Cytological Effects of 1-(2-Nitro-1-imidazolyl)-3-methoxy-2-propanol (Misonidazole) on Hypoxic Mammalian Cells in Vitro

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ABSTRACT

Hypoxic Chinese hamster V-79 cells were examined for light-microscope morphology, progression through the cell cycle, chromosomal aberrations, and viability, after incubation with the 2-nitroimidazole, misonidazole [1-(2-nitro-1-imidazolyl)-3 methoxy-2-propanol]. Cytological examination of cells up to 42 hr after incubation with the drug at 37°C indicated that increasing contact time and increasing drug concentrations interfered with cell attachment and progressively slowed cell progression through the cell cycle. Forty-two hr after a 5.5-hr treatment with 5 mM misonidazole, the majority of cells contained heteropyknotic nuclei, whereas <3% had progressed into mitosis. Of the few cells that reached mitosis by 42 hr, the level of chromosomal aberrations was 6 times that due to hypoxia alone. However, the majority of metaphases (70%) were unaltered; thus about 2% of the treated cell population passed into mitosis unaltered. After a 5.5-hr incubation with 5 mM misonidazole, 98% of the cells also had lost their ability to produce clones. It is suggested that the cytotoxic effect of this drug on hypoxic cells is largely mediated via an interphase cell death, with a minor effect due to chromosome aberrations and cell death from genetic inequality of progeny cells. The ability of misonidazole to kill hypoxic, noncycling cells, which may limit the curability of some tumors with conventional X-rays or chemotherapy agents, makes it of considerable potential interest.

INTRODUCTION

The need for chemical radiosensitizers of hypoxic cells and the properties that these radiosensitizers must possess to be effective in a clinical situation have been listed by Adams (1). Briefly stated, such agents need to be nontoxic to normal tissues, to have minimal sensitizing action on oxygenated cells, to penetrate rapidly to nonvascularized regions of a tumor, and to be relatively resistant to metabolic breakdown (1). Studies of a range of nitroaromatic and nitroheterocyclic compounds have shown that nitroimidazoles fulfill many of the above criteria (2, 3). Efforts have concentrated on the 5-nitroimidazole (Flagyl, a drug of long standing in human use for the treatment of certain forms of amebiasis and giardiasis, particularly infections of Trichomonas vaginalis), and the more efficiently radiosensitizing 2-nitroimidazole, misonidazole (formerly Ro-07-0582). Both compounds are currently under evaluation as radiotherapy adjuncts in humans (6, 23, 24). Apart from their radiosensitizing properties, these compounds have proven to be selectively cytotoxic to hypoxic cells in vitro (15, 16) and to hypoxic cells in cell spheroids (20, 22). This cytotoxicity serves to explain in vivo observations (4, 5) that these sensitizers have a chemotherapeutic action on tumors in the absence of radiation treatments. Further in vitro studies have shown that the cytotoxic action of misonidazole is greatly enhanced by modest hyperthermia (7, 8, 21).

The basis for the cytotoxic action of misonidazole is not known. The cytological effects of the drug on hypoxic Chinese hamster V-79 cells was studied to understand the mechanism of action of the drug to aid in evaluating its potential as a chemotherapeutic agent. Cells were examined up to 42 hr after incubation with misonidazole, including a drug concentration that resulted in 98% cell death as evaluated by the colony survival technique.

MATERIALS AND METHODS

Cell Culture. Chinese hamster V-79 cells were grown at 37.5°C in Falcon plastic flasks in Medium F10 (Grand Island Biological Co., Grand Island, N. Y.) supplemented with 10% fetal calf serum, antibiotics, and L-glutamine. For assessment of drug effects on aerated cells, misonidazole was added directly to cells growing in exponential phase.

Induction of Hypoxia. Cells growing in exponential phase were harvested by trypsinization, spun down, washed, and counted. The cell suspension was then subdivided and adjusted to cell concentrations of 2 x 10⁶ cells/ml, at which concentration the misonidazole, as required, was added (7). One ml of suspension (with or without the drug) was then pipetted into a series of glass ampuls. Ampuls were flushed with high-purity nitrogen plus 5% CO₂ through a long cannula (to displace air above the cell suspension) before the ampuls were heat sealed and while the gas was still flowing. Ampuls were continuously shaken and rotated end over end in a water bath at 37°C for a minimum of 1 hr. Hypoxia was induced by the respiration and metabolism of the large number of cells confined in the small volume of medium in the air-free sealed ampuls.

Drug Treatments and Cell Preparation. Misonidazole was added to aerated cells growing in log phase to concentrations of 1, 2, and 5 mM, and the cultures were returned to the incubator. Colcemid (10 µM) was added at 1.5-hr intervals up to 6 hr after drug addition. After 1.5-hr Colcemid accumulation of mitotic cells, 2 flasks at each concentration were trypsinized; then cells were spun down, washed, spun down, and made hypotonic with 0.07 M KCl for 7 min at room temperature. Cells were then fixed in
cold, fresh methanol-glacial acetic acid (3:1) and changed twice through fixative; the cell suspensions were dropped onto wet slides, 6 slides/flask. Air-dried slides were stained with Giemsa and rendered permanent with Permount.

Hypoxic cells with or without 5 mM misonidazole were maintained at 37° for 1 to 5.5 hr; this time period included the 1 hr during which hypoxia was induced by respiration and metabolism.

At appropriate intervals, ampuls from each drug time regimen were agitated on a vortex mixer and opened; aliquots of the cell suspension were replated to assay the proportion of cells retaining the ability to form colonies or were prepared for subsequent microscopic examination. For assaying clonogenicity, replated cells were incubated for 8 days before they were fixed and stained and before the colonies were counted by a projection technique. By comparison with controls the fraction of cells surviving each treatment schedule was determined.

For establishing cell populations for microscopic study, 10⁶ cells from each ampul were plated into 20 ml complete medium in Falcon plastic flasks. Replicate flasks were treated with 10 μM Colcemid from 0 to 1.5, 1.5 to 3, 3 to 4.5, and 4.5 to 6 hr posttreatment (Experiment 1) to accumulate mitotic cells. In a second and larger experiment, the 1.5-hr accumulations were extended to 15 hr, and 4 flasks from each treatment were given 10 μM Colcemid from 15 to 42 hr. Since Colcemid results in blockage of cells at mitosis, cell progression through the cell cycle can be monitored by comparison of the frequencies of mitotic figures between treatments. For cell examination after hypoxic treatments, the medium was decanted, trypsin was added for 2 min at 37.5°, and then trypsin plus the cells were added to the medium. The cells were spun down, and slides were prepared as described in the preparation of aerated cells.

**Microscopic Examination.** The proportion of cells in mitosis (mitotic index) was determined in samples of 2000 cells/treatment fixation time, whereas the frequency and spectrum of chromosome aberrations were determined from 50 well-spread metaphases/treatment fixation time when possible, except for the 15- to 42-hr accumulation period in which 200 metaphases were examined. Chromosomal aberrations were categorized according to the method of Savage (19). Chromatid deletions were scored if the acentric fragment was physically displaced by the width of 1 chromatid. Single and paired achromatic lesions were separately recorded.

**Drugs.** The 1-(2-nitro-1-imidazolyl)-3-methoxy-2-propanol (misonidazole) was donated by Roche Products, Welwyn Garden City, England.

**RESULTS**

**Aerated Cells.** Mitotic indexes over 4 sequential 1.5-hr accumulations for drug treatments and controls are shown in Chart 1. The initial comparatively low indexes can be ascribed to handling and reduced temperatures before return to the incubator. Apparently, concentrations to 5 mM have no effect on cell progression into mitosis up to 6 hr after initiation of drug treatment. There was no indication of increased chromosome aberrations in drug-treated cells; overall, 97% of metaphases were free of aberrations.

**Hypoxic Cells.** Chart 2 shows survival curves for cells exposed to hypoxia and hypoxia plus 5 mM misonidazole for various times at 37°. Data were pooled from 2 separate experiments. Surviving fractions are expressed relative to cell viability after the 1-hr period during which cells became hypoxic. Plating efficiencies were 86 and 95% for 1 hr of hypoxia and 81 and 96% for 1 hr of hypoxia plus the drug, from the 2 experiments. Thus, there is little if any effect on cell viability for 1 hr of the hypoxia induction period. Evidently, drug cytotoxicity is strongly contact time dependent, whereas prolonged hypoxia alone results in a small loss of viability.

Mitotic indexes for the 4 consecutive 1.5-hr accumulation periods up to 6 hr after treatment of hypoxic cells are shown in Chart 3; increasing drug contact time results in the progression of fewer cells into mitosis. From Chart 3, evidently, prolonged hypoxia itself influences cell progression through the mitotic cycle. The increase in mitotic index with time is less after drug treatment. Thus, both the initial
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The levels of chromosome aberrations in metaphase cells after 5 hr of hypoxia or 5 hr of hypoxia plus the drug were similar and were low in each consecutive collection period; 92 and 90.5% of the cells were aberration free in the total 6-hr collection period (compared with 97% in aerated cells). There were 0.08 and 0.10 aberrations/cell (all were chromatid type) from the respective treatments, whereas both treatments resulted in 0.06 achromatic lesions/cell. Notably, <3% of the total cell population had progressed into mitosis in the 6-hr period after drug treatment, which compares with about 10% for hypoxia alone. Since the survival study (Chart 2) indicated that about 10% of the cells survived after the 5-hr drug treatment, cells with chromosome aberrations that could result in cell lethality could have been progressing into mitosis at a later time. In the second experiment, cells were sequentially accumulated to 15 hr after treatment; then, realizing that continued short accumulations would increasingly consist of cells in their second or third mitotic division after treatment, we used continuous Colcemid accumulation up to 42 hr after treatment, which ensures that second or third division mitotic cells appear as tetraploids or octaploids. Mitotic indexes from different periods for each treatment are shown in Table 1. As is also shown in Chart 3, rendering cells hypoxic results apparently in severe perturbations before almost normal progression of the population through the cell cycle takes place. The last collection period (13.5 to 15 hr) is the first to show a mitotic index that might be expected of aerated cells (7.3%). Up to 15 hr after treatment, 32, 21, 22, and <1% of cells from the respective treated populations (Table 1) progressed into mitosis. However, the extended accumulation period from 15 to 42 hr shows that, for all except the 5.5-hr drug treatment, the majority of cells from the incident population progressed into mitosis.

Cells were observed by phase contrast prior to each fixation; notably the attachment of cells from the prolonged drug treatment was negligible for the first 6 accumulation periods, with a slightly increasing incidence of attachment through the later periods. However, whereas the cells were almost confluent from the other treatments at 42 hr, the prolonged drug treatment still showed few attached cells. Also, substantial aggregations of cellular material were suspended in the medium. When cells were concentrated, fixed, stained, and examined microscopically, the majority of cells from the 5.5-hr drug treatment (1732 of 2000 recorded from the suspended cell preparation) contained distorted heteropyknotic nuclei and appeared to be lysed. In contrast, only a minority of cells after 1 hr of the drug (153 of 2000 recorded) had a similar morphological appearance.

Throughout the ten 1.5-hr accumulation periods after the 5.5-hr drug treatment, no mitotic figures were found in the cells scored. A low frequency appeared up to 42 hr and, from these, assessments of chromosome aberrations were made, along with those for all time periods from the 1-hr drug treatment and after 5.5 hr of hypoxia. Since the incidence of chromosome aberrations was comparatively low, the data from the individual accumulation periods have been pooled for 0 to 15 hr posttreatment, as shown in Table 2. The background level of cells of higher ploidy appeared to be about 2 to 3%, whereas, after the 27-hr Colcemid accumulation, the majority of cells for other than the 5.5-hr

<table>
<thead>
<tr>
<th>Time after treatment (hr)</th>
<th>1 hr of hypoxia</th>
<th>5.5 hr of hypoxia</th>
</tr>
</thead>
<tbody>
<tr>
<td>0-1.5&lt;sup&gt;a&lt;/sup&gt;</td>
<td>2.8&lt;sup&gt;d&lt;/sup&gt;</td>
<td>1.3</td>
</tr>
<tr>
<td>1.5-3</td>
<td>1.9</td>
<td>1.9</td>
</tr>
<tr>
<td>3-4.5</td>
<td>1.9</td>
<td>2.4</td>
</tr>
<tr>
<td>4.5-6</td>
<td>2.5</td>
<td>2.1</td>
</tr>
<tr>
<td>6-7.5</td>
<td>4.4</td>
<td>3.0</td>
</tr>
<tr>
<td>7.5-9</td>
<td>2.5</td>
<td>0.3</td>
</tr>
<tr>
<td>9-10.5</td>
<td>2.7</td>
<td>2.4</td>
</tr>
<tr>
<td>10.5-12</td>
<td>2.0</td>
<td>1.7</td>
</tr>
<tr>
<td>12-13.5</td>
<td>3.9</td>
<td>2.4</td>
</tr>
<tr>
<td>13.5-15</td>
<td>7.3</td>
<td>4.3</td>
</tr>
<tr>
<td>0-15 (summed times after treatment)</td>
<td>31.9</td>
<td>21.0</td>
</tr>
<tr>
<td>15-42 (suspended cells)&lt;sup&gt;b&lt;/sup&gt;</td>
<td>84.3</td>
<td>70.9</td>
</tr>
<tr>
<td>15-42 (attached cells)&lt;sup&gt;c&lt;/sup&gt;</td>
<td>50.5</td>
<td>16.5</td>
</tr>
<tr>
<td>% of cells producing colonies</td>
<td>86.0</td>
<td>81.0</td>
</tr>
</tbody>
</table>

<sup>a</sup> Time in hypoxia refers to the time after 2 × 10<sup>6</sup> cells in 1 ml of medium are seeded in nitrogen-flushed ampuls. Cells actually enter a hypoxic state about 20 min after the ampuls are sealed.

<sup>b</sup> Misonidazole was added to cell suspensions just before hypoxia was initiated.

<sup>c</sup> Mitotic cells were accumulated with 10 μM Colcemid at 1.5-hr intervals after termination of treatment.

<sup>d</sup> The percentage of cells in mitosis (mitotic index) was determined on samples of 2000 cells from each treatment fixation time.

<sup>e</sup> Mitotic cells were accumulated with 10 μM Colcemid for 27 hr from 15 to 42 hr after termination of treatment.
drug treatment went through 2 cell cycles.

For the prolonged drug treatment, the level of chromatid interchanges was substantially enhanced from the other treatments, which contain a greater relative proportion of isochromatid deletions (Table 2). Although the close apposition of chromatids made the assessment of completeness of sister union. The high incidence of symmetrical dicentrics in tetraploid cells is compatible with those arising from isochromatid deletions with sister unions in the previous cell cycle and, in conjunction with the equivalent level of aberrations from the 2 sampling periods, indicates that further induced chromosome aberrations in the second cell cycle are negligible.

In relation to the total cell population, only 1 to 2% of the cells progressed into mitosis up to 42 hr after 5.5 hr drug treatment; of these, 11% were tetraploids, indicating that an absolute minority of cells had progressed through 2 cell cycles. However, of the tetraploid cells 31% were endoreduplicated, whereas no endoreduplicated cells were observed in the considerably more frequent tetraploid cells from the other treatments. The incidence of chromosome aberrations per cell was 6 times higher than that of the other treatments, although 76% of the cells were free of aberrations. The distributions of aberrations plus achromatic lesions in metaphase cells are shown in Table 3. Achromatic lesions were not a prominent feature at any time: an incidence of 0.11/cell occurred for the 5.5-hr drug treatment, with the remainder varying from 0.01 to 0.09/ cell. No significant difference was observed between the 1-hr drug treatment and 5.5 hr of hypoxia or between collection periods. The variance:mean ratio indicates a substantial deviation from a Poisson distribution for the 5.5-hr drug treatment. This possibly infers that the aberrant cells make up a small aberration-sensitive although progression-capable segment of the cell population, whereas the remainder of the cells either die in interphase (the vast majority) or proceed unscathed into and, presumably, out of mitosis.

The appearance of symmetrical dicentrics in the few tetraploid cells shows that repair had to take place to produce the original sister union isochromatid deletions. This is reinforced by the observation that only 22% of chromatid interchanges from this treatment were incomplete. Overall, only 5 of 427 aberrations were dicentrics (in diploid cells) or translocations, with only 2 (of 351) from drug-treated cells. Although the possibility that some of the isochromatid

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### Table 2

<table>
<thead>
<tr>
<th>Time after treatment (hr)</th>
<th>No. of metaphases analyzed</th>
<th>% of cells of higher ploidy&lt;sup&gt;a&lt;/sup&gt;</th>
<th>% of aberration-free cells</th>
<th>No. of aberrations/cell</th>
<th>% of chromatid deletions&lt;sup&gt;b&lt;/sup&gt;</th>
<th>% of chromatid interchanges</th>
<th>% of isochromatid deletions</th>
<th>% of symmetrical dicentrics&lt;sup&gt;c&lt;/sup&gt;</th>
</tr>
</thead>
<tbody>
<tr>
<td>1 hr of hypoxia + 5 mM misonidazole</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>0-15&lt;sup&gt;d&lt;/sup&gt;</td>
<td>500</td>
<td>1.5</td>
<td>90.0</td>
<td>0.11</td>
<td>38</td>
<td>0</td>
<td>62</td>
<td>0</td>
</tr>
<tr>
<td>15-42&lt;sup&gt;e&lt;/sup&gt;</td>
<td>400</td>
<td>86.0</td>
<td>90.0</td>
<td>0.13</td>
<td>6</td>
<td>12</td>
<td>6</td>
<td>71</td>
</tr>
<tr>
<td>5.5 hr of hypoxia</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>0-15</td>
<td>500</td>
<td>2.5</td>
<td>92.5</td>
<td>0.08</td>
<td>20</td>
<td>0</td>
<td>78</td>
<td>0</td>
</tr>
<tr>
<td>15-42</td>
<td>400</td>
<td>88.7</td>
<td>93.0</td>
<td>0.09</td>
<td>7</td>
<td>7</td>
<td>14</td>
<td>50</td>
</tr>
<tr>
<td>5.5 hr of hypoxia + 5 mM misonidazole</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>15-42</td>
<td>400</td>
<td>10.7</td>
<td>76.0</td>
<td>0.61</td>
<td>30</td>
<td>36</td>
<td>22</td>
<td>8</td>
</tr>
</tbody>
</table>

<sup>a</sup>Cells of higher ploidy were 97% tetraploids and 3% octaploids.

<sup>b</sup>Individual aberration types are presented as percentages of total aberrations.

<sup>c</sup>Symmetrical dicentric chromosomes were only found in tetraploid cells.

<sup>d</sup>Data from the ten 1.5-hr Colcemid accumulation periods were pooled since there was no consistent variation. Fifty well-spread metaphases were analyzed from each treatment fixation time.

<sup>e</sup>Since suspended and attached cells from the 24-hr accumulation period were handled separately, 200 metaphases were scored from each, and the data were pooled.

### Table 3

<table>
<thead>
<tr>
<th>Treatment</th>
<th>Frequency/cell&lt;sup&gt;a&lt;/sup&gt;</th>
<th>No. of cells</th>
<th>No./cell</th>
<th>% of unaltered cells</th>
<th>Variance:mean ratio</th>
</tr>
</thead>
<tbody>
<tr>
<td>1 hr of hypoxia + 5 mM misonidazole (0-15 hr)&lt;sup&gt;b&lt;/sup&gt;</td>
<td>430 63 7</td>
<td>500</td>
<td>0.15 ± 0.02&lt;sup&gt;c&lt;/sup&gt;</td>
<td>86</td>
<td>1.07</td>
</tr>
<tr>
<td>1 hr of hypoxia + 5 mM misonidazole (15-42 hr)</td>
<td>351 43 6</td>
<td>400</td>
<td>0.14 ± 0.02</td>
<td>88</td>
<td>1.09</td>
</tr>
<tr>
<td>5.5 hr of hypoxia (0-15 hr)</td>
<td>420 77 3</td>
<td>500</td>
<td>0.17 ± 0.02</td>
<td>84</td>
<td>0.89</td>
</tr>
<tr>
<td>5.5 hr of hypoxia (15-42 hr)</td>
<td>362 30 5 3</td>
<td>400</td>
<td>0.12 ± 0.02</td>
<td>90</td>
<td>1.47</td>
</tr>
<tr>
<td>5.5 hr of hypoxia + 5 mM misonidazole (15-42 hr)</td>
<td>274 65 19 14 11 6 7 4</td>
<td>400</td>
<td>0.72 ± 0.07</td>
<td>68</td>
<td>2.88</td>
</tr>
</tbody>
</table>

<sup>a</sup>The number of cells with a given number of aberrations plus achromatic lesions are recorded for each treatment.

<sup>b</sup>Time after treatment. Data were pooled from ten 1.5-hr collection periods or two 15- to 42-hr samples.

<sup>c</sup>Mean ± S.E.
deletions were actually chromosome deletions (i.e., G, induced) cannot be excluded, the finding that other chromatin-type aberrations exist in most cells with aberrations and that there is a high incidence of symmetrical dicentrics in tetraploid cells makes this most unlikely. Thus, virtually no G, phase chromosomal aberrations induced by the drug appeared in the metaphase cell population.

DISCUSSION

The pronounced cytotoxicity of misonidazole under hypoxic conditions in vitro appears in large part to be mediated via an induced interphase cell death. The proportion of cells progressing through the cell cycle into mitosis declines with both increasing drug concentration and contact time. After short drug treatments, cellular recovery and cycle progression resumes; however, a 5.5-hr treatment with 5 mM misonidazole results in the movement of <3% of the cell population into mitosis up to 42 hr after treatment (Table 1). This result in conjunction with the finding that about 98% of cells, as assessed by the colony survival technique, fail to survive (Chart 2) infers that the vast majority of those cells that enter mitosis (Table 1) about 70% are unaltered (1 to 2% of the original cell population) and, hence, are probably capable of further growth and of providing the greater part of the 2% of the treated cell population capable of proliferation. Of course, it is quite possible that a further fraction of treated cells could progress into mitosis. However, the failure of the majority of cells to attach, their aggregation into clumps of debris-like material, and their heteropyknotic state make it improbable that this fraction could be other than minor. This is particularly so if the greater part of those cells capable of proliferation have arrived at mitosis in an aberration-free state in the time period under study. Since only a very small fraction of cells survive and a very small fraction progress into mitosis, the effects of this drug are comparatively cell cycle stage nonspecific, which is supported by the finding that synchronized hypoxic cells at 3 stages of the cell cycle responded similarly to the drug (8).

Nevertheless, a minor component of induced cell death can be attributed directly to chromosomal damage, since the level of chromosomal aberrations in cells arriving at metaphase after the 5.5-hr drug treatment was 6 times that found after hypoxia alone. Although the proportion of chromosomal aberrations that do not result in cell death is not known, there can be little question that the maldistribution of genetic material in postmitotic progeny cells frequently results in cell death.

Postmitotic cell death largely due to chromosomal aberrations and, hence, genetic inequalities of postmitotic cells follows ionizing radiation treatments (31) and the use of many antineoplastic agents (9, 10, 12, 14). This contrasts with the interphase type of cell death proposed as the primary mode of action of misonidazole. Notably, the related nitroaromatic, niridazole, proved to be negative when tested for its ability to produce micronuclei in mouse marrow cells (29).

Hypoxic conditions selectively result in the reduction of the nitroimidazoles in mammalian cells (3, 26), microorganisms (11), and biochemical preparations (30). Thus, the initial compounds are altered to reduction products including hydroxylamines and amines (26), which putatively could interact with DNA. However, the hydroxylamines and amines may not be the active principals, since it has been shown that the radical scavenger, cysteamine, drastically reduces the cytotoxic effect (9) and that hypoxia-dependent free-radical nitroso derivatives have been shown to be formed during anaerobic metabolism (28). In general, it has been inferred that the target macromolecule is DNA (for example see Ref. 8). This has some support from the observations that reduction metabolites bind to DNA and, to a lesser extent, protein in microorganisms (11) and that a low level of metronidazole binds to DNA after irradiation in the test tube (30).

In these hypoxic cells, initial oxygen deprivation will result in a switch from aerobic to anaerobic processes. Thus, hypoxic cells produced by this technique metabolize themselves to an almost resting state at which crowding would result in undernutrition, competition for limiting substances, accumulation of breakdown products, and an increased intracellular acidity. This situation is possibly somewhat analogous to that found in the hypoxic region of tumors. Prolonging hypoxia alone results in slightly increasing cell death with time (Chart 2). This cannot be attributed to postmitotic cell death since the frequency of chromosomal aberrations found is insufficient (Table 2).

Since the production of highly reactive free radicals in the cytoplasm is highly probable, the effect of these free radicals is most likely to be felt initially in the most proximally sensitive cellular components, membranes of cytoplasmic organelles, the cell membrane, and the nuclear membrane. Deficient cell attachment after treatment, delay of cell progression, endotetraploids in drug-treated material and lysed cells, and altered cellular morphology are findings consistent with this view. Electron microscopic observations of thin sections of cells after drug treatment have indicated that mitochondria were distorted and that cytoplasmic membranes and nuclear membranes were disrupted (L. Roizin-Towle, personal communication). This course does not preclude direct effects on DNA, since most radicals might be expected to add to the aromatic rings of the DNA bases very rapidly; however, if the formation and binding of active radicals is principally in the cytoplasm, a nonintranuclear origin for interphase cell death could be substantial. Sensitization to radiation treatments may be a related although distinct phenomenon.

The mutagenic and carcinogenic potential of this compound is extant, since some DNA interaction obviously occurs and since the 5-nitroimidazole, metronidazole, is a known carcinogen (18) and mutagen (13, 17, 27). Also, the reduction of metronidazole under anaerobic conditions was necessary for the expression of mutagenic potential (17). Thus, it is most probable that misonidazole and related compounds have the same capacity; this could be exacerbated in body tissues that may be partially hypoxic, e.g., the testes.

Misonidazole appears to be a cell cycle stage nonspecific cytotoxic compound that could induce cell death by free-radical disruption of membranes of hypoxic cells. This asensitivity of nonproliferating hypoxic cells thus differs
from that usually found (25), in which proliferating cells are more sensitive to anticancer agents. The response of resting, hypoxic malignant cells to this cytostatic drug could conceivably be exploited as an adjunct to chemotherapeutic schedules as well as a sensitizer in radiotherapeutic schedules.

REFERENCES

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