ROLE OF DISINTEGRATING AGENTS IN VIRUS REPRODUCTION

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In cells infected with influenza or vaccinia virus, factors possessing distintegrative activity against influenza virus are synthesized (or activated). An infective subviral fraction is formed, sensitive to the action of ribonuclease. In contrast, the ribonuclease-sensitive subfraction of poliomyelitis virus is formed not only by the action of homogenates of cells infected with this virus, but also under the influence of extracts of uninfected cells.

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Active synthesis of virus-deproteinizing enzymes takes place in cells infected with vaccinia virus [5-7]. After treatment of the virus with extract of cells containing deproteinizing enzymes, a subviral fraction highly sensitive to the action of desoxyribonuclease is obtained [2, 3].

In the present investigation we studied the possibility of synthesis of virus-deproteinizing enzymes induced by viruses of different sizes and chemical composition: RNA-containing viruses (poliomyelitis and influenza) and the DNA-containing vaccinia virus.

EXPERIMENTAL METHOD

Viruses. Cultural vaccinia and type 1 poliomyelitis (Mahoney strain) viruses and also allantoic strains of type A influenza virus (strains No. 32 and WSN, adapted to a culture of chick embryonic fibroblasts) and type A1 (strain No. 3711) were used.

Tissue Cultures. Primary trypsinized cultures of chick embryonic fibroblasts and kidneys were used [1].

Preparation of Homogenates Containing Disintegrating Agents. Tissue cultures were infected with vaccinia virus, type A influenza virus (strains No. 32 and WSN), and poliomyelitis virus in doses of 1-5 virus particles per cell. After contact for 1.5-2 h at 37°, the inoculum was removed, and the cell layer was washed two or three times with phosphate-buffered physiological saline (pH 7.2). The cells were removed from the slide mechanically, suspended at the rate of 10^6 cells/ml buffer, and broken up by shaking for 5 min with glass beads in a disintegrator. The cell fragments were removed by centrifugation at 800 g. To inactivate the inducing virus (or activator) of the disintegrating agents, the cell homogenate was acidified with 0.1 N HCl to pH 2.0 [4], after which the mixture was neutralized with 5% $NaHCO_3$ solution to pH 7.2-7.4 (influenza virus) or the virus was removed by centrifugation of the cell extract twice at 18,000 g in the cold or by filtration through a Seitz filter (vaccinia virus). The homogenates obtained after fragmentation of the cells were tested for absence of inducer virus by infection of chick embryos (influenza virus) or of chick fibroblasts (vaccinia virus).

EXPERIMENTAL RESULTS

The presence of virus-disintegrating factors was concluded if treatment of the intact virus with homogenates of infected cells was followed by the appearance of a subfraction sensitive to the action of ribonuclease (RNase). Such sensitivity was completely absent in the original viruses (Table 1). In addition, in more than half of the experiments the infectivity of the virus-containing material was actually increased slightly after contact with RNase, although this did not exceed 0.5-1 log of infecting doses (ID).

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TABLE 1. Resistance of Intact Influenza and Poliomyelitis Viruses to RNase

Virus used	Expt. No.	Dose of virus treated for 30 min with RNase (in log ID_{50})	
		before contact with RNase	after contact with RNase
Influenza type A1 (strain No. 3711)	1	7.5	7.5
	2	7.5	8.0
	3	8.0	8.0
	4	1.0	2.0
Influenza type A (strain No. 32)	5	8.0	8.0
	6	7.5	8.0
	7	7.5	7.5
	8	7.0	7.5
	9	6.5	6.5
	10	6.5	7.0
	11	1.5	2.5
	12	1.0	2.0
Poliomyelitis type 1 (Mahoney strain)	13	6.7	8.3
	14	6.0	6.0
	15	5.5	6.5
	16	5.5	5.5
	17	4.5	4.7
	18	3.5	3.5
	19	1.7	3.3
	20	1.7	2.5
	21	0.5	0.5

TABLE 2. Study of Possibility of Deproteinization of Influenza Virus Type A1 by Cell Homogenates of Normal Culture of Chick Embryonic Kidney (CEK) and of a Culture Infected with Type A Influenza Virus

Preparation of CEK culture (10^6 cells/ml) used for obtaining homogenate	Expt. No.	Duration of incubation of type A1 influenza virus with homogenates (in h)	Concn. of type A1 influenza virus treated with homogenates, log ID		Dose of virus induced by ribonuclease-sensitive subfraction (in log ID)
			before contact with RNase	after contact with RNase	
Not infected	1	1½	8.0	8.0	0
	2	1½	6.0	6.5	0
	3	3	5.0	5.0	0
Infected with type A influenza virus (strain No. 32; 1-5 particles/cell)	4	1	6.5	4.5	2.5
	5	1	7.7	5.7	2.0
	6	1	7.0	5.7	1.3
	7	1½	6.3	4.5	1.8
	8	1½	4.3	1.0	3.3
	9	1½	5.3	4.0	1.3
	10	1½	4.3	3.0	1.3
Infected with type A influenza virus (strain WSN; 1-5 particles/cell)	11	1½	3.5	2.0	1.5
	12	16	5.5	4.0	1.5
	13	16	7.5	6.3	1.2
	14	16	5.5	3.0	2.5
	15	16	4.3	3.3	1.0

TABLE 3. Deproteinization of Influenza Virus Type A (strain No. 32) by Homogenates of Normal Chick Embryonic Fibroblasts and of the Same Cells Infected with Vaccinia Virus

Preparation of culture of chick embryonic fibroblasts used for obtaining homogenates	Expt. No.	Concentration of virus treated with cell homogenates (log ID)		Dose of virus induced by ribonuclease-sensitive subfraction (log ID)
		before contact with RNase	after contact with RNase	
Not infected	1	6.0	6.5	0
	2	5.0	5.0	0
	3	4.5	4.5	0
	4	5.0	5.0	0
	5	5.0	5.0	0
Infected with vaccinia virus (1-5 particles/cell)	6	3.0	2.0	1.0
	7	7.7	5.7	2.0
	8	6.7	5.5	1.2
	9	4.5	2.7	1.8
	10	7.0	6.0	1.0
	11	5.0	4.0	1.0

TABLE 4. Deproteinization of Poliomyelitis Virus by Homogenates of Normal Amnion Cells and Amnion Cells Infected with Homologous Virus

Preparation of amnion cell culture used to obtain homogenate	Expt. No.	Concentration of polio virus treated with cell homogenates (log ID)		Dose of virus induced by ribonuclease-sensitive subfraction (log ID)
		before contact with RNase	after contact with RNase	
Not infected	1	8.0	6.7	1.3
	2	8.0	6.0	2.0
	3	7.5	6.5	1.0
	4	6.5	6.7	0
	5	8.0	6.2	1.8
Infected with polio virus (1-5 particles/cell)	6	7.0	7.0	0
	7	6.8	6.1	0.7
	8	6.5	6.5	0
	9	7.5	6.5	1.0
	10	6.5	5.0	1.5
	11	7.7	6.5	1.2
	12	6.5	6.0	0.5

Resistance of the original viruses to RNase was independent of the concentration of virus in contact with the enzyme and also of the complexity of its organization.

To study the effect of disintegration, one volume of poliomyelitis or influenza virus containing 10^6-10^4 ID/ml was treated with three volumes of cell homogenate obtained as described above. After incubation for 1-2 h at 37°, part of the preparation was treated for 30 min at 37° with RNase in a concentration of 10-50 μg/ml. The dose of virus in the experimental and control (without RNase) preparations was then determined by infecting developing chick embryos in the experiments with influenza virus, or amniotic cells in the experiments with poliomyelitis virus, with the preparation.

As Table 2 shows, treatment of type A1 influenza virus with extract of cells previously infected with influenza virus of another serotype (type A, strains No. 32 or WSN) led to the formation of a ribonuclease-sensitive virus fraction which accounted to between 10 and 1000 ID of the newly synthesized type A1 influenza virus. Meanwhile, if influenza virus was treated with extract of uninfected cells (Table 2, experiments 1-3), the ribonuclease-sensitive viral subfraction did not appear. Evidently the virus-disintegrating activity of the cells did not exist beforehand but appeared in them only as a result of infection.

The disintegrating action of the homogenates reached a maximum after contact with virus for 1-1.5 h (Table 2, experiments 4-11). An increase in the time of contact of virus with homogenates to 16 h did not produce more intensive disintegration (experiments 11-15). The absence of strict specificity of the disintegrative activity can be concluded because of the possibility of its induction by vaccinia virus or by influenza virus (Table 3).

Synthesis (or activation) of virus-disintegrative agents, discovered in the early stages of virus reproduction, was induced by highly organized influenza and vaccinia viruses containing a glycolipoprotein membrane as well as internal nucleoprotein.

During the study of processes of disintegration of the small poliomyelitis virus, which is a simple nucleoprotein, it was impossible to demonstrate the presence of additional synthesis (or activation) of specific disintegrating enzymes. We recorded the regular appearance of a ribonuclease-sensitive subfraction of poliomyelitis virus after treatment with both normal and preliminarily infected homogenates of amnion cells (Table 4).

This took the form of a decrease by 10-100 times in infectivity of the virus treated with cell homogenates after contact of the virus with RNase. The results are in complete agrement with Schäfer's conclusion [8] that the small and chemically simple virus of encephalomyocarditis of mice does not require the synthesis of new enzymes in the cells for its deproteinization.

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