*REPLICATION OF POLIOVIRUS I IN CHICK EMBRYO AND HAMSTER CELLS EXPOSED TO SENDAI VIRUS**

By John F. Enders, Ann Holloway, and Elizabeth A. Grogan

RESEARCH DIVISION OF INFECTIOUS DISEASES AND CHILDREN'S CANCER RESEARCH FOUNDATION OF THE CHILDREN'S HOSPITAL MEDICAL CENTER, AND DEPARTMENT OF BACTERIOLOGY AND IMMUNOLOGY, HARVARD MEDICAL SCHOOL

Communicated January 19, 1967

Evidence is accumulating that replication of animal viruses may take place in naturally resistant cells provided measures are taken to circumvent the barrier to infection presented at the cell surface and thus to place the virus directly in contact with the biosynthetic factory of the cell. The only technique by which this has so far been accomplished consists in exposing resistant cells to viral nucleic acid (NA), which, unlike complete infectious particles, attains, under appropriate conditions, an intracellular position.

Holland and his associates[1] presented the first example of the capacity of viral NA to initiate infection in resistant cells when they showed that poliovirus RNA induced in chick embryonic cells replication of complete virus. That cells even very distantly related to those of the natural host may also support virus replication when exposed to viral NA was subsequently demonstrated in three reports of 1964.[2-4] These findings are significant from the point of view of general biology, since they support the concept of the universality of the genetic code. They also have practical implications, since they suggest the possibility that populations of cells which can be easily and economically propagated in large quantities and which are naturally resistant to certain agents such as poliovirus might be employed in the mass production of vaccine or as a means of isolating and growing agents of diseases like infectious hepatitis which so far have defied all attempts at cultivation. But for such purposes the requirement imposed by the necessity of using viral NA renders direct application of the method impractical. We have therefore considered how it might be possible to introduce complete virus into resistant cells under conditions that permit cell survival.

A simple way of doing this was suggested by recent demonstrations that certain myxoviruses when added in high concentration to cell suspensions rapidly bring about fusion of the cytoplasm of the individual elements, which results in the formation of viable multinucleate cells (for summary of literature see ref. 5). We speculated that if such fusion is brought about in the presence of complete virions, those which by chance are in contact with the cell surfaces where coalescence occurs might be incorporated within the cytoplasm of the polynucleate complex. To explore the possibility that virus could in this manner be placed within cells considered to be resistant and there might replicate, we selected, as virus, Poliovirus I and, as cell systems, secondary chick embryo cells and an established line of hamster embryo cells. We adopted Sendai virus, which Harris and his associates[6] have applied in their studies of artificial heterokaryons, as the fusion-inducing agent.

Materials and Techniques.—Viruses: Stock Poliovirus I, strain Brunhilde, was grown in primary human amnion cell cultures with bovine amniotic fluid medium.[7] Two stock preparations were employed consisting of pooled supernatant fluids from such cultures. Infectivity titers in monkey kidney cells were log 4.8 and 5.8 TID_{50}/0.1 ml, respectively. Sendai virus was propagated

in chick embryos and then concentrated and exposed to ultraviolet irradiation for 3 min, following procedures described by Harris and his co-workers.[6] The concentrates exhibited hemagglutinin titers of 1:80,000 and 1:20,000, respectively. The second lot was tested for residual infectious Sendai virus in 10-day chick embryos. As with material used by Harris,[6] a residue of infectious virus was detected.

Cells: (a) *Chick embryo cells:* Primary cultures were grown in prescription bottles from trypsinized cells derived from 7–10-day white leghorn chick embryos fed with Eagle's[8] medium containing 2% fetal calf serum. After 3 days' incubation at 37°C, cells were removed with Puck's trypsin (0.05% in Puck's saline A), centrifuged at 1000 rpm for 5 min, and suspended in Hanks' solution without glucose. Cell counts were made and the volume was adjusted with Hanks' solution to give the required number of cells/ml. (b) *Hamster embryonic cells:* Cells from the 69th to the 78th passage of the "I" line established by Dr. G. Diamandopoulos[9] were propagated in Puck's medium containing 10% fetal bovine serum and 10% horse serum and handled in the same manner as chick embryo cells. (c) *African green monkey kidney cells:* Monolayer cultures of cells from the AH-I line established in this laboratory,[10] propagated in the modified Puck's medium, and maintained in Eagle's medium containing 2% fetal calf serum were employed in all infectivity titrations.

Poliovirus infectivity titrations: Two AH-I cell cultures were each inoculated with 0.1 ml of each dilution of the fluid under test. Complete destruction of cells as observed on the seventh day after inoculation was taken as criterion for the presence of virus. ID_{50} was calculated by the method of Reed and Muench.

Inoculation and fusion of cell suspensions: As routine, cell suspensions were first exposed to poliovirus. Usually 0.5 ml of undiluted or diluted stock virus pool was employed. Virus was added to cells in suspension. Cell-virus mixtures were incubated at 37°C for 1 hr in a water bath. They were frequently shaken gently by hand and then sedimented by centrifugation at 1000 rpm for 5 min. Supernatant fluid was removed and Sendai virus was added. Various concentrations of Sendai virus were employed as noted hereafter. Mixtures, after shaking, were allowed to stand for 15 min at 4°C and were then shaken for 20 min at 37°C in a mechanical shaker operating horizontally at about 100 excursions per min. Cells were sedimented at 1000 rpm for 5 min and suspended in 1 ml of Eagle's medium containing 2% fetal calf serum. These preparations were incubated at 37°C in the slightly inclined position usually adopted for stationary monolayer tube cultures. As controls, poliovirus-exposed cell suspensions without Sendai virus and cell suspensions to which only Sendai virus was added were treated in the same manner. Hank's solution without glucose was added in place of the missing component. Microscopic examination of hamster cell cultures after 18 hr showed that many of the cells which by this time were adherent to the glass had coalesced, forming large giant cells containing numerous nuclei. Multilayered clumps of cells were also frequently seen. In chick embryo cell populations the number of polynucleate cells was much smaller, as was the number of included nuclei.

Demonstration of viral replication in fused-cell cultures: Procedure 1. Fluids were removed 18–24 hr after the cultures were established, and cells were washed 5 or 6 times with neutralized Hanks' solution, employing 2-ml aliquots for each washing. Final washings were reserved for viral assay. Fresh medium (1 ml) was added to the cultures and incubation continued. At 3–4-day intervals thereafter, the procedures just described were repeated. Control cultures were treated in the same manner. Before infectivity titrations were done, all fluids were centrifuged at 2000 rpm for 15 min. The dilution factor of the virus originally introduced was calculated on the assumption that 0.2 ml of the inoculum remained initially and that this was diluted 5 or 10 times with each removal of culture fluid or washing, depending upon whether aliquots of 1 or 2 ml were employed. *Procedure 2.* In certain experiments Procedure 1 was modified as follows. About 14 hr after cultures were established, one or more of a replicate set containing poliovirus-exposed fused cells (FC), as well as the same number of cultures of control sets of poliovirus-exposed unfused cells (NFC) were washed 6 times, frozen rapidly 3 times in alcohol at about −70°C, and thawed in water at 37°C. Tubes were then centrifuged at 2000 rpm for 15 min and supernatant fluids titrated for infectivity. Incubation of the remaining cultures in each set was continued until the fourth day or later, when they were frozen and thawed 3 times, centrifuged, and the supernatant fluids titrated.

TABLE 1

CHICK EMBRYO AND HAMSTER CELLS EXPOSED TO POLIOVIRUS I WITH AND WITHOUT ADDITION OF SENDAI VIRUS: POLIOVIRUS INFECTIVITY TITERS OF FLUIDS AND FINAL WASHINGS AT VARIOUS INTERVALS AFTER EXPOSURE (PROCEDURE I)

		Hamster Cells			Chick Cells		
Day	Material	Polio only[a]	Polio and Sendai[b]	Dilution inoc.[c]	Polio only	Polio and Sendai	Dilution inoc.
1	Wash[d]	2.[e]	2.		Nil[f]	= >2.	
3	Fluid[g]	2.	5.	4.9	2	4.8	4.9
3	Wash	ND	ND		Nil	<2.	
7	Fluid	0.5	4.2	5.6	<2	2.8	7.7
7	Wash	ND	0.5			Nil	
10	Fluid	Nil	3.8	9.1	Nil	1.8	11.2
10	Wash	Nil	<2.		Nil	Nil	
18	Fluid	ND	1.4	12.6	ND	0.5	14.7

[a] Approximately 10^6 cells exposed 1 ml poliovirus, log inf. titer = 4.8/0.1 ml.
[b] Cells and poliovirus as in footnote *a*; in addition 0.5 ml Sendai virus, HA titer = 1:80,000.
[c] Calculated log reciprocal dilution poliovirus inoculum in culture fluid removed on day indicated, assuming a dilution factor of 5× at each washing and fluid change.
[d] Last of series of washings done on 1st day after exposure of cells to poliovirus. As routine, cultures were washed 5× with 1-ml aliquots.
[e] Log ID_{50} poliovirus/0.1 ml.
[f] No virus detected in undiluted specimen.
[g] Culture fluid removed on 3rd day after exposure to poliovirus.

Fixing and staining: Cultures were fixed in Bouin's fluid, embedded in collodian, and stained with hematoxylin and eosin.[11]

Experimental.—Demonstration of replication of poliovirus in cells treated with Sendai virus: Procedure 1. The results of two experiments in which Procedure 1 was followed are presented in Table 1. Similar findings have been repeatedly recorded in essentially comparable experiments. From the data it is apparent that in both FC hamster and chick cell systems infectivity titers of culture fluids removed at various intervals thereafter were consistently higher than those of the corresponding fluids from comparable NFC cultures. It will be noted in particular that the titers of the 4-day-old fluids from FC cultures are equivalent to those of the stock virus (ID_{50}/4.8) employed as inoculum and which at this time was by calculation diluted $10^{4.9}$ times. It will also be seen from Table 1 that considerable quantities of virus were present in fluids removed at longer intervals from FC cultures, whereas at the same time very little or no virus was demonstrated in fluids from NFC cultures. Calculated dilutions of the viral inoculum represented by fluids removed from FC cultures after these longer intervals far exceed its original endpoint of infectivity.

It is noteworthy that the amounts of virus demonstrated in the three- and seven-day fluids from NFC cultures (Table 1), although significantly smaller than in the corresponding fused systems, are nevertheless definitely larger than expected on the basis of dilution alone. In most other experiments done according to Procedure 1, similar findings have been recorded. Especially in the hamster cell system, the quantity of virus found has exceeded significantly the calculated residuum of the inoculum, assuming that no viral replication took place. Possible explanations for the presence of unexpected amounts of virus under these conditions will be discussed subsequently.

Procedure 2. By means of this technical modification the increase in the amount of cell-associated virus present in comparable cultures soon after inoculation and the total amount of virus present at later intervals was measured in FC and NFC preparations of chick and hamster cells in order to eliminate the possibility that virus introduced as the inoculum combined temporarily with the cellular phase and was

TABLE 2

POLIOVIRUS INFECTIVITY TITERS OF FROZEN AND THAWED CULTURES OF HAMSTER CELLS AT VARIOUS INTERVALS AFTER EXPOSURE TO POLIO AND SENDAI VIRUSES AND POLIOVIRUS ONLY (PROCEDURE 2)

		Log ID_{50}/0.1 ml			
		Day 1		Day 4	Day 8
Viruses	Expt.	Whole cult.*	W6†	Whole cult.	Whole cult.
Polio	I	2.5	1.5	5.5	ND
+		2.5	0	3.5	4.0
Sendai	II				
		(2.5)‡		(1.5)	(Nil)
Polio	I	2.5	0	2.5	ND
	II	1.5	0.5	2.5	0
		(2)		(Nil)	(Nil)

* Aliquots of cell suspension ($10^{5.8}$ cells/ml) were each exposed to 0.5 ml stock Poliovirus I (ID_{50} = 5.8/0.1 ml) diluted 10^{-1}. Concentrated Sendai virus (HA tit. = 1:80,000) was added to one half of these preparations in aliquots of 0.25 ml. On the following day cultures were washed 6×. One ml medium was then added to each. One culture exposed to polio and Sendai virus and one exposed to poliovirus alone were then frozen and thawed, centrifuged at slow speed, and the supernatants reserved for infectivity titrations. Remaining cultures were incubated at 37°C for additional periods as indicated, when the whole cultures were frozen and thawed, centrifuged, supernatant fluids collected, and infectivity titers of specimens determined. The cultures incubated for 8 days included in expt. 2 were also washed 6× on the 4th day after removal of the medium.

† Sixth wash fluid of first day.

‡ Figures in parentheses indicate titers of cultures frozen and thawed on day 1 and afterwards incubated at 37°C.

TABLE 3

POLIOVIRUS INFECTIVITY TITERS OF FROZEN AND THAWED CULTURES OF CHICK EMBRYO CELLS AT VARIOUS INTERVALS AFTER EXPOSURE TO POLIO AND SENDAI VIRUSES AND POLIOVIRUS ALONE (PROCEDURE 2)*

		Log ID_{50}/0.1 ml			
		Day 1		Day 4	Day 8
Viruses	Expt.	Whole cult.	W6†	Whole cult.	Whole cult.
Polio	I	3	1.	3.5	ND
+					
Sendai	II	2.5	1.	3.5	4.
Polio	I	1.5	0.	2.5	ND
	II	3	0.	1.5	1

* Procedures were identical to those summarized in the first footnote to Table 2 except that in expt. 2 undiluted stock poliovirus was employed.

† Sixth wash fluid of 1st day.

gradually released thereafter. The results served to confirm and extend those obtained by Procedure 1. From the data of two experiments summarized in Tables 2 and 3 which are representative, it is apparent that both in FC and NFC cultures significant quantities of poliovirus are associated with the cellular phase on the first day after establishment of the cultures, since the cells had been thoroughly washed just before they were extracted by freezing and thawing. The higher concentrations of virus found on the fourth and eighth days in the whole frozen-and-thawed FC cultures compared with those present on the first day demonstrate that multiplication occurred during each of these intervals. Again it is to be remarked that the titers found for certain of these cultures are comparable to those obtained when this agent is propagated in poliovirus-susceptible cells. The infectivity titers of NFC preparations in these experiments either increased or decreased somewhat between the first and fourth days, but declined markedly by the eighth day, indicating that replication of poliovirus in the absence of Sendai virus was slight or failed to occur.

Additional evidence for viral replication in FC cells was obtained during the course of these experiments. Thus in the second experiment with the hamster system, one FC and one NFC culture were frozen and thawed three times on day 1 after they had been washed six times. The frozen and thawed cells were then

sedimented and resuspended in the original culture fluid. These preparations were thereafter incubated along with other unfrozen cultures, and aliquots of fluid were removed from them on the fourth and eighth days and titrated for infectivity. The titers are recorded between parentheses in Table 2. It is evident that infectivity decayed rapidly in the systems in which the cells were killed on day 1. The elevated infectivity titers, therefore, found on the fourth and eight days in the cultures of FC cells that had not previously been frozen cannot be attributed to persistence of virus originally introduced as inoculum, but to its continuing replication.

Determination of minimal dose of poliovirus in FC cultures: The amount of poliovirus capable of multiplying in FC hamster and chick embryo cultures was determined by Procedure 1. Dilutions of stock virus were prepared and added to aliquots of cell suspension. The results of infectivity titration of four-day culture fluids of FC and NFC cultures are presented in Table 4. It is apparent from the data that the amount of virus required to initiate replication in these fused culture systems exceeds by a factor of about 10^4 the minimal infective dose.

Quantity of Sendai virus required: The minimal quantity of Sendai virus necessary to promote the growth of poliovirus has not been consistently studied. One experiment, however, has been done which indicates that a high concentration is required. Employing Procedure 1, aliquots of a suspension of hamster cells were exposed to $10^{4.8}$ ID_{50} of poliovirus. Aliquots (0.25 ml) of a series of tenfold dilutions of concentrated Sendai virus (HA titer = 1:20,000) as well as the undiluted material itself were each added to an aliquot of the virus-exposed cell suspension. A significant difference in poliovirus replication as compared with a control preparation without Sendai virus was recorded only in the system treated with undiluted virus. It is of interest that in this experiment the cell-fusing effect of Sendai virus was still distinguishable, although much reduced, when the virus was diluted 100 times. When the virus was diluted 1000 times, fusion was not apparent. Further investigation will be necessary to determine whether the disparity in activities thus suggested is consistently demonstrated.

Failure of increased replication of poliovirus when added after induction of cell fusion: Evidence consistent with the hypothesis that the virus is incorporated in cells duriug the process of fusion was obtained in an experiment in which poliovirus was added after fusion was induced. In general the procedure consisted in bringing about fusion by Sendai virus in replicate cultures and exposing the fused cells to poliovirus on the next day. On the day after addition of poliovirus the cells were washed repeatedly. As controls, cultures of unfused cells were treated in the same manner. After addition of fresh medium, one of two FC cultures and one of two NFC cultures were frozen and thawed and titrated for infectivity. Three days later, i.e., on the fifth day after initiation of the experiment, each of the two remaining cultures of FC and NFC cells was treated in a similar manner. From Table 5 it is evident that the infectivity titer of the FC culture did not exceed that of the NFC culture at a time when it has consistently been higher in experiments in which fusion was induced in the presence of poliovirus.

Cytomorphological observations: Extended studies have not yet been carried out on stained preparations, so that we cannot state unequivocally that replication of poliovirus in Sendai virus-treated cultures is associated with specific cytopathic effects. We have, nevertheless, repeatedly observed as incubation was continued

TABLE 4

DETERMINATION OF POLIOVIRUS MINIMAL INFECTIVE DOSE IN FUSED CHICK AND HAMSTER CELLS (PROCEDURE 1)

Log ID_{50} poliovirus inoculated*	Fluids from Cells Exposed to—Poliovirus Only		Fluids from Cells Exposed to—Poliovirus + Sendai†	
	Hamster	Chick	Hamster	Chick
6.5	3‡	1.5	5.	3.5
5.5	3	0	5.5	2.5
4.5	0	Nil	4.0	2.0
3.5	0	Nil	Nil	Nil
2.5	Nil	Nil	Nil	Nil

* Stock poliovirus, ID_{50} 5.8/0.1 ml : 0.5 ml of indicated dilutions added to approx. 700,000 cells.
† Concentrated Sendai virus, HA titer 1:20,000, added; hamster cell suspensions treated with 0.25 ml and chick cell suspensions with 0.5 ml of this concentrate.
‡ Log ID_{50}/0.1 ml.

TABLE 5

FAILURE OF REPLICATION WHEN POLIOVIRUS ADDED AFTER FUSION INDUCTION (PROCEDURE 2)

Viruses	Infectivity Titer of Materials Tested—Whole cult. 2†	W_2‡	Whole cult. 5§	W_2‡
Polio + Sendai	1.5*	0	2.5	Nil
Polio	1.0	0	2.5	Nil

* Log ID_{50}/0.1 ml.
† Culture frozen and thawed 3× in 1 ml medium on 2nd day.
‡ Cultures washed 6× on 2nd day. Titer on last washing.
§ Whole culture frozen and thawed 3× on 5th day; fluid after removal of cellular debris.

that in hamster systems in which enhanced viral proliferation took place, the number of cells often appeared to be less than in cultures exposed only to Sendai virus. Furthermore, we have an impression that in cultures in which poliovirus replication is actively occurring, degenerative changes develop sooner and progress to cell destruction more rapidly than in controls exposed to Sendai virus alone. Such indications of greater cellular injury and loss have frequently been associated, as incubation is extended, with a greater decrease in acid production in cultures showing replication of poliovirus. The practical importance of determining whether these observed differences are in fact specifically related to replication of poliovirus is obvious. Experiments toward this objective are in progress.

Failure to demonstrate an increased capacity of poliovirus propagated in fused cells to multiply in unfused cells: It seemed possible that poliovirus propagated in FC cultures might acquire and retain an ncreased capacity to multiply in unfused cells of the same species. Accordingly, virus was propagated in FC hamster cultures by Procedure 2 and was subsequently grown in AH-I cells. It was then tested for its capacity to multiply in NFC hamster cultures. No evidence of an inheritable enhanced capacity of the virus to multiply in this system was obtained.

Passage of poliovirus propagated in fused cells: We have found that poliovirus propagated in FC hamster cells can be passed in the same system provided the infectivity titer is sufficiently high. For example, in one experiment virus in the form of pooled fluids removed on the fourth day from FC hamster cell cultures treated according to Procedure 1 was employed as inoculum for a second passage using the same technique. The titer of the inoculum was 5.5 ID_{50}/0.1 ml, of which 0.5 ml was added. Two aliquots of hamster cell suspension after exposure to this virus were fused by addition of Sendai virus and washed six times on the next day. The fluids removed from these cultures on the fourth day each exhibited infectivity titers of 5.5 ID_{50}/0.1 ml. In other similar experiments in which virus with titers ranging from log 3 to 4 ID_{50}/0.1 ml were employed as inocula, replication of the agent was not demonstrated either in hamster or chick cell systems, as might be expected from the results summarized in Table 4.

Discussion.—The results show that in cultures of hamster and chick cells which by themselves have little or no capacity to support growth of Poliovirus I, the replication of this agent occurs when high concentrations of irradiated Sendai virus were subsequently added. Two different procedures were employed to demonstrate this

phenomenon. In both procedures the concentration of poliovirus in "fused" cells was found to be consistently and significantly higher as compared with "unfused" controls.

In FC chick and hamster cell systems the infectivity titers occasionally approached or equalled those obtained in a line of susceptible grivet monkey cells. At other times lower levels were recorded which were more frequently encountered in chick cell cultures. The factors responsible for this variation in viral yield have not yet been defined.

The persistence of small quantities of poliovirus in NFC cultures of both hamster and chick cells after sufficient washing to remove all that was introduced as inoculum raises the question whether these cells are completely resistant to this agent. A conclusive answer has not yet been obtained. In favor of complete resistance is our repeated failure, as well as that of others, to demonstrate continuous viral multiplication in serial passages in chick embryonic cell cultures or in living chick embryos and our more recent failure to propagate the present strain in serial monolayer cultures of the "I" line of hamster cells. Furthermore, little or no evidence that poliovirus multiplication occurred in cultures without Sendai virus was obtained in the present experiments in which the virus content of the whole culture was measured after varying intervals. Finally, in experiments done by Procedure 1 in which determinations of the virus content of the fluid phase were made at intervals over a total period of 18 days, no virus was detected after the seventh day in NFC cultures although it was found in significant concentrations on the tenth and 18th days in FC cultures. The weight of available evidence, therefore, supports the view that the cell systems employed are solidly resistant to Poliovirus I infection. However, since in inoculated cultures in which cells were afterwards killed by freezing and thawing, viral infectivity decayed more rapidly than in unfrozen NFC cultures, the possibility cannot be excluded at present that a few susceptible cells among a preponderantly resistant population may provide the source of the small amounts of virus encountered in NFC cultures soon after inoculation.

The validity of the initial hypothesis that during fusion poliovirus is passively or mechanically incorporated within the newly forming polynucleate cells has not been unequivocally confirmed. In support of this concept is the demonstration that enhanced replication of poliovirus did not occur when exposure was effected after induction of fusion. Furthermore, such enhancement was observed only when high concentrations of Sendai virus capable of inducing fusion were employed. An exact correlation, however, between the concentration of Sendai virus required to enhance viral replication and that required to induce recognizable fusion, together with the marked difference in the extent of fusion in chick and hamster cell systems induced by the same concentration of virus, suggests that another mechanism may be responsible. As an alternative, one can visualize focal impairment of the integrity of the lipoprotein constituent of the membrane of the mononucleate cell brought about by the Sendai fusion factor itself or an independent viral constituent through which poliovirus particles might pass.

The procedures described were adopted because of the original hypotheses regarding the manner in which virions might be incorporated. It seemed logical to provide conditions which might allow most efficient contact between cell and virus at the time fusion was induced. Accordingly, cell suspensions were employed.

Recently, however, Dr. John Neff in this laboratory has shown that fusion of monolayer cell cultures previously exposed to poliovirus is followed by enhancement of viral replication comparable to that described here. Since this modification is less laborious and somewhat more economical, it would appear to be preferable for most purposes in future work.

Whether or not the techniques described may prove effective in the propagation of other agents, in particular those such as SH and IH viruses which have so far defied attempts to cultivate them *in vitro*, remains to be determined. In addition the possibility of inducing the replication of complete virus in fused cell complexes in which one component may harbor the viral genome in a partially or completely suppressed state and the other be capable of supporting its replication in the infective form might be profitably explored.

The demonstration that viral nucleic acid is infective for the naturally resistant cell does not eliminate the possibility that resistance to the intact virion may depend upon the absence of inhibition of intracellular factors essential for decapsidizat on or "uncoating" of the viral particle. Our findings provide evidence that such factors are indeed present and operative in the resistant cells studied and that the barrier to infection lies at the cell surface rather than internally.

Conclusion.—In cultures of naturally resistant hamster and chick embryo cells exposed to Poliovirus I, replication of this agent occurs when high concentrations of ultraviolet-irradiated Sendai virus are subsequently added.

* Supported by research grant AI-01992-09, U.S. Public Health Service, Department of Health, Education and Welfare.

[1] Holland, J. J., L. C. McLaren, and J. T. Syverton, *J. Exptl. Med.*, **110**, 65 (1959).

[2] Sander, E.-M., personal communication to P. Abel cited by P. Abel, in *Cold Spring Harbor Symposia on Quantitative Biology*, vol. 29 (1964).

[3] Abel, P., and T. A. Trautner, *Z. Vererbungsl.*, **95**, 66 (1964).

[4] Bayreuther, K., and R. Romig, *Science*, **146**, 778 (1964).

[5] Kohn, A., *Virology*, **26**, 228 (1965).

[6] Harris, H., and J. F. Watkins, *Nature*, **205**, 640 (1965).

[7] Enders, J. F., *Proc. Soc. Exptl. Biol. Med.*, **82**, 100 (1953).

[8] Eagle, H., *Science*, **122**, 501 (1955).

[9] Diamandopoulos, G. Th., and J. F. Enders, *Am. J. Pathol.*, **49**, 397 (1966).

[10] Günalp, A., *Proc. Soc. Exptl. Biol. Med.*, **118**, 85 (1965).

[11] Enders, J. F., and T. C. Peebles, *Proc. Soc. Exptl. Biol. Med.*, **86**, 277 (1954).