Exogenous Deoxyribonucleoside Triphosphates: Their Effect on Pulverized Human Chromosomes at the G_2 Phase (34015)

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(Introduced by N. Gengozian)
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Nichols *et al.* (1) used the term "pulverization" for the extreme fragmentation of chromosomes seen in association with measles virus. This phenomenon was seen in cells from patients who had recently been vaccinated (2) and in cultured cells treated with several types of virus (3–6). The mechanism that produces pulverization in chromosomes is unknown; it is thought to be different from that responsible for a single chromosomal break (6).

In our studies of radiation-induced chromosomal breakages (7, 8), several observations suggested that there might be many mechanisms by which human chromosomal breakages have been produced. The present experiments tested the assumption that one of the mechanisms was a defect in the DNA synthesis.

Materials and Methods. Ninety ml of blood were drawn from patient F (7, 8) on November 7, 1966, 1 week after he had received an injection of polyvalent formaldehyde-killed influenza virus vaccine. Eight 40-ml leukocyte cultures were made from this patient and two 20-ml cultures were prepared from the blood of a normal donor; we used the standard techniques for this laboratory (7). The same bottle of nutrient medium was used in preparing all the cultures to preclude the possibility of variations in the concentration of the ingredients in the medium. Sixty-eight hr after incubation at 37°, each of the eight 40-ml cultures was divided into two 20-ml cultures. To four of these cultures, four deoxyribonucleotides[1] were added singly; and to 11 cultures, the four compounds were added in different combinations (see Table I). The final concentration of each compound in the culture medium was $1 \times 10^{-5}M$. Nothing was added to the patient's sixteenth culture. All the cultures were then coded and incubated for another 4 hr before the cells were prepared for cytogenetic studies (7). The G_2 phase of human lymphocytes is believed to be about 4–6 hr (9).

The slides were examined microscopically at 970×. The nonpulverized metaphases were karyotyped in detail and chromosomal breakages and abnormal chromosomes were recorded. When possible, 100 metaphases were analyzed per culture with the use of at least two slides from each culture.

The mitotic rate was defined as mitosis per 100 interphases. It was determined from analyzing at 450× magnification at least 3000 nuclei from two slides. The fields for analyzing the frequency of metaphases and interphases for computing the mitotic rate were selectively chosen from the microscopic stage. These fields were used in all slides for the analysis of the mitotic rate.

Results. The metaphases of the normal person showed a normal chromosomal pattern. There were no pulverized chromosomes, chromosomal breakages (X_1), chromosomal fragments, or abnormal chromosomes in 100 metaphases from each of his two cultures. The mitotic rate was 1.4%.

Table I shows the analyses of the patient's control culture and the 15 experimental cultures into which the deoxyribonucleotides had been added during the G_2 period.

In the patient's control culture, 71 of the 100 metaphases were pulverized (Fig. 1). In the 29 nonpulverized metaphases, 5 had chro-

[1] Deoxyadenosine 5′-triphosphate (sodium) = dATP; deoxyguanosine 5′-triphosphate (sodium) = dGTP; deoxycytidine 5′-triphosphate (sodium) = dCTP; Thymidine-5 triphosphate (trisodium) = TTP; These chemicals were obtained from Nutritional Biochemicals Corporation, Cleveland, Ohio. The final concentration in the culture medium was 1×10^{-5} *M*.

TABLE I. Cytogenetic Analyses of the Control and Deoxyribonucleotides Added at G_2 Cultures.

Culture	Compounds[a]	Total metaphases	2N[b] (%)	Pulverized metaphases (%)	X_1 cells[c] (%)	Other abnormal cells[d] (%)	Smaller G[e] (%)	Mitotic rate[f]
1	dATP	100	93	0	7	10	5	1.68
2	dGTP	100	89	2	15	12	5	1.63
3	dCTP	100	85	0	12	9	4	2.02
4	TTP	100	85	0	12	13	5	2.27
5	dATP + dGTP	100	84	0	10	10	6	1.80
6	dGTP + dCTP	100	82	0	8	19	7	1.75
7	dCTP + TTP	65[g]	88	0	7.7	12.3	3.1	2.19
8	dGTP + TTP	100	85	0	8	14	2	2.68
9	dATP + dCTP	100	79	0	9	13	3	1.60
10	dATP + TTP	100	80	0	8	7	1	1.40
11	dATP + dGTP + dCTP	100	86	0	8	11	4	1.67
12	dATP + dGTP + TTP	100	90	0	15	10	4	1.94
13	dATP + dCTP + TTP	100	88	0	10	15	5	1.69
14	dGTP + dCTP + TTP	100	68	4	15	18	2	1.62
15	dATP + dGTP + dCTP + TTP	86[g]	81	1	10	10	5.8	1.42
		1451	87	0.5	10.4	12.3	4.1	1.82
16	Control	100	?	71	5	2	?	1.17

[a] Deoxyadenosine 5′-triphosphate (sodium) = dATP; deoxyguanosine 5′-triphosphate (sodium) = dGTP; deoxycytidine 5′-triphosphate (sodium) = dCTP; thymidine-5 triphosphate (trisodium) = TTP.

[b] Percentage of metaphases having 46 chromosomes (normal diploid).

[c] Percentage of metaphases with direct chromosomal breakages or acentric fragments.

[d] Percentage of metaphases having abnormal chromosomes (translocations, dicentrics, rings, deletions).

[e] Percentage of metaphases having a deleted long arm of a G (Gq—) chromosome.

[f] Mitotic figures per 100 interphase nuclei.

[g] All the available well-spread metaphases.

mosomal breakages and two had abnormal chromosomes. The frequency of the diploid metaphases or metaphases having a smaller G (Gq—) chromosome resulting from a deletion of part of its long arm (10), could not be determined in this culture. The mitotic rate was 1.17%.

There were only seven (0.5%) pulverized metaphases in three of the experimental cultures. Although the pulverized metaphases were greatly reduced in the experimental cultures, as shown in Table I, the frequency of other chromosomal abnormalities was about the same as the patient's control. Metaphases with a terminal chromosomal breakage or an acentric fragment were seen in 10.4% (range 7–15%) and methaphases with translocations, dicentrics, rings, or deleted chromosomes were seen in 12.3% (range 7–19%). Four percent (range 1–7%) of the 1451 metaphases in the experimental cultures had a Gq— chromosome, morphologically resembling the Ph^1 chromosome of chronic myelocytic leukemia (CML) (11). The average mitotic rate in these experimental cultures was 1.82% (range 1.4–2.68%).

Comments. Morphologically, chromosomal breakages are seen either as a nonstainable segment of chromosome (both sister chromatids at metaphase) with a dislocation of the distal segment or as an acentric fragment (Fig. 2). The technique used for somatic chromosomal studies at present includes (a) arrest of mitotic cells at metaphases with chemicals; (b) adding hypotonic solution to swell the cells; and (c) adding several fixatives. During each of these procedures, centrifugations are necessary. Possibly these procedures could produce morphological chromosomal breakages, if there is weakness

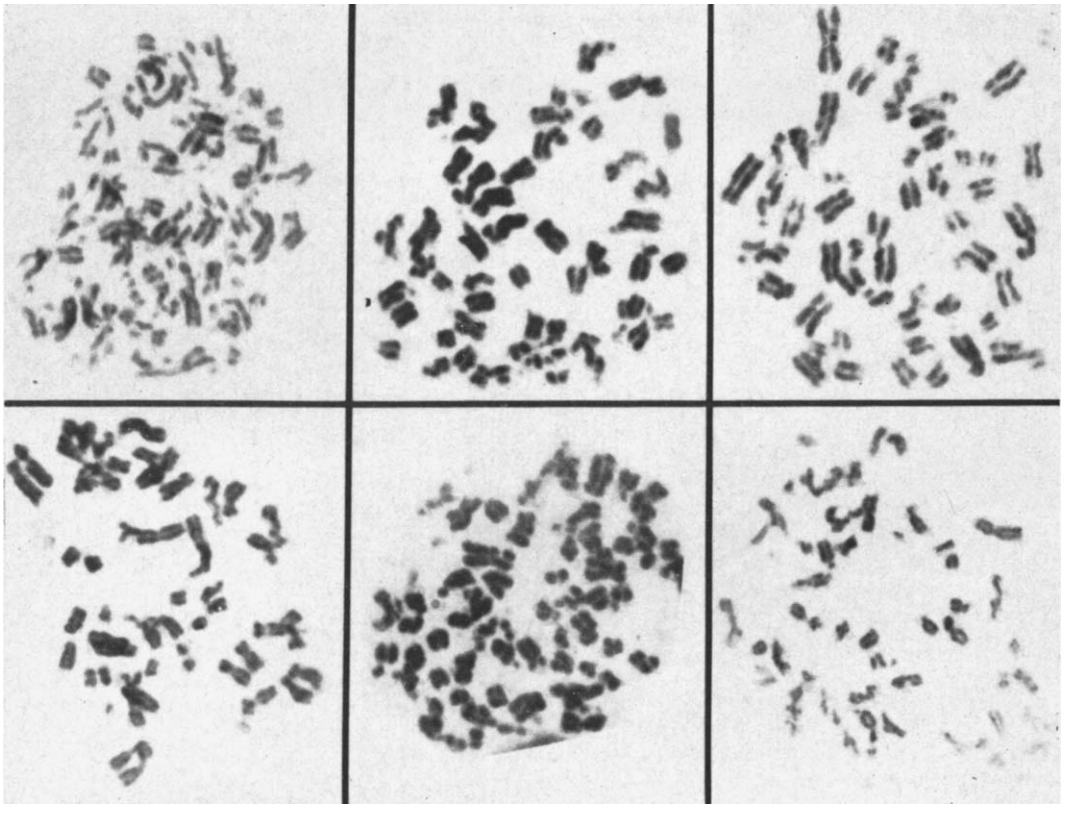

FIG. 1. Six metaphases from the patient's control culture showing partial pulverization of the chromosomes.

of the chromosomal backbone, which chemically consists mainly of DNA, RNA, protein, and histone.

Ahnström and Natarajan (12) proposed that the chromosomal damage may be caused by an enzyme, DNA polymerase, which in time and space coincides with the duplication of chromosome material and that this enzyme actively removes nucleotides from the already formed chromosomal DNA. This removal produces a weakness of the chromosomal backbone and results in breakages.

The chemical equation for the DNA polymerization is as follows:

$$\begin{matrix} \text{dATP} \\ \text{dGTP} \\ \text{dCTP} \\ \text{TTP} \end{matrix} \xrightarrow[\text{DNA polymerase}]{\text{primer DNA}} \text{DNA polymer} + \text{pyrophosphate}$$

Recently Natarajan and Ahnström (13) showed that chromosomal breakages and despiralizations could be produced by adding exogenous inorganic pyrophosphate (PP) under anaerobic conditions. The additional PP probably upset the normal chemical equilibrium of the equation and forced the reaction toward the left.

In the present experiments, pulverized metaphases were greatly reduced in the cultures where exogenous deoxyribonucleoside triphosphates were added at the G_2 phase. It is not yet possible to say how these compounds are transported through the cell membrane, whether as nucleotides or nucleosides. However, the addition of these substrates at this point apparently forced the chemical reaction toward the DNA polymers. Hence we can prevent the removal of the already formed chromosomal DNA.

The addition of exogenous deoxyribonucleotides appears either to prevent the pulverization of the chromosomes or to heal the pulverized chromosomes rather than to inhib-

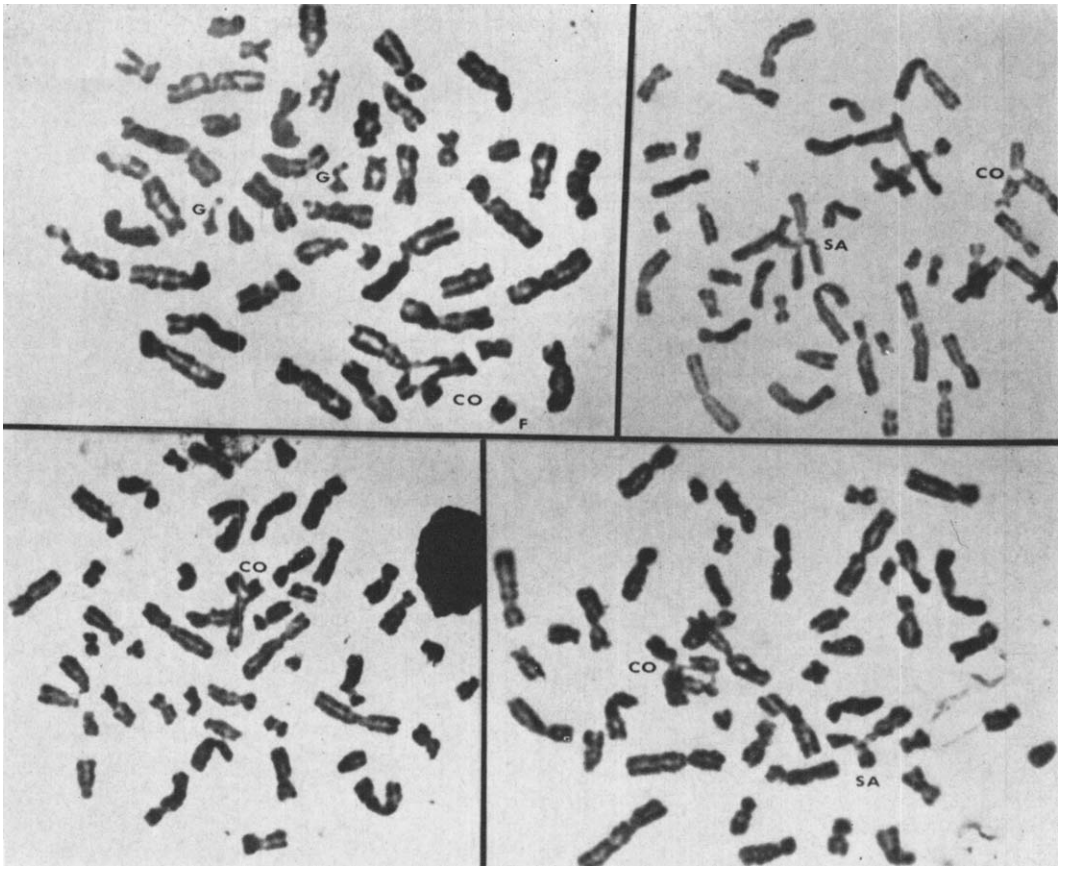

FIG. 2. Four metaphases from the experimental cultures where dGTP + dCTP + TTP were added during the G_2 period: CO = crossover; SA = satellite association; and F = acentric fragment.

it cells from undergoing further process in preparing for mitosis. This conclusion is supported by the finding that the mitotic rate was not suppressed in the experimental cultures. This indirect evidence also suggests that the exogenous compounds did not destroy the pulverized chromosomes already present.

The finding of frequent crossed-over chromosomes (Fig. 2) and other chromsomal abnormalities in the experimental cultures where the exogenous substrates were added at the G_2 phase favors the possibility that these substrates help in healing the pulverized chromosomes. If this interpretation is correct, it would indicate that the healing of the pulverized chromosomes takes place in the G_2 phase and could also account for the autoradiographic labeling of the pulverized chromosomes found by Kato and Sandberg (5) and Nichols *et al.* (6).

It is not possible to explain the variation in the frequency of the different types of abnormal chromosomes in different experimental cultures. However, the lowest frequency of Gq— was in the culture to which exogenous dATP + TTP had been added and the highest frequency was in the culture to which exogenous dGTP + dCTP had been added. Possibly the dATP and TTP, a natural pair of DNA polymers, could stimulate the healing of breakage of the long arm of a G chromosome while the dGTP and dCTP, another natural pair, could prevent it. An alternative explanation could be that dCTP and dGTP stabilize the distal and proximal

ends of the broken G chromosome and hence prevent healing.

Summary. A patient who had been accidentally irradiated in 1958 was vaccinated with polyvalent killed influenza virus in 1966. A week after he had been vaccinated 16 peripheral leukocyte cultures were made. Two cultures were made from a normal person. To 15 patient cultures, different and combinations of different deoxyribonucleotides were added 4 hr before termination of the cultures. The sixteenth patient culture (control) had no added deoxyribonucleotides. The normal person had a normal chromosomal pattern. Seventy-one of the 100 control metaphases of the sixteenth culture and seven (0.5%) of the 1451 metaphases from the patient's experimental culture were pulverized. Various (1–7%) frequencies of smaller G (Gq—) chromosomes were found in the experimental cultures. The mitotic rate was 1.17% in the control and 1.82% (range 1.4–2.68%) in the experimental cultures. The findings suggested that exogenous deoxyribonucleoside triphosphates either prevent or heal the pulverized chromosomes. The finding of frequent other chromosomal abnormalities in the experimental cultures favors the latter possibility.

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Received Mar. 20, 1969. P.S.E.B.M., 1969, Vol. 131.