U.V. STABILIZATION OF RESCUED POLIOVIRUS*

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Numerous experiments show that enteroviruses which are sensitive to guanidine hydrochloride can be rescued in the presence of this inhibitor provided cells are also infected with a strain of virus that is resistant to the drug (Agol and Shirman, 1964; Holland and Cords, 1964; Ikegami, Eggers and Tamm, 1964; Wecker and Lederhilger, 1964). Similarly, guanidine dependent virus can be rescued in the absence of drug by virus which is not dependent on the compound for growth. The appearance of both infectious nucleic acid and mature virus of the rescued type has lead to the conclusion that helper virus supplies enzymes for the development of the rescued virus. Although it seems highly plausible that the rescued virus underwent a replicative process another explanation must also be considered. It is probable that rescued virus represents input viral nucleic acid that is activated and subsequently encapsulated with the protein of helper virus. Two considerations make this a likely explanation. First, the recovered virus has a protein coat similar to helper virus (Holland and Cords, 1964; Ikegami, Eggers and Tamm, 1964) and secondly, non-replicating DNA of bacteriophage lambda can be enclosed in the protein coat of replicating virus (Arber and Dussoix, 1962).

In the present report the development of rescued virus was monitored by testing for changes in UV sensitivity of virus synthesizing centers following infection. If rescued virus represents encapsulated input viral RNA then changes in UV sensitivity would not be anticipated following

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infection. The data will show that the rescued virus undergoes a normal process of UV stabilization suggesting replication of the incoming genome.

Materials. LSC-g^s (guanidine sensitive) and Loddo-g^d (guanidine dependent) strains of type 1 poliovirus were employed. Guanidine dependent virus was a gift of Dr. Hans J. Eggers. Inhibitor was always used at a concentration of 100 μg/ml.

HeLa cells were employed and were grown in Hanks balanced salt solution which contained per liter: 120 ml bovine serum, 5 gm lactalbumin hydrolysate, 10 mg streptomycin, 10^4 units of penicillin, 2.5×10^3 units of polymyxin and 5×10^3 units of mycostatin.

A Westinghouse G15T8 sterilamp which emitted 59.4 ergs/mm^2/sec in the UV range was employed. Calibration was made with a Weston 8PV1 DAA microampere meter.

Methods. The methods for infecting and irradiating cells and titrating virus have been thoroughly described in a previous report (Tershak, 1962). Only one modification was used in the present study. After UV treatment the survival of virus yield rather than infectious centers was determined. Three ml samples of cells at a concentration of 2.5×10^5 cells/ml were distributed into 100 mm tubes that were tightly capped and incubated in a water bath at 36°C. The time of incubation always totaled 7 hours from time of infection unless otherwise specified. After incubation the cultures were frozen and thawed and the final virus yields were determined.

Results and Discussion. Initially studies were performed to establish the efficiency of rescue of drug sensitive virus (LSC-g^s) by drug dependent virus (Loddo-g^d) in the presence of inhibitor. Cultures were infected in quadruplicate with LSC-g^s, Loddo-g^d and a mixture of the two viruses in the presence or absence of guanidine hydrochloride. The final virus yields were determined by plaque assay with normal overlay and overlay containing 100 μg/ml guanidine hydrochloride.

Table I, column I shows that guanidine inhibited the growth of

LSC-g^s about 3.7 $logs_{10}$ (1.1×10^8 PFU/ml vs. 2.1×10^4 PFU/ml). However, simultaneous infection with Loddo-g^d boosted the LSC-g^s titer about a hundred fold (2.6×10^6 PFU/ml vs. 2.1×10^4 PFU/ml). This represents about 2 per cent of the LSC-g^s yield in the absence of inhibitor. In other experiments rescue has been as high as 10 per cent. The data in column II show that 100 µg/ml guanidine in the overlay caused a 4 log_{10} inhibition of LSC-g^s but increased the titer of Loddo-g^d about 3.4 log_{10} units. This shows that in a mixed infection LSC-g^s can be titrated free of Loddo-g^d by employing normal overlay medium. Conversely Loddo-g^d can be titrated by adding inhibitor to the overlay medium.

Rescue of Guanidine Sensitive Poliovirus
by Guanidine Dependent Virus

Conditions of Infection	PFU/ml Control Overlay	PFU/ml 100 µg/ml Guanidine in Overlay
	Column I	Column II
LSC-g^s, Control medium	1.1×10^8	1.2×10^4
LSC-g^s, 100 µg/ml guanidine in medium	2.1×10^4	
Loddo-g^d, 100 µg/ml guanidine in medium	1.4×10^4	8.6×10^7
Loddo-g^d plus LSC-g^s, 100 µg/ml guanidine present	2.6×10^6	6.5×10^7

Table 1. Cultures containing 2×10^6 HeLa cells were infected in quadruplicate with LSC-g^s at a ratio of 2 PFU/cell, Loddo-g^d at a ratio of 7 PFU/cell, and a mixture of both viruses. After one hour at 36°C the cultures were washed three times with medium and 5 ml of medium were added to each bottle. After an additional 9 hours of incubation the cultures were frozen, thawed and like samples were pooled and titrated with normal overlay and overlay containing 100 µg/ml inhibitor. When drug was used in the experiment it was present at all times including the period of washing.

It is apparent that LSC-g^s must be titrated in the presence of Loddo-g^d under conditions where the latter would not form visible plaques. In the radiation experiments to be reported below as many as 500 PFU of drug dependent virus is present in each titration bottle. To determine

whether this affects the titration of drug sensitive virus experiments were conducted wherein the sensitive virus was titrated in bottles containing 10^4 PFU of dependent virus. Table 2 shows that the presence of 10^4 PFU of dependent virus, either unirradiated or irradiated to one per cent survival, had no effect upon the titration of LSC-g^s under normal overlay medium.

Titration of LSC-g^s in the Presence of Loddo-g^d

Experimental	Titer of LSC-g^s PFU/ml
LSC-g^s	6.8×10^7
LSC-g^s + 10^4 PFU Loddo-g^d	6.9×10^7
LSC-g^s + 10^4 PFU Loddo-g^d irradiated to one per cent survival	6.3×10^7

Table 2. Guanidine sensitive virus was titrated in control bottles and bottles containing 10^4 PFU of guanidine dependent virus. Overlay medium lacking the inhibitor was used.

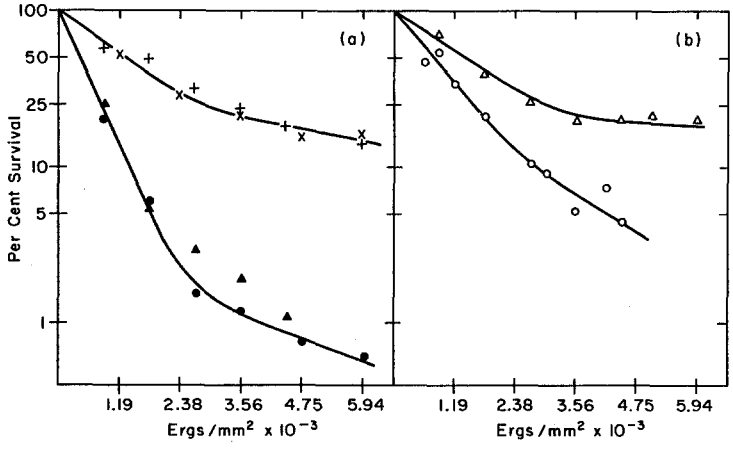

Fig. 1a. UV survival of cell free LSC-g^s (●); LSC-g^s 2.75 hrs. after infection in the presence of 100 μg/ml guanidine hydrochloride (▲); LSC-g^s 2.75 hrs. after infection in drug free medium (X); and LSC-g^s 2.75 hrs. after infection in the presence of drug$_d$ and Loddo-g^d (+). LSC-g^s was used at a ratio of 2 PFU/cell, Loddo-g^d was used at a ratio of 7 PFU/cell.

Fig. 1b. UV survival of Loddo-g^d in a rescue experiment 2.75 hrs after inoculation (Δ); and LSC-g^s alone 2.75 hrs. after infection in the presence of 100 μg/ml inhibitor and at a ratio of 10 PFU/cell (○).

The effects of UV light on LSC-g^s before and after infection are presented in Figure 1a. About 98% of the cell free virus is inactivated exponentially (•). Thereafter a more resistant population appears. Although this has not been examined any further it will be assumed to result from clumping of virus. The UV sensitivity of drug sensitive virus does not change 2.75 hours after infection in the presence of 100 μg/ml of inhibitor (▲). However in the absence of inhibitor a marked degree of UV stabilization is noticeable (X).

The data indicates that guanidine hydrochloride at a concentration of 100 μg/ml can completely halt UV stabilization and hence development of drug sensitive virus. Many growth curve experiments were performed and although the data are not presented here they show that inhibitor can block virus synthesis at any stage of infection.

The UV sensitivity of rescued virus (+) 2.75 hours after infection is analogous to the UV sensitivity of LSC-g^s 2.75 hours after infection in the absence of guanidine. This suggests that the rescued virus is indeed replicating. However, before this assumption can be accepted one complication must be examined. It must be shown that the high level of UV resistance of rescued virus is not due to multiplicity reactivation.

To examine the role of multiplicity reactivation cells were infected with LSC-g^s virus at a ratio of 10 PFU/cell in the presence of guanidine. Two hours and 45 minutes later the cultures were irradiated and incubated an additional 7 hours without drug before freezing.

Figure 1b (o) shows that at high multiplicity LSC-g^s infected cells are more resistant to UV light than cells infected at low multiplicity in the presence of guanidine. However, the degree of UV resistance is not comparable to that of rescued virus or virus that developed in the absence of drug. The degree of UV resistance under high multiplicity of infection is a maximum assuming that Loddo-g^d could cross-reactivate LSC-g^s as efficiently as LSC-g^s could reactivate itself.

Previous experiments have shown that the UV stabilization of one virus cannot be transferred to a second virus (Tershak, 1962). The second virus must undergo development to attain UV resistance. Therefore, it appears that the phenotypic products of Loddo-g^d enabled LSC-g^s to replicate and establish radiation resistance.

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