INTERACTION OF GUANIDINE-SENSITIVE AND GUANIDINE-DEPENDENT VARIANTS OF POLIOVIRUS IN MIXEDLY INFECTED CELLS

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During the period of active synthesis of poliovirus RNA in infected cells, the synthesis of cellular RNA is drastically inhibited (Holland, 1963). The synthesis of poliovirus RNA seems to be accomplished by a new virus-induced polymerase (Baltimore et al., 1963). These data taken together indicate that in the infected cell, exclusively viral RNA can be used as a template by the virus-induced RNA-polymerase. Hence this enzyme in the cell possesses a high degree of specificity. Partially purified viral RNA-polymerase, however, at least in some cases (e.g. polymerase induced by f2-su11 phage) is not specific and can replicate different types of RNA's (Shapiro and August, 1964)[1]. Therefore, one may assume that viral RNA-polymerase in the cell has a more restricted specificity than under in vitro conditions.

In connection with the problem of specificity of viral RNA-polymerase in the cell, the following question may be raised: can RNA of a virus be replicated with the help of RNA-polymerase induced by another virus? In this respect

[1] A preparation of viral RNA-polymerase induced by MSø2 phage, described in an earlier report (Haruna et al.,1963), possesses, however,a very high degree of specificity.

guanidine mutants of poliovirus may probably be of great use. Guanidine was shown to prevent the formation of viral RNA-polymerase by guanidine-sensitive (g^s) variants of poliovirus; on the other hand, it is an obligate prerequisite for the formation of this enzyme by guanidine-dependent (g^d) variants (Baltimore et al., 1963). Therefore in the cells infected by both variants in the presence of guanidine, only g^d variant should induce the formation of the polymerase. If this polymerase has an absolute specificity in the cell, there can be no reproduction of g^s variant (or more precisely, there can be no increase in the extent of its reproduction as compared with cells infected by g^s variant only). If however, RNA-polymerase induced by g^d variant, can use as a template the RNA of g^s virus, then some number of virus particles with the g^s genome is expected to be found among the progeny. On the other hand, when mixed infection takes place in the absence of guanidine, the appearance of g^d variant in the harvest, may indicate that RNA-polymerase induced by g^s virus, is responsible for the formation of g^d variant.

As a g^s variant of poliovirus a line M-I-2p, a twice plaque purified derivative of Mahoney strain (Agol et al., 1962) was used. The number of plaques formed by this variant in the presence of as little as 15 μg of guanidine per ml of agar overlay, decreased more than 1000 times as compared with the number of plaques formed in the absence of the drug. A plaque-purified guanidine-dependent variant was obtained by consecutive passages of M-I-2p in the Macacus cercopithecus kidney cell cultures in the presence of increasing concentrations of guanidine; this variant was designated M-g^d-180. The number of plaques formed by M-g^d-180 in the

presence of 100-200 ug guanidine per ml of agar overlay was more than 1000 times higher than that formed under a drug-free overlay. Viruses were grown on monkey kidney cell or HeLa cell cultures, concentrated by pervaporation (Polson and Hampton, 1957), and dialyzed against Earle salt solution. Cells of M.cercopithecus or M.rhesus kidneys were grown in 50 mm Petri dishes in a humidified air incubator at 37° in the presence of 5% CO_2. Cells were grown in a medium containing 0.5% lactalbumin hydrolysate in the Hanks salt solution, which was replaced by the Eagle medium on the fourth day. 7-8 day old cultures were used. Virus titrations were done by plaque method. As agar overlay a mixture with skimmed milk (Wallis and Melnick, 1961) was used, modified final concentrations of $NaHCO_3$ and agar being 0.22% and 1%, respectively. A preparation of guanidine carbonate was used, which was neutralized with HCl before experiments. All the concentrations of guanidine in this report are expressed as the concentrations of guanidine carbonate.

The design and the results of two typical experiments are presented in Tables 1 and 2. The yield of g^s virus from mixedly infected cells incubated in the presence of guanidine, was some ten times as high as the yield of this variant from cells infected with g^s virus only. Moreover, the titer of g^s variant was almost the same in the samples taken at the zero time and at 9 hours after infection with g^s variant in guanidine-containing medium. Thus, g^s virus in these cells represents apparently the non-washed-off and non-eclipsed part of the input virus. Thus, under our conditions, practically all the g^s virus formed in mixedly infected cell, is reproduced at the expense of its partner. The difference be-

Table 1. Reproduction of g^s Variant in Cells Mixedly Infected in the Presence of Guanidine (200 ug/ml)

Conditions of Infection		Virus Yield[++] in PFU/ml x 10^{-5}	
Infecting Virus[+]	PFU/Plate	Zero Time	after 9 hours
M-1-2p + M-g^d-180	4.1×10^8 + 1.7×10^8	1.9	39[+++]
M-I-2p	4.1×10^8	3.9	5.4
M-I-2p	8.2×10^8	6.6	4.0
M-g^d-180	1.7×10^8	-	3.0

[+] Each virus or their mixture (in 0.5 ml of Earle solution) were added to 4 plates. After adsorption for 1 hour at 37°, unadsorbed virus was washed off, then monolayer was incubated with polio type 1-specific antiserum (diluted 1:100) for 30 min at 37°, washed two more times, and to each plate 5 ml of Earle salt solution containing 0.5% lactalbumine hydrolysate were added.

[++] After adsorption, plates were incubated for indicated time at 37°, then frozen at -70°, and after thawing fluid from duplicate plates was pooled and stored at -20° until used. Viruses were titrated on the cultures of kidney tissue of green monkeys, using guanidine-free agar overlay. The cultural fluid was diluted 10^3 and 10^4-fold, and four plates were used for each dilution, the volume of inoculum being 0.2 ml.

[+++] When titrated in the presence of guanidine, the harvest after mixed infection was about 10^8 PFU/ml.

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tween yield from mixedly and singly infected cells is even more pronounced for g^d variant grown in the absence of guanidine: the yield of g^d variant in the presence of g^s virus is about 100 times higher than that found in the absence of g^s variant. Thus, two variants seems to interact much better in the absence of guanidine than in its presence. Such a situation should be expected, for guanidine may exert an inhibitory action on some other reactions besides its effect on the synthesis of RNA-polymerase (Eggers and Tamm, 1964).

The most likely explanation of the described results is the assumption which led to the planning of the present ex-

Table 2. Reproduction of g^d variant in Cells Mixedly Infected in the Absence of Guanidine

Conditions of Infection		Virus Yield[+] in PFU/ml x 10^{-5}	
Infecting Virus	PFU/Plate	Zero Time	after 9 hours
M-I-2p + M-g^d-180	4.1×10^8 + 1.7×10^8	0.2	28[++]
M-g^d-180	1.7×10^8	0.3	0.2
M-g^d-180	3.4×10^8	0.3	0.5
M-I-2p	4.1×10^8	-	0.1

[+] Titration was performed on the kidney cell cultures of M. rhesus in the presence of 200 ug guanidine per ml of the overlay. $10^{-2.5}$ and $10^{-3.5}$ dilutions were used for inoculation of plates.

[++] When titrated in the absence of guanidine, the harvest after mixed infection was about $10^{8.7}$/plate.

For other explanations see Table 1.

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periments: the RNA-polymerase induced by a virus may use as a template the RNA of another virus. Nevertheless, some other possibilities cannot be excluded. For example, if guanidine prevents the synthesis of a precursor of RNA-polymerase rather than synthesis of the enzyme itself, the results obtained may indicate non-specific nature of that precursor. The direct interaction of two viruses on the genome level cannot be excluded either, though it is not easy to put forward a simple hypothesis based on such an assumption and compatible with the results observed. Any multiplicity phenomena can hardly be involved in the mechanism of mutual enhancement of reproduction of g^d and g^s variants, as evidenced by the results obtained with doubled infecting doses of both viruses.

When these experiments were almost completed, a report of Cords and Holland (1964) became avalable to us. These authors did not reveal the ability of a g^s variant of polio-

virus to stimulate the reproduction of a guanidine-resistant (g^r) variant in mixedly infected cells. However, the experiments of Cords and Holland differed from ours by some technical details which could be responsible for the different results: (a) g^r rather than g^d variant was used, (b) the cells were infected by the both viruses not simultaneously, (c) infectious RNA rather than mature virus was assayed.

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