CHANGES IN MITOSIS IN RES (CLONE 1) INFECTED WITH SENDAI VIRUS

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Sendai virus causes characteristic quantitative and qualitative changes in mitosis in RES (clone 1) cultures. In the early periods of infection, a marked increase of mitotic activity is observed in the infected cultures. This coincides in time with intranuclear synthesis of virus-specific RNAs. A wave of abnormal mitoses appears somewhat later. The spindle of division and centrioles are damaged. Cells with micronuclei appear.

Investigations of pathological mitoses in cell cultures infected by different viruses have recently been published [1-8]. Some of these investigators found that qualitative disturbances in mitosis are one of the early manifestations of the cytopathic action of viruses.

This paper describes changes in the mitotic cycle in RES (clone 1) cultures infected with Sendai virus.

EXPERIMENTAL METHOD

The test objects were cultures of a transplantable line of RES hog embryonic kidney cells (clone 1), grown on medium No. 199 with 10% bovine serum in penicillin flasks with cover slips. Cultures aged 2-3 days were used. After 1-48 h, specimens were fixed in Carnoy's mixture and stained with hematoxylin and eosin. The liquid fraction of the cultures was discarded, and the cells were washed with Hanks' solution and then removed with versene solution, ground in a mortar, and centrifuged to obtain a supernatant (cell fraction). The liquid and cell fractions were used for titration of the virus.

Allantoic cultures of Sendai virus, strain No. 960, obtained by infecting 10-11-day chick embryos, were used. The embryos were examined after incubation for 3 days at 37°. The infective titer of the virus was determined by infecting 10-11-day chick embryos with successive tenfold dilutions of virus. The hemagglutination test was performed with a 1% suspension of chicken erythrocytes.

TABLE 1. Mitotic Activity of RES (Clone 1) Cultures Infected with Sendai Virus (M±m)

Time after infection (in h)	Mitotic activity in ‰		P	Mitotic activity in ‰		P
	infecting dose 800 EDI_{50}/cell	control		infecting dose 800 EDI_{50}/cell	control	
0,5	23,0±2,1	24,6±1,8	>0,1	22,0±1,6	24,6±1,8	>0,1
2	62,6±1,5	24,8±1,1	<0,001	49,2±1,0	24,8±1,1	<0,001
4	52,0±0,5	24,4±0,8	<0,001	45,8±2,2	24,4±0,8	<0,001
8	50,0±3,3	21,4±0,7	<0,001	37,6±1,4	21,4±0,7	<0,001
10	28,6±0,7	27,6±1,2	>0,1	35,4±1,3	27,8±1,2	<0,001
22	6,6±1,0	28,4±1,3	<0,001	31,2±0,8	28,4±1,3	<0,001
24	0,6	22,3±0,8	<0,001	1,0	22,3±0,8	<0,001

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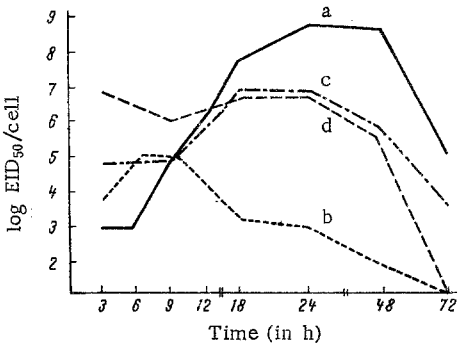

Fig. 1

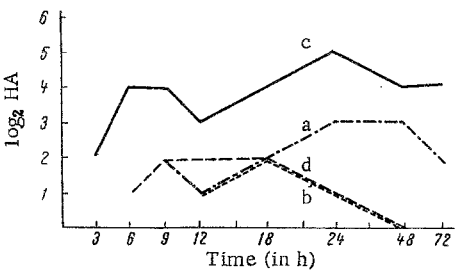

Fig. 2

Fig. 1. Infective titers of Sendai virus in RES (clone 1) culture after infection with doses of 80 (a, b) and 800 (c, d) EID_{50}/cell. Here and in Fig. 2: a, c) liquid fraction of cultures; b, d) cell fraction.

Fig. 2. Accumulation of HA in RES (clone 1) cell culture after infection with Sendai virus in doses of 80 (a, b) and 800 (c, d) EID_{50}/cell.

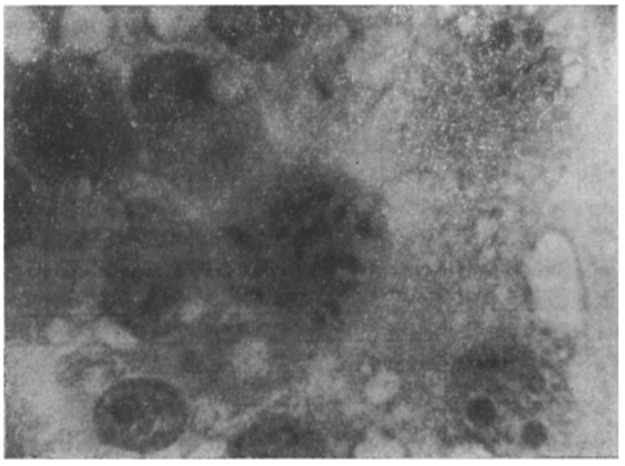

Fig. 3. Formation of cell with micronuclei in RES (clone 1) cultures infected with Sendai virus. Chromosomes which have not gone through the uncoiling stage are visible; 6 h after introduction of virus. Hematoxylin-eosin, 600×.

EXPERIMENTAL METHOD

The dynamics of accumulation of infective virus in the RES (clone 1) cell culture is illustrated in Fig. 1. Virus accumulation in the liquid fraction of these cultures reached a maximum in a dose of 80 EID_{50}/cell. A lower titer of infective virus was obtained with a multiplicity of 800 EID_{50}/cell. Accumulation of hemagglutinins (HA) in the liquid and cell fractions was directly proportional to the infecting dose (Fig. 2). The HA titer in the liquid fraction increased parallel to that in the cell fraction, but exceeded it at all times of observation. Neither infective activity nor HA could be detected in the liquid and cell fractions of control cultures.

Cytopathic changes in the infected RES (clone 1) cultures were expressed as syncytium-formation and destruction of the cell layer. Starting from 3-4 h after infection with a multiplicity of 800 EID_{50}/cell and from 8-10 h after infection with 80 EID_{50}/cell, syncytia appeared in the cultures. In the early stages they usually contained 3-8 nuclei in a common cytoplasm. In later stages (18-24 h) large syncytia were formed, containing dozens of nuclei. Between 12 and 48 h after infection, degeneration of the cell took place. Degenerating mononuclear cells and syncytia fell from the surface of the cover slip. In the early stages of infection (until 12 h), large numbers of cells containing numerous micronuclei appeared in the infected cultures.

Changes in the mitotic activity of RES (clone 1) cultures infected with different doses of virus are shown in Table 1. A sharp increase in mitotic activity was observed in cultures infected with a high dose of virus (800 EID_{50}/cell) between 2 and 8 h after infection. By 10 h, mitotic activity had decreased approximately to the control level. By the end of the first day, marked inhibition of mitotic activity was present. Similar changes in mitotic activity were observed in cultures infected with a dose of 80 EID_{50}/cell.

The number of pathological mitoses in the infected cultures depended on the infecting dose (Table 2). They reached a maximum 6 h after introduction of the virus.

The predominant forms of pathological divisions were pathological metaphases (up to 90% of the total number of abnormalities of mitosis). Colchicine-like metaphases were seen, showing swelling, agglutination,

TABLE 2. Percentage of Pathological Mitoses among Dividing Cells of RES (Clone 1) Cultures Infected with Sendai Virus

Time after infection (in h)	Percent of pathological mitoses		
	Infecting dose		Control
	800 EID_{50}/cell	80 EID_{50}/cell	
2	21	13	4
4	35	13	8
6	54	22	6
8	30	16	9
10	27	18	7

or pulverization of the chromosomes, metaphases with single or multiple residual chromosomes, groups-of-3 metaphases, and multipolar metaphases with swollen chromosomes. These pictures were frequently combined with the appearance of multiple micronuclei. In some cases, chromosomes which had not gone through the uncoiling process were clearly seen in the micronuclei (Fig. 3).

Previous investigations have shown that qualitatively different changes in the mitotic cycle take place in cell cultures infected with various viruses. For example, in DKLCh-4 and PS cultures, infected with herpes simplex virus, colchicine-like metaphases appear, and division is delayed in metaphase [1, 7]. In cultures infected with human adenoviruses, the predominant forms of pathology of mitosis were multigroup and multipolar metaphases [4]. Deletion of single chromosomes in metaphase was characteristic of cultures infected with viruses of the smallpox group [2, 6].

The data described in this paper show that characteristic quantitative and qualitative changes in mitosis arise in cultures infected with Sendai virus. A marked increase in mitotic activity was observed 2 h after infection. It coincided in time with intranuclear synthesis of virus-specific RNAs. A little later (after 4-6 h) a wave of pathological mitoses was observed. Severe damage was done to the chromosomes, in agreement with published data [8]. In addition, the spindle of division and centrioles were damaged. Probably in most cases the changes in mitosis which were observed were lethal: evidence of this was given by the pictures of degeneration of the cells dividing by pathological mitoses, degeneration of cells with micronuclei, and disappearance of those cells 12-14 h after infection. Evidently the pathological mitoses now observed in RES (clone 1) cultures infected with Sendai virus are one of the early manifestations of the cytopathic action of this virus on the chosen cell system.

LITERATURE CTIED

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