Induction of Endoreduplication in Cultured Mammalian Cells by Some Chemical Mutagens

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SUMMARY

Chinese hamster cells treated with such chemical mutagens as 4-nitroquinoline 1-oxide, captan, acridine dyes, and Cytoxan developed diplochromosomes, showing the induction of endoreduplication. This indicates that these mutagens not only cause damage to genes but also cause changes in ploidy. In this study, the maximum ratio of endoreduplicated cells, 6.6%, was obtained when cells were treated with Cytoxan, 5 mg/ml for 6 hr. Ethyl methanesulfonate, triethylene phosphoramide, and N-methyl-N'-nitro-N-nitrosoguanidine, all potent mutagens, showed little or no activity at the doses used. There seems to be no direct relationship between the induction of endoreduplication and mutagenic activity.

Further analyses were conducted using 4-nitroquinoline 1-oxide. Treatment with 4-nitroquinoline 1-oxide, 1.5 µg/ml, 7.9 X 10^-6 M, for 1 hr induced endoreduplication in 4.3% of cells. Cells synchronized by the double thymidine procedure gave the maximum ratio of endoreduplication when they were treated just prior to M phase. Autoradiography experiments indicated that endoreduplication was, after the first S phase (S1), mainly induced in G2 phase and then, omitting cell division unless otherwise specified, proceeded to the second S phase (S2). The S2 phase took a particularly long period, comparable to two S periods of an ordinary cell cycle.

Various chromosomal aberrations observed in diplochromosomes are presented, one of which shows that a symmetrical dicentric chromosome can be formed from an "arch" structure.

INTRODUCTION

It is of extreme importance for the normal development of higher organisms to keep definite chromosome sets in diploid form. However, tissues in adult humans and animals sometimes have polyploid cells. Endoreduplication seems to be involved in the formation of these cells, although it is not clear whether endoreduplication plays a main or a subsidiary role. Endoreduplication occurs spontaneously, as shown in human euploid fibroblast cultures (23), primary culture of hamster cells (22), and cells of mouse embryo (12). Tumors, e.g., human stomach tumors (6) and mouse ascites tumors (11), sometimes give rise to cells having diplochromosomes. Physical treatments such as low temperature (3) and X-rays (2, 7) can cause endoreduplication. As for chemical compounds, sulfhydryl compounds are well-known inducers. a-Mercaptopethanol (24), β-mercaptoethanol (8), and β-mercapto pyruvate (9) induce endoreduplication in cultured human leukocytes. Various other chemical compounds such as 8-azaguanine (19), colchicine (18), Cytoxan (15), nitrogen mustard (14), 6-mercaptopurine (14), and a piperazine derivative (16) have been reported to have inducing activity.

Endoreduplication was originally described as a form of dual chromosome reproduction, involving the omission of anaphase separation so that additional chromosome replication produced 4, instead of the normal 2, chromatids/metaphase chromosome (11). Rizzoni and Palitti (18) have recently analyzed the cell cycle of endoreduplication. They induced endoreduplication in Chinese hamster cells with colchicine and showed the existence of 2 chromosome reproducing periods, S1 and S2, separated by G7. Experiments with 4NQO2 in this report largely confirm their results. This report also describes that some other chemical compounds that display mutagenic activity can induce endoreduplication in cultured mammalian cells.

MATERIALS AND METHODS

Cells and Medium. An aneuploid Chinese hamster cell line, Don, established by Hsu and Zenzes (5) from a lung of a male animal was obtained through Dr. H. Kato at the National Institute of Genetics (Japan). Eagle’s minimal essential medium containing kanamycin, 60 µg/ml (Nissui Co., Ltd., Tokyo, Japan), was used, supplemented with sodium pyruvate (Wako Pure Chemicals Co., Osaka, Japan), 0.110 g/liter; L-serine (Wako), 0.021 g/liter; and fetal calf serum [Flow Laboratories, Rockville, Md., except in the experiments in Table 4 where serum from Grand Island Biological Co., Grand Island, N.Y., was used. No difference was observed.], 10%.

Cells were grown as monolayers in plastic Petri dishes 60 mm in diameter (Toyoshima Seisakusho, Tokyo, Japan) in a CO2 incubator at 37° unless otherwise mentioned.

Chromosome Preparations. Cells were treated with Colcemid (Ciba-Geigy (Japan), Ltd., Osaka, Japan) at a concentration of 0.05 µg/ml for 1 or 2 hr (see legends to tables and charts) prior to chromosome preparation. Then the cells were harvested by trypsinization and washed once with Dulbecco’s phosphate-buffered saline. The cells were suspended in 0.5 to 1.0 ml of hypotonic solution, 1.0% sodium citrate, for 10 min

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2 The abbreviation used is: 4NQO, 4-nitroquinoline 1-oxide.
at 37° and thereafter fixed in methanol:glacial acetic acid (3:1). After another fixation, the cells were spread on a glass slide and stained with Giemsa's fluid.

** Autoradiography.** Labeling was carried out with tritiated thymidine (thymidine-6-3H; specific activity, 5 Ci/mmmole; Radiochemical Centre, Amersham, England). Each slide with labeled cells was dipped in molten photoemulsion (Sakura NR-M2, Konishiroku Photo Industry Co., Ltd., Tokyo, Japan) and exposed for 5 days. Cells were stained with Giemsa's fluid after developing and fixing.

**Chemicals.** Chemical compounds tested in this study were as follows: 4NQO (K & K Laboratories Inc., Plainview, N. Y.), acridine orange (Merck AG, Darmstadt, West Germany), acridine yellow (Wako), ethyl methanesulfonate (Eastman Kodak Co., Rochester, N. Y.), N-methyl-N'-nitro-N-nitrosoguanidine (Aldrich Chemical Co., Milwaukee, Wis.), acriflavin (Tokyo Kasei Co., Tokyo, Japan), triethylenephosphoramide (Schuchardt U.S.A., Brewster, N.Y.), captan (Stauffer Chemical Co., Richmond, Calif.), and Cytoxan (Mead Johnson & Co., Evansville, Ind.).

**RESULTS**

**Experiments with Synchronized Cells.** Before investigation of the cell cycle of endoreduplication, the normal cell cycle of Chinese hamster cell line Don was determined by the usual method of labeled mitoses. The total generation time, G1, S, G2, and M periods, were found to be 12.0, 2.3, 6.6, 2.8, and 0.3 hr, respectively. When the generation time was calculated from a growth curve obtained by daily counting of the number of cells, it was found to be 12 hr. Thus, the generation times obtained by 2 different methods coincided well.

One of the authors of this paper previously showed that the treatment of cells with 4NQO, 0.5 µg/ml, for 4 hr induced endoreduplication in 3.3% of asynchronous cells (26). This treatment was applied to synchronized cells, since synchronized cells seemed useful for investigating the critical period of the cell cycle leading to endoreduplication and to obtain a large number of endoreduplicated cells. At first, attempts were made to use cells synchronized in G1 by the method of Terashima and Tolmack (27) and cells synchronized in S and G2 by the method of Pederson and Robbins (17). However, it was difficult to obtain a large number of synchronized cells and, moreover, 4NQO showed highly toxic effects. This seemed to be due to its population density effect (1), i.e., the smaller the population density, the more toxic 4NQO becomes at the same dose level. Therefore, the double thymidine treatment procedure of Tobey et al. (28) was used for synchronization. In order to reduce the toxic effects of thymidine, a concentration of 2 mM was used instead of 5 mM, as originally used (28). The synchrony obtained by this method is shown in Chart 1. The chart shows that the S period, (5.0 hr) is shorter than that obtained with asynchronous cultures (6.6 hr). As S became short, the maximum number of cells in M occurred 6 hr after the removal of the thymidine block.

Cells were treated with 4NQO, 0.5 µg/ml, for 4 hr immediately after the removal of the 2nd thymidine block in I experiment and 4 hr after its removal in the other. The appearance of cells with diplochromosomes was examined about 1 and 2 generation times after the 4NQO treatment. The results are given in Table 1. Since few cells with diplochromosomes appeared either 1 or 2 generation times after treatment, the sensitive period occurs between G2 and G1. Even in this case, cells with diplochromosomes did not appear within 1 generation time. Synchronized cells unexpectedly did not give a high ratio of endoreduplicated cells to normal cells as compared with the ratio obtained with asynchronous cultures (see Tables 2 and 3).

Treating cells with 4NQO for 4 hr does not permit pinpointing the exact area of the cell cycle specific for the induction of endoreduplication. An experiment was conducted to test whether or not shorter treatments would be effective. Table 2 shows that shorter periods of treatment are also able to induce endoreduplication. The ratio of endoreduplicated cells reached the maximum, 4.3%, when cells were treated with 4NQO, 1.5 µg/ml, for 1.0 hr. The same treatment is likely to have different inductive and/or toxic effects on a different cell density, as mentioned previously. Treating cells for 0.5 hr is also effective for induction. Although cells were washed twice after the chemical treatment, 4NQO molecules incorporated into the cells and bound to cellular components such as proteins and nucleic acids are thought to be difficult to remove. The binding of 4NQO to proteins and nucleic acids has already been reported (1).

On the basis of the experiments in Tables 1 and 2, further analysis was carried out using cells synchronized by the double thymidine procedure. In this experiment, treating time was reduced to 1 hr. The results are given in Table 3. The

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**Chart 1. Synchronization of cells by the double thymidine procedure.** Five X 10⁴ cells were plated in a glass Petri dish 9 cm in diameter, which included 10 sheets of coverglass. After 1 day, cells were cultured in minimal essential medium containing 2 mM thymidine for 15 hr, then in fresh minimal essential medium for 10 hr, and again in 2 mM thymidine minimal essential medium for 12 hr. Cells on the coverglass were taken out at 1-hr intervals, labeled with thymidine-3H, 1.0 µCi/ml, for 15 min, and fixed in methanol:glacial acetic acid (20:1). About 1000 cells were observed under a microscope and cells with more than 5 grains were counted as labeled cells.
Table 1

**Cell cycle specificity and expression time for the induction of endoreduplication with 4NQO**

Per dish, 10³ cells were plated. After 2 days, cells were synchronized by the double thymidine method: culturing cells in 2 mM MEM for 15 hr, in fresh MEM for 10 hr, and again in 2 mM MEM for 12 hr. Two cultures were then treated with 4NQO, 0.5 μg/ml for 4 hr, and 3 cultures underwent the same treatment 4 hr after release from the thymidine block. Another culture was used as control. Cells were cultured in the presence of Colcemid, 0.05 μg/ml during the final 2 hr. The cell cycles of harvested mitoses were presumed from Chart 1.

<table>
<thead>
<tr>
<th>4NQO-treating period</th>
<th>Time span between O time and fixation (hr)</th>
<th>Presumed cell cycle of harvested mitoses</th>
<th>No. of mitoses examined</th>
<th>No. of endoreduplicated cells</th>
</tr>
</thead>
<tbody>
<tr>
<td>Control</td>
<td>28</td>
<td>3rd M</td>
<td>1000</td>
<td>0</td>
</tr>
<tr>
<td>0–4 hr (around 1st S)</td>
<td>20</td>
<td>2nd M</td>
<td>890</td>
<td>0</td>
</tr>
<tr>
<td></td>
<td>35</td>
<td>3rd M</td>
<td>1080</td>
<td>1</td>
</tr>
<tr>
<td>4–8 hr (around 1st M)</td>
<td>20</td>
<td>2nd M</td>
<td>906</td>
<td>1</td>
</tr>
<tr>
<td></td>
<td>28</td>
<td>3rd M</td>
<td>1181</td>
<td>17</td>
</tr>
<tr>
<td></td>
<td>35</td>
<td>3rd M</td>
<td>595</td>
<td>8</td>
</tr>
</tbody>
</table>

a MEM, minimum essential medium.

b Time when double thymidine treatment was finished.

**Table 2**

*Induction of endoreduplication by 4NQO (short treatment)*

Per dish, 10³ cells were plated. After 2 days, cells were treated with 4NQO. Cells were cultured in the presence of Colcemid, 0.05 μg/ml, during the final 2 hr before fixation. The time between the cessation of 4NQO treatment and the fixation was 27 hr.

<table>
<thead>
<tr>
<th>Treating time (hr)</th>
<th>Concentration (μg/ml)</th>
<th>No. of mitoses examined</th>
<th>No. of endoreduplicated cells</th>
</tr>
</thead>
<tbody>
<tr>
<td>0.5</td>
<td>1.0</td>
<td>1000</td>
<td>10</td>
</tr>
<tr>
<td>0.5</td>
<td>1.5</td>
<td>1000</td>
<td>17</td>
</tr>
<tr>
<td>0.5</td>
<td>2.0</td>
<td>1000</td>
<td>25</td>
</tr>
<tr>
<td>1.0</td>
<td>1.0</td>
<td>1000</td>
<td>23</td>
</tr>
<tr>
<td>1.0</td>
<td>1.5</td>
<td>1000</td>
<td>43</td>
</tr>
<tr>
<td>1.0</td>
<td>2.0</td>
<td>1000</td>
<td>20</td>
</tr>
</tbody>
</table>

**Table 3**

*Cell cycle specificity for the induction of endoreduplication*

Per dish, 10³ cells were plated. Synchronization by the double thymidine procedure began after 26 hr. Cells were treated with 4NQO, 1.0 μg/ml, for 1 hr at 1-hr intervals 4 hr after the removal of the second batch of thymidine, i.e., 4 hr after 0 time. Other conditions were as given in the legend to Table 1.

<table>
<thead>
<tr>
<th>Treating period (hr)</th>
<th>No. of mitoses examined</th>
<th>No. of endoreduplicated cells</th>
</tr>
</thead>
<tbody>
<tr>
<td>Control</td>
<td>1000</td>
<td>0</td>
</tr>
<tr>
<td>4–5</td>
<td>1000</td>
<td>20</td>
</tr>
<tr>
<td>5–6</td>
<td>1000</td>
<td>20</td>
</tr>
<tr>
<td>6–7</td>
<td>1000</td>
<td>23</td>
</tr>
<tr>
<td>7–8</td>
<td>1000</td>
<td>20</td>
</tr>
<tr>
<td>8–9</td>
<td>1000</td>
<td>3</td>
</tr>
<tr>
<td>9–10</td>
<td>1000</td>
<td>0</td>
</tr>
</tbody>
</table>

Maximum sensitivity for induction of endoreduplication was shown by the cells treated 5 to 6 hr after the removal of the thymidine block. Chart 1 indicates that the maximum number of cells in G₂ occurs 5 to 6 hr after the removal. Therefore, G₂ seems to be the most sensitive period. However, the sensitive period is not confined to 5 to 6 hr, but is rather broad. Thus, there remains the possibility that S, M, and G₁, as well as G₂, are also sensitive to induction. Synchronization by the double thymidine procedure has the limitation that, although a large number of synchronized cells can be obtained, high synchrony cannot be realized. However, Table 3 indicates that G₂ may be the most sensitive. Here again, synchronized cells did not give a high ratio of endoreduplicated cells (Table 1).

**Autoradiography Experiments.** Cells synchronized by the double thymidine procedure did not fully clarify the cell cycle of endoreduplication, so the cycle was analyzed autoradiographically with asynchronous cultures. In the following experiments, cultures were treated with 4NQO at the same time and fixed simultaneously 27 hr after the treatment. Pulse labeling was carried out at various intervals before and after the 4NQO treatment. Therefore, diplochromosome labels directly follow the cell cycle of endoreduplication, showing G₁ to S to G₂. In order to diminish the time lag caused by dealing with many cultures at once, experiments were divided into 3 parts. The 1st results are shown in Chart 2, which indicates that a long period of DNA synthesis (approximately 18 hr) is necessary for the formation of diplochromosomes and that almost 2 ordinary cell cycles occur during 1 cell cycle of endoreduplication. This is why it was impossible to get endoreduplicated cells within 1 generation time in the experiments in Table 1. When labeling of more than 10 grains, instead of 20, on diplochromosomes of a cell was adopted as a criterion of a labeled cell, the ratio of labeled cells at 0 time (84%) was higher than that at 2 hr
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(76%). Subsequently, the ratio increased and reached 100% at 6 hr. The high labeling at 0 time seems to be a result of unscheduled DNA syntheses to repair the damage caused by 4NQO. Chart 2 shows that the G₂ period in the cycle of endoreduplication is slightly longer than normal. The difference is not as prominent as in the S period, however. Grains on diplochromosomes observed in the experiment in Chart 2 were randomly distributed.

Chart 3 shows that the S period prior to the induction of endoreduplication is 7.5 hr and seems normal. It also indicates that the specific period of the cell cycle for induction is mainly G₂. Cells in late S phase are also sensitive.

Chart 4 shows the labeling pattern in diplochromosomes before and after 4NQO treatment. The chart indicates that cells in G₂, mainly, and in late S phase, partially, are sensitive to the induction of endoreduplication, as shown in Chart 3, and that 4NQO-treated cells remain inactive in DNA synthesis for a fairly long period (7.8 hr). This is enough time for cells to divide, go into G₁, and enter S, if prolongation of the cell cycle does not occur following 4NQO treatment.

Therefore, it is not possible to conclude on the basis of Chart 4 whether subsequent mitoses occur in endoreduplication-induced cells in their late S or G₂ phases, giving rise to daughter cells that have incorrect information and that carry out 2 successive DNA replications during the long S period, or whether endoreduplication-induced cells, omitting the subsequent mitoses, enter a long S phase to carry out only the 2nd DNA replication. However, the labeling pattern shown in Fig. 1 supports the latter case. Grains are distributed mainly along 1 chromatid of each pairing chromosome, which is characteristic of the 1st DNA replication of 2 continual replications in the semiconservative replication model. It was not easy to obtain these fairly orderly arrays of grains in cells labeled with radioisotope prior to 4NQO treatment. The difficulty seems mainly to stem from the repair mechanisms that are actively operating in cells during and after 4NQO treatment.

Chromosomal Aberrations Observed in Diplochromosomes. Chromosomal aberrations in diplochromosomes are shown in Fig. 2. Gaps, breaks, and rings of both chromatid and chromosome types and exchanges are seen. Ring diplochromosomes shown in Fig. 2 include a single ring, a chromatid type (f), and a double ring, a chromosome type (g). Endoreduplicated minute chromosomes were not rare, as shown in Fig. 3. Although a minute chromosome is small in a condensed form, the DNA folded in it is at least long enough to constitute a unit of replication, a replicon. Fig. 4 shows the crossing over of chromatids between a pair of chromosomes. It is not clear...
whether the 2 chromatids are cut at the crossing point to exchange a part of each chromatid or whether neither cutting nor exchange occurs, thanks to the smooth separation of pairing chromosomes, which is partially shown in Fig. 6. Fairly frequent appearance of arch structures has been reported previously (26). An arch structure is shown in Fig. 5. A consequence of this structure after the breakdown of the centromere connection is the formation of a symmetrical dicentric chromosome, as shown in Fig. 6. At a cell division, this will either be broken at some region between the 2 centromeres or separated into 2 dicentric chromatids.

Endoreduplication-inducing Agents. 4NQO is not only carcinogenic but also mutagenic. Some other chemical mutagens were tested for activity to induce endoreduplication. The results are given in Table 4, which shows that 4NQO, acridine yellow, acridine orange, Cytotoxan, and captan possess such activity. The activity of β-mercaptoethanol, which is not a mutagen, was confirmed and is listed in Table 4. However, ethyl methanesulfonate, N-methyl-N'-nitro-N'-nitrosoguanidine, and triethylenephosphoramide, all potent mutagens, showed little or no activity to induce endoreduplication. The treating doses were determined preliminarily by toxicity tests, in which 200 cells were treated with test compounds for certain periods, and the colonies formed after 1 week were counted. According to survival curves thus obtained, 90% and 50% lethal doses were chosen as treating doses. Since dose ranges to induce endoreduplication seem rather narrow, the agents that are shown as negative might have activity at other treating doses. 4NQO and acridine dyes, which seemed to show population density effects, were tested at higher doses than the 90 and 50% lethal doses obtained in the toxicity tests with 200 cells.

These results indicate that some mutagens not only cause damage to DNA but also induce changes in ploidy. However, the fact that certain potent mutagens do not induce endoreduplication suggests that there is no exact correlation between mutagenesis and endoreduplication.

DISCUSSION

In the previous report (26) one of the authors has shown that the maximum number of endoreduplicated cells appeared about 2 cell generation times after 4NQO treatment, while none appeared after 1 generation time. This was confirmed in this report (Table 1; Chart 2). Further analyses revealed that endoreduplication was induced in cells in late S and, mainly, in G2 (Charts 3 and 4) and that, after a gap period, the triggered cells entered an extraordinarily long S period that was comparable to 2 ordinary S periods (Chart 2). The long S period is responsible for the delayed appearance of endoreduplicated cells. The results largely coincide with those obtained by Rizzoni and Palitti (18), who have shown that the endoreduplication cycle is made up of 2 S periods, S1 and S2, separated by an intervening period that they call G2. However, the gap period that we found (7.8 hr) (Chart 4) is rather longer than theirs (6.5 hr), and our endoreduplicating cells did not enter the long S2 as synchronously as did theirs. The differences are probably due to the higher specificity of colchicine in inducing endoreduplication than that of 4NQO, for colchicine has been reported to induce up to 10 to 20% of cells, while 4NQO induced only 3 to 4% (Table 2).

S1, according to the terminology of Rizzoni and Palitti, is quite normal (Chart 3), but the S2 period is extraordinarily

<table>
<thead>
<tr>
<th>Compounds</th>
<th>Concentration (µg/ml)</th>
<th>Treatment time (hr)</th>
<th>Expression time (hr)</th>
<th>No. of observed mitoses</th>
<th>No. of endoreduplicated cells</th>
</tr>
</thead>
<tbody>
<tr>
<td>4NQO</td>
<td>0.5</td>
<td>6.0</td>
<td>27</td>
<td>1027</td>
<td>25</td>
</tr>
<tr>
<td></td>
<td>1.5</td>
<td>1.0</td>
<td>27</td>
<td>1043</td>
<td>43</td>
</tr>
<tr>
<td>Acridine orange</td>
<td>0.5</td>
<td>2.0</td>
<td>26</td>
<td>1000</td>
<td>0</td>
</tr>
<tr>
<td></td>
<td>1.0</td>
<td>2.0</td>
<td>26</td>
<td>1003</td>
<td>3</td>
</tr>
<tr>
<td></td>
<td>5.0</td>
<td>2.0</td>
<td>20</td>
<td>1009</td>
<td>9</td>
</tr>
<tr>
<td>Acridine yellow</td>
<td>0.33</td>
<td>3.0</td>
<td>24</td>
<td>568</td>
<td>16</td>
</tr>
<tr>
<td>Acriflavine</td>
<td>0.75</td>
<td>3.0</td>
<td>24</td>
<td>1001</td>
<td>1</td>
</tr>
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<td>Ethane methanesulfonate</td>
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<td>2.0</td>
<td>26</td>
<td>1000</td>
<td>0</td>
</tr>
<tr>
<td>N-Methyl-N'-nitro-N'-nitrosoguanidine</td>
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<td>2.0</td>
<td>26</td>
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<td>1</td>
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<td>Triethylenephosphoramide</td>
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<td>27</td>
<td>1085</td>
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<td>Cytoxan</td>
<td>2500</td>
<td>6.0</td>
<td>27</td>
<td>1079</td>
<td>2</td>
</tr>
<tr>
<td></td>
<td>5000</td>
<td>6.0</td>
<td>27</td>
<td>1177</td>
<td>73</td>
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<tr>
<td>Captan</td>
<td>5.0</td>
<td>4.0</td>
<td>27</td>
<td>1021</td>
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<td>β-Mercaptoethanol</td>
<td>112</td>
<td>12</td>
<td>26</td>
<td>1074</td>
<td>1</td>
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<td></td>
<td>224</td>
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<td>26</td>
<td>1083</td>
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<td></td>
<td>1120</td>
<td>12</td>
<td>26</td>
<td>787</td>
<td>16</td>
</tr>
</tbody>
</table>

*One mg of 4NQO was dissolved in 2 ml of ethanol and diluted with minimal essential medium to prepare the treatment solutions.

*One mg of Captan was dissolved in 2 ml of propylene glycol and diluted with minimum essential medium to 5 µg/ml.
long (Chart 2). Shortage of precursors required to produce twice as much DNA content in diplochromosomes as in ordinary chromosomes may be the reason for the long S period.

The gap G2 has G2 character, as it is a period following S, S1; and, at the same time, it has G1 character, as it is a period before G1. When G2 is divided into early G2, mid G2, and late G2, these periods correspond to G2, M, and G1, respectively, in a normal cell cycle. Although at the present time no biochemical evidence is available as to whether or not G2 functions as both G2 and G1, the length of time (7.8 hr) is sufficient for cells to go through both G2 and G1. The parameters of the normal cell cycle of the Chinese hamster cell line Don show a combined time of G2 and G1 of 5.1 hr.

Our data are not clear as to whether or not the condensation of chromosomes takes place during G2. However, crossing over of chromatids, as shown in Fig. 4, suggests that no condensation occurs. The process of formation of such a crossing over is considered as follows: (a) a pair of double-stranded DNA’s is formed in S1; (b) 4NQO treatment induces endoreduplication and causes crossing over between the DNA pair in late S1 or early G2; (c) DNA’s are replicated in S2; and (d) the condensation of DNA’s results in the formation of crossing over of chromatids. If the condensation of chromosomes occurs in mid G2, crossing of single-stranded DNA’s will break.

Thus, our view is that G2 is a short linking of G2 with G1 and the failure of mitotic events results in the induction of endoreduplication. A sequence of biochemical events is considered to be involved in the control mechanisms of a cell cycle. For example, a requirement for both RNA and protein synthesis during G2 phase has been established as necessary for mitoses (10). On the other hand, many chemical compounds are able to induce endoreduplication (see Table 4). If one of the component reactions that is necessary for mitosis, for example, the condensation of nucleoproteins, is damaged in some way by the action of the chemicals, then this may result in the induction of endoreduplication. The facts that various kinds of chemicals have inducing activity and that the sensitive periods are not only G2 but also late S seem to indicate that there are different sites or molecular events that are sensitive to endoreduplication. Colchicine, even if it is considered to be a specific inhibitor of mitosis on the basis of its reactivity with microtubules (29), has at least 2 effects, mitotic inhibition at lower concentrations and endoreduplication induction at higher concentrations (18). The condensation of nucleoproteins is permitted in the former case, but not in the latter.

In the experiments in Tables 1 and 3, the ratio of endoreduplicated cells to normal cells did not increase in synchronized cells in comparison with asynchronous cells, even though cells synchronized by the double thymidine procedure were used. When the parameters of the cell cycle were compared between asynchronous cells and synchronized cells, the S period in Chart 1 was found to be considerably compressed. This phenomenon has already been reported (21). The authors (21) have suggested that the blocking effect of excess thymidine does not prevent, just before S period, the preparation by cells of some components necessary for the following cell cycle. The low sensitivity of cells synchronized by the double thymidine procedure to the induction of endoreduplication may be due to the synchrony with respect to DNA syntheses and to the asynchronous conditions of the other cellular processes.

4NQO (13) and captan (4) have been reported to react with sulphydryl compounds. β-Mercaptoethanol is a sulphydryl compound. A cyclic fluctuation of sulphydryl proteins has been reported not only in well-studied sea urchin eggs (20) but also in cultured mammalian cells (21). Acridine dyes readily bind to proteins. Although it is far from clear to what extent the interconversion between sulphydryl and disulfide groups participates in the control and initiation of mitosis, some regulatory sulphydryl proteins are likely to be causative of failure of mitosis and, perhaps consequently, causative of the induction of endoreduplication.

Stubblefield (25) proposed that there were at least 2 autonomous replication cycles, the centriole cycle and the chromosome replication cycle. He suggested that G2-blocking agents may have anticancer activity, because during the elongated G2 period overmatured centrioles show multipolar division to form nonviable daughter cells. Schmid (22) has shown that multipolar division occurs after endoreduplication. As shown by Rizzoni and Palitti (18) and in this report, the endoreduplication cycle requires a long S period, S1 + S2, and this may permit centrioles to overmature. If this is so, endoreduplicated cells may give birth to some nonviable cells.

Endoreduplication leads at first to exact karyotype doubling. If there is no discord between chromosome quadruplication and centriole duplication in the endoreduplication cell cycle, such polyploids resume normal mitosis and usually reproduce more slowly than the diploids. However, this is followed readily by mitotic nondisjunction and consequent selection of better-adapted hypotetraploid variant cells.

In tumors, these aneuploids may emerge as a new tumor stem-cell line and are often more malignant than the original stem cells that they have outgrown. Endoreduplication has been observed in tumor tissues (6,11).

Some of the agents capable of inducing endoreduplication are mutagenic (Table 4). Thus, they not only cause damage to genes but also make changes in ploidy. Since mutagenesis is closely related to carcinogenesis, these agents that are both mutagenic and cause endoreduplication may be especially hazardous.

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Endoreduplication by Chemical Mutagens


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Fig. 1. Distribution of silver grains on diplochromosomes. This shows cells labeled with thymidine-3 H 3 hr before 4NQO treatment (Chart 4). Scale in all Figures, 10 μm.

Fig. 2. Various diplochromosomal aberrations observed in an endoreduplicated cell. Gaps of chromatid type (a) and chromosome type (b); breaks of chromatid type (c) and chromosome type (d); exchanges (e); and rings of chromatid type (f) and chromosome type (g).

Fig. 3. Endoreduplication of a minute chromosome (arrow).

Fig. 4. Crossing over of chromatids between a pair of chromosomes (arrow).

Fig. 5. An arch structure (arrow).

Fig. 6. A symmetrical dicentric chromosome formed from an arch structure (arrow).
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