot #	No. of 32 oz cultures	Vol of cell extract, ml	PFU/ml	PFU total
1	10	3	1.08×10^{10}	3.2×10^{10}
4	18	5	.92 "	4.6 "
5	10	3	1.12 "	3.36 "
8*	7	2.43	.91 "	2.21 "

* Material was ultracentrifuged in sucrose density gradient prior to assay (Zone SDG^D).

to 6 hours at 32°C and good harvest can be obtained at any time during that interval.

Preparation of high potency virus material from infected cells incubated at room temperature. Five to twenty 32 oz bottle cultures of FL cells were inoculated at one time, each with 25 ml of maintenance fluid containing sufficient virus to provide a multiplicity of 20-30 PFU/cell. Cultures were incubated at 37°C for 2 to 3 hours. The maintenance fluid containing the inoculum was removed and 25 ml of fresh maintenance fluid was added. The cultures were then placed on top of a laboratory bench for 18-20 hours. A recording thermometer in the same location indicated a temperature range 26° to 32°C. At time of harvest the tissue culture fluid was discarded; the cells were scraped off, pooled, and packed by centrifugation at 1000 rpm for 4 minutes. The packed cells were stored in a glass vial at −55°C until needed.

Several lots of infected cells were thus prepared. All lots were made with an inoculum of virus prepared from cells infected and maintained at 37°C. On several occasions the virus inoculum was reused after replenishing for virus lost by adsorption to cells or by inactivation. Assays made on extracts from 4 lots of infected cells are shown in Table I. Lot #8 was fractionated by ultracentrifugation in a sucrose density gradient(2,4). CF tests of the SDG^D fraction of the sucrose density gradient with a selected monoreactive convalescent serum of *D* specificity indicated the presence of approximately 1000 CF units of *D* antigen(4). From the numerical relationship established previously(1,2) between CF activity of the antigen in the SDG^D zone and physical virus particles(2,3), it was computed that SDG^D contained 10^{13} physical virus particles, of which at least 2.21×10^{10}, *i.e.*, about 1 in 400, were infectious for rhesus monkey kidney monolayer cultures. Because of low sensitivity of this assay system as compared with other assays(5,6), as well as for other reasons stated elsewhere(1), the infectious virus content is probably much higher.

Summary. Rate of formation and release of poliovirus Type 1 were studied at 32° and 37°C in FL cell cultures. Virus accumulated almost as rapidly and reached practically the same intracellular levels at 32°C as at 37°C. However, release of virus was much slower at 32° than at 37°C and as a result, at 32°C the intracellular concentration of virus remained at or near peak levels for 4 to 6 hours. From these observations, highly potent virus for use in immunological studies was prepared by mechanical disruption of infected cells maintained at room temperature (26-32°C) for 18-20 hours.

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