# Virus-Induced Chromosome Pulverization in Human Diploid Cells[1] (34892)

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In 1964, Nichols *et al.* (1) reported observing chromosome pulverization in cultured human cells infected with measles virus; in the same year, Benyesh-Melnick *et al.* (2) reported a similar phenomenon in human fibroblasts infected with herpes zoster, which they described as chromosome fragmentation. Subsequently, Cantell *et al.* (3) found that pulverization could be induced with Sendai, mumps, and Newcastle disease viruses but not with influenza viruses. Other viruses which may produce pulverization or similar effects include herpes simplex (4), hog cholera virus (5), adenovirus type 12 (6), and adenoviruses types 4 and 18 (7).

It has been shown that this abnormality also occurs spontaneously in a number of permanent or established mammalian cell lines (8, 9) and in hamster tumors induced by adenovirus type 12 (10). We have also observed this abnormality occurring spontaneously in direct preparations of human cancer cells in studies which have not been reported as yet. In the permanent mammalian cell lines, it has also been shown that the pulverized material replicates late or exhibits DNA synthesis which is out of phase with the normal chromosomes (8, 9).

One suggested explanation (11) is that pulverization might result from cell fusion and the formation of syncytia, since this abnormality often appears in polyploid cells and since one of the effects of measles virus is syncytia formation. Consistent with this view, other viruses which may produce pulverization also induce cell fusion (Sendai, h. simplex, mumps, and Newcastle disease). Furthermore, as shown recently by Kato and Sandberg (12), with Sendai-induced pulverization, increases in the incidence of chromosome pulverization parallel increases in polyploidy.

The present report describes virus-induced pulverization in human diploid cells. As with spontaneously occurring pulverization, the virus-induced effect in our experiments exhibited out-of-phase DNA synthesis, and the pulverized chromosomes were asynchronous with the normal chromosomes. Cells in the S and in the G1 or G2 phases of nuclear replication were equally susceptible to this viral effect.

*Materials and Methods.* Only human diploid strains in tissue culture were employed in order to avoid ambiguities arising from the use of permanent cell lines, which may display spontaneous pulverization of chromosomes (8, 9). Phytohemagglutinin-stimulated peripheral leukocytes (PL) from the blood of various laboratory donors were cultured for 3 days. (The blood of a 6-month-old infant secured prior to measles vaccination was used until it was observed that nonimmune blood was not necessary to achieve positive results.) The sources of fibroblasts employed were human adult skin (HAS), isolated from a benign surgical specimen, and human embryonic lung cells (HEL), obtained from Flow Laboratories.

The Edmonston strain of measles virus from vaccine (Eli Lilly, Inc.) was propagated in HEL cells to titers of $10^5$–$10^6$ $TCID_{50}$/ml.

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Sendai virus, a gift of Dr. W. F. Shearer, Cornell Medical College, had a titer of $8 \times 10^3$ hemagglutination units (HAU)/ml. Mrs. A. Bailey of the Utah State Department of Health kindly furnished a strain of h. simplex virus (HSV) type 1; she also provided rubella virus which had been growing in a permanent line of monkey kidney cells without cytopathic effect; media subjected to two cycles of freezing and thawing were passed from the monkey kidney cells to HEL, where the virus was allowed to propagate, with almost no cytopathic effect. Strains of HSV type 1 and HSV type 2 were gifts of Dr. W. E. Rawls, Baylor University College of Medicine, and were grown in HEL to titers of $10^7$–$10^8$ $TCID_{50}$/ml. Adenovirus type 12 and h. zoster virus (HZV) were kindly supplied by Dr. F. Rapp, Baylor University College of Medicine. HZV was propagated in HAS and in HEL by passage of dispersed infected cells to uninfected cell cultures (13).

The methods of chromosome analysis in conjunction with autoradiography used in these experiments were described previously (9). The only deviation in the present experiments was that virus preparations were added to the cultures at various times before harvesting cells. Exposure times are shown in Table I. Control preparations were employed in each experiment.

Relative susceptibility of cell nuclei in different phases of the cell cycle was investigated by means of two kinds of autoradiographic experiments. The first was performed to determine if the pulverized chromosomes replicate late. Cell cultures were exposed to

TABLE I. Virus-Induced Chromosome Pulverization in Human Diploid Cells.

| Virus | Titer | Exposure (hr) | Cell culture | Total no. of cells | Cells with pulverization No. | Cells with pulverization % |
|---|---|---|---|---|---|---|
| HZV | | 24 | HAS | 150 | 3 | 2 |
| Control | | | HAS | 150 | 0 | 0 |
| Sendai | $8 \times 10^3$ HAU/ml | 4 | PL | 500 | 4 | 0.8 |
| Control | | | PL | 500 | 0 | 0 |
| Sendai | $8 \times 10^3$ HAU/ml | 1.5 | HEL | 250 | 7 | 2.8 |
| Control | | | HEL | 250 | 0 | 0 |
| Sendai | $8 \times 10^3$ HAU/ml | 0.5 | HEL | 600 | 42 | 7 |
| Measles | $10^{6.3}$ $TCID_{50}$/ml | 4 | PL | 565 | 17 | 3.2 |
| Control | | | PL | 782 | 0 | 0 |
| Measles | $10^5$ $TCID_{50}$/ml | 72 | HEL | 300 | 0 | 0 |
| Control | | | HEL | 300 | 0 | 0 |
| Measles | $10^5$ $TCID_{50}$/ml | 0.5 | HEL | 600 | 5 | 0.83 |
| HSV-1[a] | $10^8$ $TCID_{50}$/ml | 4 | PL | 987 | 1 | 0.1 |
| Control | | | PL | 829 | 1 | 0.1 |
| HSV-1[b] | $10^8$ $TCID_{50}$/ml | 4 | HEL | 700 | 0 | 0 |
| HSV-1[a] | $10^7$ $TCID_{50}$/ml | 0.5 | HEL | 600 | 0 | 0 |
| HSV-2 | $10^{7.67}$ $TCID_{50}$/ml | 4 | HEL | 898 | 4 | 0.44 |
| Control | | | HEL | 1,200 | 0 | 0 |
| Adeno-12 | $10^6$ $TCID_{50}$/ml | 24 | HEL | 225 | 0 | 0 |
| Control | | | HEL | 225 | 0 | 0 |
| Rubella | | chronic infect. | HEL | 300 | 0 | 0 |
| Control | | | HEL | 300 | 0 | 0 |

[a] Strain of HSV type 1 from the Utah State Department of Health.
[b] Strain of HSV type 1 from Baylor University College of Medicine.

either Sendai or HZV for periods ranging from 0.5 to 24 hr. The tritiated thymidine (6.7 Ci/-mmole) was added to the cultures at a final concentration of 0.5 μCi/ml at times ranging from 15 min to 2 hr before the cells were harvested for chromosome analysis.

The second group of experiments was performed to determine if the cells which exhibited virus-induced chromosome pulverization were in the S phase of the cell cycle at the time the virus was added to the culture. Leukocyte or HEL cultures were incubated with tritiated thymidine (0.5 μCi/ml) for 15 min; cultures were then washed in media once. In two experiments, fresh media containing the virus suspension (Sendai or measles) and cold thymidine (3.6 μg/ml) were added to the leukocyte cultures, which were then incubated for 4 hr. In a third experiment, fresh media containing Sendai virus but without cold thymidine were added to HEL cultures, which were then incubated for ½ hr.

*Results.* The incidence of chromosome pulverization is summarized in Table I. The measles and Sendai viruses induced pulverization in the chromosomes of the leukocytes and HEL fibroblasts (HAS and HEL). HSV produced pulverization in the leukocytes as well. Table I also shows the absence of pulverization in the control preparations.

In general, the shorter the exposure to virus, the higher the incidence of pulverization. For example, when HEL cultures were exposed to measles for 3 days, pulverization was not observed, whereas a 30-min exposure yielded positive results. Likewise, when cultures were treated with Sendai virus for 0.5, 1.5, and 4 hr, there was an inverse correlation between incidence of chromosome pulverization and length of exposure to the virus. Similar findings with Sendai-treated hamster cells were reported by Kato and Sandberg (12).

Pulverization was not observed in HEL cells chronically infected with rubella virus, although other chromosome abnormalities were observed. Similarly, although HSV type 1 produced other chromosome abnormalities, pulverization was extremely rare, even when the exposure time to the virus was reduced to 30 min. Neither pulverization nor any other chromosome abnormality was observed in HEL cells treated with adenovirus type 12. (Adenovirus does not grow well in HEL cells or in similar human fibroblast lines.)

Morphologically, virus-induced pulverization and spontaneous pulverization were similar. The abnormality was almost always observed in polyploid cells in which one diploid set of chromosomes appeared to be pulverized while the other diploid set remained intact (Fig. 1). Only rarely could the effect be detected in diploid cells (Fig. 2).

The data for the first group of experiments employing labeling with tritiated thymidine showed that the normal and pulverized chromosomes were asynchronous with one another; the pulverized chromosomes replicated

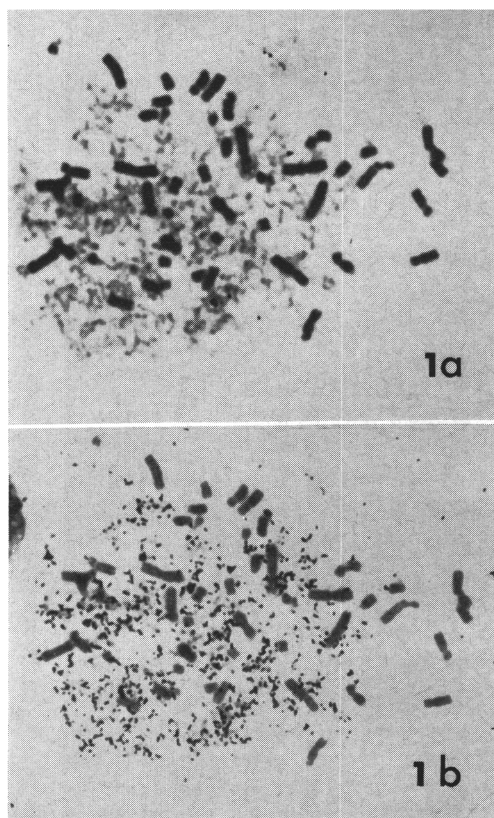


FIG. 1. H. zoster in HAS cells. (a) Polyploid cell at metaphase, and (b) autoradiograph showing pulverization. There are approximately 46 intact chromosomes. Only the pulverized material appears labeled. (1- and ¾-hr terminal label).

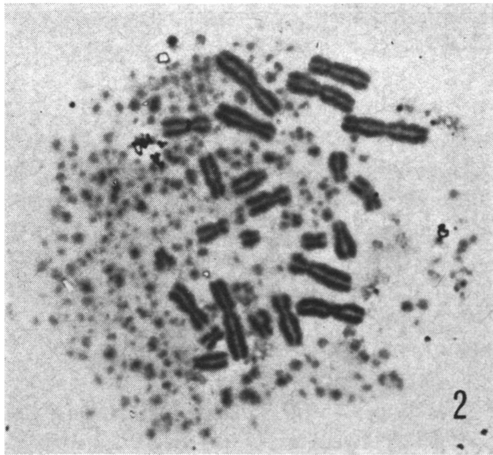

FIG. 2. Sendai virus in HEL cells. There are approximately 24 intact chromosomes. The general configuration of the mitotic figure suggests an unbroken cell and, judging from the total amount of chromatin, the cell is probably diploid.

late. Pulverization induced in the fibroblast or leukocyte cultures by either Sendai virus or HZV exhibited late labeling (Table II, Fig. 1). Since the normal chromosomes were not labeled (having completed DNA synthesis), the pulverized chromosomes incorporated the label in what was the G2 phase for the intact chromosomes. The incidence of labeled pulverized chromatin ranged from about 7% for the Sendai-treated leukocyte cultures to about 32% for the HZV-treated fibroblast cultures (Table II). No normal chromosomes were labeled.

In the second group of autoradiographic experiments, when leukocyte or HEL cultures were pulsed with tritiated thymidine and subsequently exposed to either Sendai or measles, the pooled data showed that most of the cells exhibiting pulverization were completely unlabeled, and thus their nuclei were not in the S phase of the cell cycle at the time the virus was added to the culture (Table III).

*Discussion.* Our observations confirm reports by others that measles (1, 3), Sendai (3, 12, 15), and h. zoster (2) viruses can produce chromosome pulverization. All of the experiments reported here employed benign tissue culture strains and short-term leukocyte cultures, thus eliminating any ambiguity arising from observations of spontaneous chromosome pulverization in permanent mammalian cell lines (8, 9, 14).

Virus-induced pulverization appears to be similar to that occurring spontaneously, not only in morphologic appearance, but because DNA synthesis is out of phase with that of the unaffected chromosomes. Similar results have been reported by other investigators (15, 16).

As pointed out previously (11, 12), the chromosome pulverization induced by measles and Sendai viruses appears to be closely associated with cell fusion, or syncytia formation. Since pulverization induced by measles, Sendai, HZV, and HSV type 2 in our experiments seemed to involve polyploid cells in which one set of chromosomes remained intact and one set was pulverized, our evidence supports the theory that pulverization is secondary to cell fusion. This is further demonstrated by the autoradiographic data showing that the normal and pulverized chromosomes are asynchronous with one another, that is, the pulverized chromosomes

TABLE II. Virus-Induced Chromosome Pulverization: Late Labeling with Tritiated Thymidine.

| Virus | Duration of exposures: Virus (hr) | Duration of exposures: $^3$H TdR[a] (hr) | Cell culture | Total no. pulverized cells scored | Pulverized cells with label: No. | Pulverized cells with label: % |
|---|---|---|---|---|---|---|
| Sendai | 4 | 2 | PL | 29 | 2 | 7 |
| Sendai | 1.5 | 1.25 | HEL | 20 | 4 | 20 |
| Sendai | 0.5 | 0.25 | HEL | 51 | 11 | 21 |
| HZV | 24 | 1.75 | HAS | 25 | 8 | 32 |

[a] 6.7 Ci/mmole at a final concentration of 0.5 $\mu$Ci/ml.

TABLE III. Virus-Induced Chromosome Pulverization: Labeling of Chromosomes Previously Pulse-Chased with Tritiated Thymidine.

| Virus | Total no. of cells | No. with $^3$H TdR label | | | |
|---|---|---|---|---|---|
| | | Pulverized | Normal | Both | None |
| Sendai[a] | 34 | 1 | 8 | 0 | 25 |
| Measles[a] | 15 | 6 | 1 | 1 | 7 |
| Sendai[b] | 54 | 15 | 0 | 0 | 39 |
| Totals | 103 | 22 | 9 | 1 | 71 |

[a] These experiments employed human peripheral leukocyte cultures which were exposed to virus for 4 hr.

[b] Human embryonic lung cells were treated with Sendai virus for 30 min.

replicate late (8, 9, 15, 16). Such chromosome asynchrony could result from a fusion of cells containing asynchronous nuclei. It is interesting to note, however, that small amounts of pulverization are occasionally observed in metaphase cells of virus-infected cultures (14). Similar observations are depicted in a report by MacKinnon *et al.* (6), who employed adenovirus type 12 to infect hamster cell cultures. Spontaneous pulverization of small numbers of chromosomes (including the long arm of chromosome No. 1) has also been observed in permanent human leukocyte cell lines (8, 9). Since in these instances only one or a few chromosomes are affected, it is more difficult to postulate cell fusion as a mediator of this type of pulverization.

Since most pulverizations did not occur in cells in the S phase (unlabeled) of the cell cycle (Table III), it can be concluded that cells in G2 or perhaps G1 are equally susceptible to virus-induced pulverization. This conclusion is in agreement with the findings of Kato and Sandberg (16) but not with those of Nichols *et al.* (15) and Stenman and Saksela (17), who performed similar experiments but concluded that the pulverized chromosomes represented S-phase nuclei. However, these reports employed established cell lines such as HeLa and Don, while the experiments reported here utilized human diploid cell strains.

Two problems remain unsolved. One is the question as to whether the pulverized chromosomes are, in fact, broken or damaged, since it has been argued that they are incompletely condensed chromosomes (8) or simply represent S-phase nuclei (15). As reported previously (9), the chromosomes affected appear to be fragmented, and this evidence is precisely the same as that in other experimental situations when breaks occurring in an apparently less extreme form are designated isochromatid breaks. Electron microscopy is one possible method of resolving this problem. If the fragments apparent with the light microscope are attached by extremely attenuated segments, it may be possible to describe and identify such segments by means of electron microscopic examination. In collaboration with Dr. C. G. Jensen at the University of Utah, partially pulverized cells have been examined with the electron microscope. Thus far, we have not been able to identify connections between the apparent fragments; however, since the technique has some limitations, we are unable to conclude with complete assurance that connections do not exist.

The second problem which remains unsolved is the mechanism of chromosome pulverization. Viral lysosome labilization appears to have been ruled out by the recent experiments of Aula and Nichols (18), which show no relation between lysosomal labilization and pulverization or chromosome breakage. Since the hemolytic fraction of measles virus is also able to produce pulverization (19), it seems doubtful that a viral-coded DNase could be responsible for the effect.

In the light of the cytogenetic and autoradiographic evidence suggesting that pulverization results from fusion of cells with nuclei

in different stages of replication, we offer the following hypothesis: After the fusion of cells with asynchronous nuclei, the nucleus which is farther along in the G2 phase of the cycle (that is, closer to mitosis) will enter mitosis first. A specific substance producing chromosome condensation may be diffusible within the boundaries of the plasma membrane; this substance will cause chromosome condensation not only in the mitotic nucleus but also in the nucleus which is in interphase. The chromosomes of the interphase nucleus may be susceptible to damage because they are engaged in DNA or RNA synthesis and, as a result of the forced condensation, these chromosomes become fragmented or pulverized. In instances in which only a small number or even only a part of one chromosome is affected, there may be a mechanism which induces delay of synthesis in these chromosomes or segments. Such segments would then also be susceptible to damage from premature condensation.

*Summary*. Human diploid cells (peripheral leukocytes and fibroblasts) infected with measles, Sendai, h. zoster, and h. simplex type 2 viruses showed chromosome pulverization, usually in polyploid cells. Autoradiography indicated that the pulverized chromatin replicated later than the normal chromosomes, and that susceptible nuclei may be in the S, G2 (or G1) phase of cell replication. It is hypothesized that pulverization may result from premature chromosome condensation induced by a diffusible substance.

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