*REPLICATION OF POLIOVIRUS RNA INDUCED BY HETEROLOGOUS VIRUS**

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During a previous study of interference among enteroviruses, no evidence could be obtained that the growth of one enterovirus in human cells could accelerate or support the growth of another superinfecting virus.[1] The present report demonstrates that the ribonucleic acid (RNA) of a drug-dependent poliovirus can be replicated by enzymes induced in HeLa cells through simultaneous infection with another (drug-independent) enterovirus. Similar findings have been obtained independently by Wecker and Lederhilger and will be reported elsewhere.[2]

Materials and Methods.—Cell culture: HeLa cells were routinely cultivated as monolayers in glass bottles with a medium consisting of 7–10 per cent calf serum in Hanks' balanced salt solution with 0.1 per cent yeast extract and 0.1 per cent proteose peptone no. 3 (Difco).

Viruses: Type 1 poliovirus (Mahoney), type 2 poliovirus (MEF-1), and type B_1 Coxsackie virus (Conn. 5) were all prepared as cell pools after repeated passage in HeLa cells. Guanidine-dependent type 1 poliovirus was derived by repeated passage of the virus in the presence of 10^{-2} *M* guanidine. Virus photosensitized by incorporation of proflavine was prepared by propagation of guanidine-dependent type 1 poliovirus in medium containing 2 μg/ml proflavin monohydrogen sulfate.[3] Photoinactivation of excess, noneclipsed inoculum virus was carried out by floodlamp illumination for 10 min of washed, infected monolayers 2 hr after adsorption of virus in the dark at 37°.

Assays: Mature virus was assayed by the monolayer technique described elsewhere,[4] and infectious RNA was extracted and assayed as previously described.[1] Virus-induced RNA polymerase was measured by a modification[5] of the method of Baltimore and Franklin.[6]

Results.—Table 1 shows that the infectious RNA of guanidine-dependent type 1 poliovirus does not replicate in the absence of guanidine. However, if such cells are simultaneously infected with a homologous virus (type 1 poliovirus) or heterologous virus (type 2 poliovirus or type B_1 Coxsackie virus), the dependent viral RNA is replicated to about 5–10 per cent of the titers attained by the supporting virus. These results indicate that "early enzymes" produced in doubly infected cells are able to replicate the genome of the dependent virus which cannot replicate itself without guanidine.

It has been shown elsewhere[1] that cells infected for more than 2 $^1/_2$ hr with high multiplicities of one enterovirus did not allow replication of equal multiplicities of superinfecting virus RNA. Such interference was not observed in the present study because simultaneous infection at equal multiplicities does not lead to interference.[1] Furthermore, interference against high multiplicities of superinfecting virus is not established for several hours after an original infection with low multiplicities of virus.

It is not certain how many enzymes induced by the supporting virus participated

TABLE 1

EFFECT OF ENTEROVIRUS OF HOMOLOGOUS OR HETEROLOGOUS TYPE ON THE REPLICATION OF GUANIDINE-DEPENDENT TYPE 1 POLIOVIRUS RNA IN THE ABSENCE OF GUANIDINE*

Expt. no.	Supporting virus†	Dependent virus‡	PFU of Infectious RNA Produced by Doubly Infected Cells§	
			Supporting virus RNA	Dependent virus RNA
1	None (control)	Type 1 poliovirus	—	< 10
2	Type 1 poliovirus	Type 1 poliovirus	3.2×10^4	5.1×10^3
3	Type 2 poliovirus	Type 1 poliovirus	6.2×10^4	3.1×10^3
4	Type 2 poliovirus	Type 1 poliovirus	4.8×10^4	2.2×10^3
5	Type B_1 Coxsackie	Type 1 poliovirus	6.2×10^4	1.1×10^3

* HeLa cell monolayers containing approximately 2×10^6 cells were employed for all experiments. Supporting and dependent viruses were adsorbed simultaneously for 0.5 hr at 37°, washed, and reincubated under medium without guanidine. Infectious RNA was extracted and assayed 6 hr following adsorption.

† Infection with supporting polioviruses was carried out at PFU/cell multiplicities of approximately 100, and with Coxsackie B_1 virus at a multiplicity of approximately 5. All supporting viruses were guanidine-independent.

‡ Infection with dependent virus was carried out at PFU/cell multiplicity of approximately 500–1,000. In order to keep the background infectivity at a low level, the guanidine-dependent type 1 poliovirus was photosensitized by prior incorporation of proflavin. Two hours after adsorption and incubation in the dark, excess uneclipsed inoculum was inactivated by illumination with a floodlamp.

§ Assay in the presence of 3×10^{-3} *M* guanidine suppressed replication of supporting virus RNA and allowed selective detection of dependent virus RNA. Assay in the absence of guanidine suppressed the dependent virus RNA and allowed detection of supporting virus RNA.

in the replication of the dependent virus. However, it has been shown by Baltimore *et al.*[7] that cells infected with guanidine-dependent poliovirus do not synthesize virus-induced RNA polymerase[6] in the absence of guanidine. It can be seen in Table 2 that the guanidine-dependent type 1 poliovirus employed here did not induce viral polymerase production in the absence of guanidine, although supporting type 2 poliovirus induced significant quantities of polymerase both in singly and doubly infected cells. It can reasonably be assumed that this enzyme contributed to the replication of the dependent virus RNA seen in Table 1.

Discussion.—The findings presented above indicate that enzymes produced in HeLa cells as a result of infection by one virus are able to contribute to the replication of the RNA of another superinfecting virus which (because of drug dependence) cannot replicate itself. Certainly the virus-induced RNA polymerase is one of the enzymes involved (Table 2), but there may well be others. In fact, this approach may help in determining whether there are other enzymes induced as a result of enterovirus infection.

These results prove that guanidine does not act on enterovirus RNA directly to change its configuration or charge so that it can replicate (in guanidine dependence) or cannot replicate (in guanidine sensitivity). Preliminary results indicate that supporting virus can overcome guanidine inhibition of sensitive virus as well as the

TABLE 2

LEVELS OF VIRUS-INDUCED RNA POLYMERASE ACTIVITY IN HELA CELLS INFECTED WITH GUANIDINE-DEPENDENT AND GUANIDINE-INDEPENDENT VIRUS IN THE ABSENCE OF THE DRUG

Enzyme obtained from HeLa cells infected 6 hr with:	$\mu\mu$moles P^{32} UTP incorporated/mg protein
No virus (control cells)	5
Type 2 poliovirus	66
Guanidine-dependent type 1 poliovirus	3
Type 2 poliovirus and guanidine-dependent type 1 poliovirus	59

Reaction mixture modified from Baltimore and Franklin[6] contained in 0.5 ml: 30 μmoles tris·HCl pH 8.5; 1.8 μmoles magnesium acetate; 0.4 μmoles UTP, GTP, CTP, ATP with UTP labeled in the alpha position with P^{32}; approximately 1 mg cytoplasmic protein as the source of enzyme (total cytoplasmic material which sedimented at 105,000 *g* after removal of nuclei). Incubation was carried out at 37° for 15 min.

guanidine requirement of dependent virus. This suggests that guanidine acts on virus-induced proteins by influencing their synthesis, configuration and/or function.

The accompanying paper[8] shows that supporting virus can also cause maturation of dependent virus RNA within heterologous capsid protein.

Summary.—Evidence is presented that the early enzymes induced in HeLa cells by infection with type 1 or type 2 poliovirus or Coxsackie B_1 virus can support replication of the RNA of guanidine-dependent type 1 poliovirus which superinfects the same cells.

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*MATURATION OF POLIOVIRUS RNA WITH CAPSID PROTEIN CODED BY HETEROLOGOUS ENTEROVIRUSES**

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The preceding paper[1] presents evidence which indicates that virus-induced enzymes produced in HeLa cells during replication of enteroviruses will, under certain conditions, support the replication of drug-dependent heterologous enteroviruses superinfecting the same cells. The present report demonstrates that it is possible to coat the RNA genome of guanidine-dependent type 1 poliovirus with the protein capsid of guanidine-independent type 2 poliovirus or Coxsackie B_1 virus. Wecker and Lederhilger have independently obtained similar results which will be published elsewhere.[2] The only other known occurrence of enclosure of an animal virus genome within the capsid of a heterologous virus is the apparent maturation of Rous sarcoma virus with protein encoded by helper virus superinfecting tumor cells which carry the Rous virus genome.[3]

Materials and Methods.—Cell culture methods, viruses, and virus preparation are described in the preceding paper.[1] Specific antiviral antiserum was prepared by repeated inoculation of live virus into monkeys. Antisera to the three viruses were highly specific, showing no cross-neutralization of plaque-forming ability.

Results.—Early attempts to coat the RNA of one enterovirus with the protein of another enterovirus superinfecting the same cell were unsuccessful. Cells were infected with RNA from a guanidine-resistant virus during the time when they were already synthesizing guanidine-sensitive virus and its capsid protein at a maximal