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Exogenous Deoxyribonucleoside Triphosphates II. Their Effect on Pulverized Human Chromosomes at the S Phase[1] (37928)

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(Introduced by T. Franklin Williams)
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The term "pulverization" has been used for the extreme fragmentation of chromosomes seen in association with some virus infection (1). In our earlier studies in radiation-induced chromosomal breakages, several of our observations suggested that chromosomal breakages may be a result of several mechanisms (2), among them, a defect in the DNA synthesis. Based upon this hypothesis, we performed several experiments in which exogenous deoxyribonucleoside triphosphates were added either singly or in combination in cultures at G_2 phase. The cells were known to have a high frequency of pulverization (3). We now report the effect of exogenous deoxyribonucleoside triphosphates upon pulverized chromosome at S phase.

Material and Methods. Patient F, who was exposed to 68.5 rads of total-body irradiation from fast neutrons and gamma rays in June 16, 1958 (4), was given an injection of polyvalent formaldehyde-killed influenza virus vaccine on November 1, 1966. Seven days later, 90 ml of blood were drawn, and the leukocytes were cultured according to the standard technique used in our laboratory. Eight 40-ml cultures were made from this patient and two 20-ml cultures from a control donor as described (3). All materials used for the cultures were obtained from a single bottle of each material to preclude the possibility of variations in the concentration of the ingredients. Instead of incubation for 4 hr after the addition of deoxyribonucleoside triphosphates[4] as in our previous experiments (3), the cultures were coded and incubated for 24 hr. The metaphases seen in these cultures would have been at S phase of their mitotic cycle when deoxyribonucleoside triphosphates were added.

After the cells were prepared and fixed for cytogenetic studies, they were spread on slides with the air-dry method and stained with 2% orcein. The slides were examined microscopically. Pulverized and nonpulverized metaphases were differentiated, and their frequencies were computed. In addition, the nonpulverized metaphases were karyotyped either under the direct microscopic examination (2) or from photographed metaphases (Fig. 1). Chromosomal breakages and abnormal chromosomes were recorded. Mitotic rate was determined from each culture. The methods used to deter-

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[4] Deoxyadenosine 5′-triphosphate (sodium), dATP; deoxyguanosine 5-triphosphate (sodium), dGTP; deoxycytidine 5′-triphosphate (sodium), dCTP; Thymidine 5-triphosphate (trisodium), TTP. These compounds were obtained from Nutritional Biochemicals Corp., Cleveland, OH. The final concentration in the medium was $1 \times 10^{-5} M$.

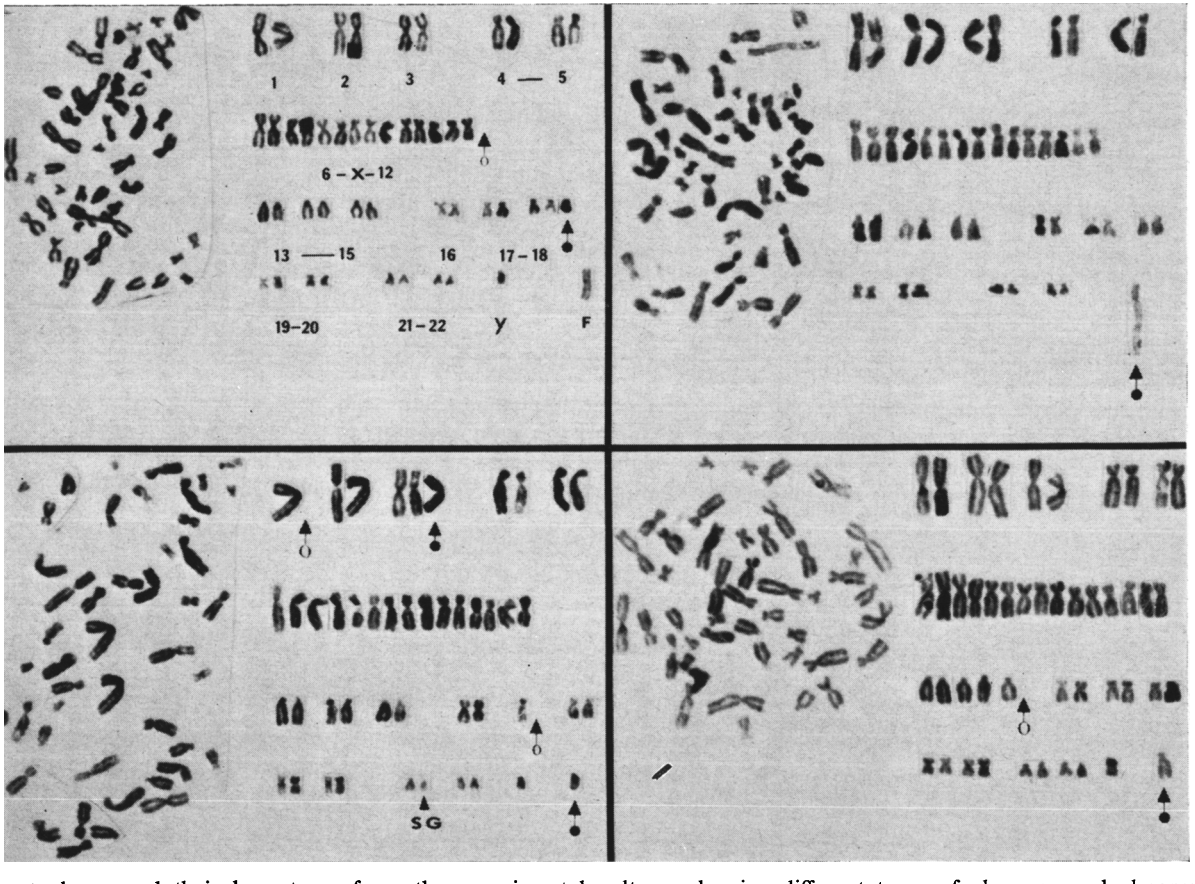

FIG. 1. Four metaphases and their karyotypes from the experimental cultures showing different types of chromosomal abnormalities. An arrow with a black circle indicates additional chromosome or abnormal chromosomes, an arrow with a white circle indicates the position of missing chromosomes, F indicates a pair of acentric fragments, and SG indicates small G chromosome.

TABLE I. Analyses of 100 Metaphases of the Control and Deoxyribonucleotides Added at S Period.

Culture	Compounds[a]	2N[b]	Pulverized metaphases	X_1 cells[c]	Other abnormal cells[d]	Small G[e]	Mitotic rates[f]
1	dATP	76	0	9	26	12	2.12
2	dGTP	79	0	12	32	8	1.94
3	dCTP	93	0	10	33	19	1.80
4	TTP	93	0	5	56	19	2.50
5	dATP + dGTP	89	0	17	10	2	2.60
6	dGTP + dCTP	91	0	8	8	2	2.20
7	dCTP + TTP	95	0	6	8	2	1.86
8	dATP + dCTP	?	100	—	—	?	2.24
9	dATP + TTP	91	0	14	32	9	2.48
10	dGTP + TTP	?	100	—	—	?	2.32
11	dATP + dGTP + dCTP	94	0	4	7	1	2.82
12	dATP + dGTP + TTP	96	0	11	4	0	2.16
13	dATP + dCTP + TTP	86	8	30	2	0	2.50
14	dGTP + dCTP + TTP	91	9	27	0	0	2.18
15	dATP + dGTP + dCTP + TTP	92	0	8	42	14	1.88
16	Control	?	79	1	2	?	2.10

[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 (translocation, dicentrics, rings, deletions).

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

[f] Number of mitotic cells per 100 interphases.

mine these were similar to those described (3). The cultures were decoded after these analyses were completed.

Results. The normal donor has normal chromosomal pattern without any pulverized chromosome. There were two chromosomal breakages and no abnormal chromosome in 100 metaphases analyzed. The mitotic rate was 1.9%. Table I shows the results of the patient's experimental control and the 15 experimental cultures into which the deoxyribonucleosides had been added at the S phase. In the experimental control culture, 79 of the 100 metaphases were pulverized. The frequencies of the diploid metaphases or metaphases having a small G were impossible to be determined. The mitotic rate was 2.1%.

There were 217 pulverized metaphases in the cultures where deoxyribonucleosides were added during the S period. Eight pulverized metaphases were found in the culture where additional dATP, dCTP, and TTP were added and nine pulverized metaphases in the culture where dGTP, dCTP, and TTP were added. In two experimental cultures, all metaphases seen were pulverized. The pulverization in these two cultures appeared more severe than the pulverization found in the experimental control (Fig. 2). The deoxyribonucleosides added to these cultures were dATP and dCTP in one and dGTP and TTP in the other.

Although there was a great reduction of the pulverized metaphases in 13 of the 15 cultures where exogenous deoxyribonucleoside triphosphates were added at the S phase, there was an increase in the frequency of metaphases either having abnormal morphologic chromosome or having chromosomal break. The average mitotic rate in these cultures was 2.24% (range 1.80–2.82%).

Comments. The pulverization found in this patient suggests that it was the result of the synergism between the patient's previous total-body irradiation and his influenza vaccination. Synergism in producing chromosomal aberrations have been reported by Koo (5) with 5-bromodeoxyuridine and

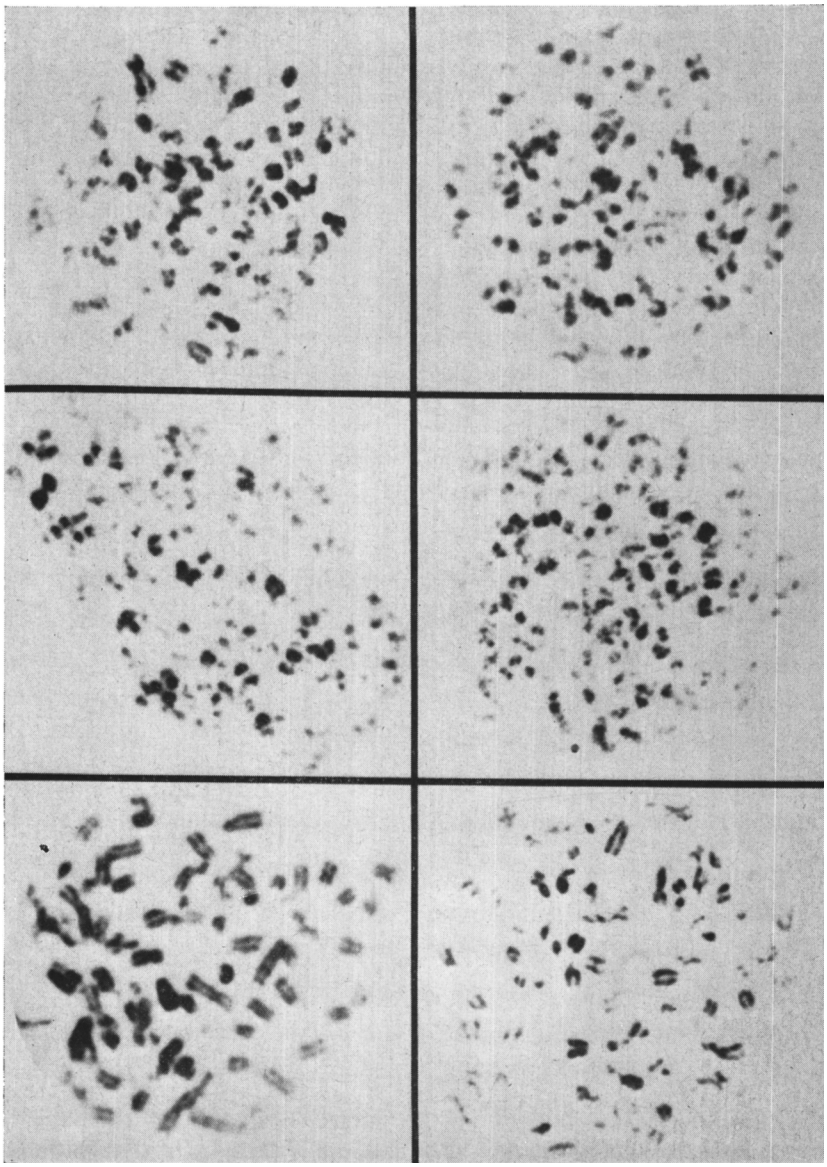

FIG. 2. Six pulverized chromosomes. The two metaphases in the extreme left were from experimental controls, the middle two from culture where dATP and dCTP were added, and the two in the extreme right were from cultures where dGTP and TTP were added.

gamma rays, by Nichols *et al.* (6) with Rous sarcoma virus and cytidine triphosphate, and recently by O'Neill and Rapp (7) with herpes simplex virus and cytosine arabinoside.

In our previous communication (3), we reported decreased frequencies of pulverized chromosomes, no change in mitotic rate, and an increased frequency of crossed-over chromosomes in the experimental cultures where exogenous deoxyribonucleoside triphosphates were added at G_2. Inhibition of cell growth and chromosomal aberrations can be seen in culture by addition of high concentration of normal DNA constituents, i.e., deoxyguanosine (8). However, the concentration used in our experiments was lower than the concentration used by Schachtschabel and Cunze (8). These suggested that the nucleosides probably help in restoring pulverized chromosome rather than inhibiting the pulverized cells from undergoing further processing in preparing for mitosis. The restoring of pulverized chromosomes with the exogenous nucleosides may account for the prolongation of survival time in irradiated mice treated with Nucleton (alkaline-hydrolyzed produce of yeast RNA) or mixture of nucleosides reported by Sugahara *et al.* (9).

Although our present experiments differ from our previous report (3) in that the metaphases analyzed in this report were exposed to nucleosides at the S rather than the G_2 phase of their mitotic cycle, the results confirmed our previous report. The nucleosides added either singly or in various combinations can restore pulverized chromosomes whether they were added at G_2 (3) or at S phase. In the S phase, however, no restoring effect was observed in two cultures. In addition, all metaphases examined in these two cultures were pulverized, and the pulverizations were more severe than the patient's experimental control. The nucleosides involved were dATP and dCTP in one and dGTP and TTP in the other. The paired bases of these two nucleosides were different from the natural occurring pairs of AT and GC. Thus, it is very tempting to explain our observations on the basis that the combination of these nucleosides might further injure the chromosomes. However, the combination of these compounds were added in similar cultures at G_2 phase in our previous experiments, and no such effect was observed (3). Thus it is unlikely that the observations were the results of the imbalance of the substrates. Perhaps our observations were the results of the imbalance of the metabolic end-products of these nucleosides, such as the inorganic pyrophosphate (PP), in the medium. It is known that PP can cause erosion and despiralization in the secondary roots of *Vicia faba* chromosomes (10). These cause weakening of the chromosomal backbone and result in chromosomal breakages (11). The pulverized metaphases seen in our two cultures are very similar, morphologically, to Figs. 1 and 2 published by Natarajan and Ahnstrom (10). The morphological difference could simply be the difference in the degree of erosion and despiralization.

Summary. Sixteen peripheral blood leukocyte cultures were made from a patient with pulverized chromosomes. To 15 of his cultures, different and combinations of different deoxyribonucleotides were added 24 hr (S phase) before termination of the cultures. No deoxyribonucleotide was added to the sixteenth culture (experimental control). Seventy-nine of the one-hundred metaphases from the experimental control and 217 of the 1500 metaphases from the experimental cultures were pulverized. Most pulverized chromosomes in the experimental cultures belonged to two cultures. The nucleotides added were dATP and dCTP in one and dGTP and TTP in the other. The mitotic rates were within normal range in all cultures. This S phase experiment confirmed our G_2 phase experiment in that exogenous deoxyribonucleoside triphosphates can restore pulverized chromosomes. However, in two S phase experimental cultures, further pulverization was seen. This is thought to be due to the imbalance of the metabolic end-products of these nucleosides.

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1. Nichols, W. W., Levan, A., Aula, P., and Norrby, E., Hereditas **54**, 101 (1965).

2. Goh, K. O., Radiat. Res. **35**, 155 (1968).

3. Goh, K. O., Proc. Soc. Exp. Biol. Med **131**, 942 (1969).

4. Brucer, M., *in* "The Acute Radiation Syndrome: A Medical Report on the Y-12 Accident, June 16, 1958," p. 190. Technical Services, Department of Commerce, Washington, DC (1959).

5. Koo, F. K. S., Science **141**, 261 (1963).

6. Nichols, W. W., Levan, A., Heneen, W. K., and Peluse, M., Hereditas **54**, 213 (1965).

7. O'Neill, F. J., and Rapp, F., J. Virol. **7**, 692 (1971).

8. Schachtschabel, D. O., and Cunze, P., Humangenetic **10**, 127 (1970).

9. Sugahara, T., Nagata, H., and Tanaka, T., Radiat. Res. **29**, 516 (1966).

10. Natarajan, A. T., and Ahnstrom, G., Hereditas **59**, 229 (1968).

11. Goh, K. O., Cytologia **35**, 465 (1970).

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