A Probable Role for Protein Synthesis in Intestinal Epithelial Cell Damage Induced in Vivo by Cytosine Arabinoside, Nitrogen Mustard, or X-irradiation

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SUMMARY

Cell death induced in vivo in the continuously dividing intestinal crypt epithelial cells by 1-β-D-arabinofuranosylcytosine, nitrogen mustard, or X-irradiation can be prevented by two agents which inhibit protein synthesis: cycloheximide and tenuazonic acid. Both pretreatment and posttreatment (up to 45 min), in doses sufficient to produce an 80% drop in the rate of protein synthesis, are effective. Protection against hydroxyurea-induced cell death is similar but more variable. Our data suggest that, in this system, the active metabolic response of the cell (protein synthesis), to injurious agents, rather than mere passive attrition, is necessary for cell death. While protective to the intestinal crypt epithelium, inhibitors of protein synthesis enhance damage to lymphoid cells. It is suggested that these agents may be useful adjuncts to the X-ray and chemical treatment of lymphomas.

INTRODUCTION

Cell death, despite its obvious importance not only in our understanding of metabolic organization and control of cells but also in radiotherapy and in chemotherapy, remains poorly understood from the mechanistic viewpoint. During a systematic investigation of the cellular consequences of inhibition of protein synthesis in vivo (24, 25, 43–46), observations were made which led to a new perspective on this problem.

The administration of the metabolic inhibitor cycloheximide in doses sufficient to induce almost complete inhibition of protein synthesis interrupted the cell cycle of the intestinal epithelial cells without causing necrosis (43, 44). These observations were particularly surprising since inhibition of protein synthesis was always associated with marked inhibition of DNA synthesis in the intestine and in other proliferating tissues (43, 44) and since inhibitors of DNA synthesis such as hydroxyurea (30, 36) and ara-C3 (5) (H. S. Schwartz, personal communication) are among the potent agents inducing cell death in both normal and neoplastic proliferating tissues. Thus, it appeared that inhibition of DNA synthesis alone leads to cell death in some proliferating cells, while inhibition of DNA plus protein synthesis does not. These observations suggested that the synthesis of some protein(s) may play an important role in the lethal response to interference with DNA metabolism. It also indicated the possibility that inhibiting protein synthesis may protect some cells against death under these circumstances. This communication reports that 2 inhibitors of protein synthesis, cycloheximide and tenuazonic acid (37, 40), protect cells in the intact animal against cell death induced by ara-C, nitrogen mustard, or X-irradiation. This protective effect is selective in that it is readily apparent in the epithelial cells of the intestinal crypts but is not seen in the neighboring lymphoid tissue. The possible implications of these observations for our understanding of the metabolic organization of cells and the molecular mechanisms of cell death, as well as for our understanding of some practical aspects of cancer chemotherapy, are discussed.

MATERIALS AND METHODS

Male white Wistar rats (Carworth Farms, New York, N. Y.) weighing 160 to 200 g or male Fischer rats weighing 150 to 170 g were fed Wayne Lab Blox until 16 hr before the beginning of the experiment, when food but not water was removed. The following compounds were administered i.p. between 9:00 and 10:00 a.m.: cycloheximide (0.1 to 1.5 mg/kg body weight) freshly prepared in 0.9% NaCl solution.
(1 mg/ml); ara-C (250 mg/kg body weight) as the hydrochloride made up in distilled water (50 mg/ml) and adjusted to pH 5.5 with a few drops of 10% NaOH; HN2 (2 mg/kg body weight) as the hydrochloride made up in 0.9% NaCl solution (1 mg/ml) immediately before injection; hydroxyurea (250 mg/kg body weight) made up in distilled water (50 mg/ml); and tenuazonic acid (30 to 75 mg/kg body weight) as the sodium salt made up in 0.9% NaCl solution (10 mg/ml). The ara-C (NSC 63878), hydroxyurea (NSC 32065), and tenuazonic acid (NSC 525816) were obtained from the Cancer Chemotherapy Service Center of the National Cancer Institute, Bethesda, Md. The HN2 was a commercial preparation from Merck, Sharp and Dohme, Rahway, N. J. The irradiated animals received 1000 rads whole-body radiation from a 250 kV peak X-ray generator (Westinghouse Coronado radiation therapy unit) or from a 60Co irradiator.

The animals for histological study were killed by decapitation 3 or 3.5 hr after the injection of the necrotizing agent or after irradiation, times at which necrosis of the crypt cells of the intestinal mucosa and of the cells in the germinal centers of Peyer’s patches is well developed under the experimental conditions used. Different animals were killed at various intervals from 1 to 24 hr after the damaging treatment. Although portions of the whole gastrointestinal tract were examined on occasion, the changes in the first 15-cm segment of the small intestine were representative. Therefore, this segment was removed routinely and fixed in Stieve’s solution for histological examination. Sections of paraffin-embedded tissue were stained with hematoxylin and eosin and occasionally with the Feulgen method for DNA. Protein, DNA, and RNA synthesis, as measured by the incorporation of an appropriate precursor and corrected for changes in the precursor pool, were determined as previously described (44).

RESULTS

Protection by Cycloheximide against ara-C or Nitrogen Mustard. The first series of experiments were concerned with the possible protective effect of cycloheximide against cell damage induced by ara-C or HN2. Cycloheximide was administered 15 or 30 min before the injection of ara-C or HN2. It became evident early in the study that cycloheximide provided virtually complete protection to the crypt cells against the necrogenic effects of these 2 compounds (Figs. 1 and 2). Instead of the crypt epithelial cells showing obvious stigmata of cell death, such as karyorrhexis and coagulated areas of cell cytoplasm and nucleus, the cells in this region were intact and normal. However, cycloheximide did induce a disappearance of mitotic figures (44) and some transitory nuclear swelling.

In contrast to the crypt epithelial cells, there was no protection afforded to the cells of the germinal centers of the lymphoid tissue. Many of the large cells in the germinal centers of all lymphoid tissue (intestine, lymph nodes, spleen, etc.) showed a rapid induction of cell death and necrosis (Fig. 8). Although this latter phenomenon was observed in animals treated with each of the above compounds alone, the degree of necrosis was, in general, somewhat more extensive when cycloheximide was used in conjunction with either ara-C or HN2. Thus, whereas the crypt epithelial cells show protection, the contiguous lymphoid tissue does not.

X-irradiated Animals. These results raised several questions, not only about the possible mechanism of action of cycloheximide, but also about the general applicability of the protective phenomenon to other situations, such as that in irradiated animals or animals receiving other inhibitors of DNA synthesis, such as hydroxyurea. The results with hydroxyurea are more complicated and will be discussed later in this report. The findings with irradiation, insofar as they go, are clear cut. Rats pretreated as above with cycloheximide and exposed to 1000 rads show the same selective protective effect on the crypt epithelial cells (Figs. 6 and 7). Again the lymphoid tissue shows no protection (Fig. 9). Control X-irradiated animals were found to have extensive crypt cell and lymphoid tissue necrosis at 2.5 to 3 hr following exposure. Thus, the selective preventive action of cycloheximide occurs with an alkylating agent, a potent inhibitor of DNA synthesis and X-irradiation, suggesting some common effect on the response pattern of the involved cells.

Time Sequence of Cellular Damage and Protection. Several possibilities arise in regard to the overall mechanism of action of cycloheximide. Its protective effect may be a more or less direct consequence of the inhibition of protein synthesis. Alternatively, it may represent an indirect effect related to the interference with the primary action of the various necrogenic agents on susceptible cells or to the interruption of the normal passage of cells through the different phases of the cell cycle (44). Although the precise manner by which this agent exerts its influence cannot as yet be identified, the results of some recent experiments suggest the likelihood of a direct effect.

One approach was a carefully timed study observing both the biochemical and morphological effects of ara-C and HN2 and the possible protective effect of cycloheximide at different times after administration of the damaging compound. This was designed to determine the rapidity with which the necrogenic agent interacted chemically with the target tissue and to observe whether cycloheximide is effective after the occurrence of such biochemical lesions, as well as before. In experiments on DNA synthesis, animals were given ara-C or HN2 at 0 time and thymidine-2'14C (7 to 14 μCi/animal) 10, 15, 20, or 30 min later. The animals were allowed to incorporate the thymidine for 5, 10, or 15 min before being killed. As is evident from Chart 1, the ara-C-treated animals showed almost complete inhibition of DNA synthesis in the intestine within 10 to 15 min. With HN2, inhibition of thymidine incorporation into DNA was evident within 10 min after its administration, but the degree of inhibition was much less than with ara-C (Chart 1).

It is well known that the half-life of nitrogen mustard in biological systems is very short and that most, if not all, of its primary biochemical effects are completed within a few minutes after its administration. The same is believed to be the case with X-irradiation. Thus, with all 3 agents, their
primary biochemical effects are probably completed within 15 min, although morphological evidence of cell death does not become manifest by light microscopy until 2 to 2.5 hr. Yet cycloheximide was found to be as effective in counteracting the acute cell damage when administered 30 min after HN2 or 45 min after ara-C administration as it was when given prior to or along with the noxious stimuli. However, this protective effect was no longer noted when the introduction of cycloheximide was delayed beyond these respective times. Rather than interfering with the primary biochemical effects of the necrogenic agents, cycloheximide appears to alter the response pattern of the crypt cells to these effects. This concept is of fundamental importance, since it implies that the lethal consequences of these agents are not the immediate or direct result of their primary biochemical injurious action, but rather the result of an active secondary metabolic response of the cell to the initial biochemical lesion.

As part of this phase of the study, the morphological appearance of the intestinal wall was observed at various time intervals up to 24 hr after ara-C, HN2, or X-irradiation with or without the added injection of cycloheximide. With ara-C, there occurs a fairly rapid induction of crypt cell death, evident morphologically between 2 and 2.5 hr. The dead cells are rapidly removed, so that by about 7 to 8 hr later the crypt epithelium was almost normal, with only an occasional dead cell or only a few cells containing a cytoplasmic vacuole. Presumably, the dead cells have been replaced by the proliferation of the surrounding intact cells. No evidence of any further episodes of cell death is seen within a 24-hr period, the epithelium at the end of this period being of normal thickness and appearance (Fig. 3). The crypt cell changes after a single dose of hydroxyurea, in confirmation of the previous work of Philips et al. (30), were indistinguishable from those with ara-C. The administration of cycloheximide even 30 to 45 min after the ara-C prevented any occurrence of crypt necrosis, at least up to 24 hr, thus indicating that this inhibitor of protein synthesis did not merely delay the onset of cell damage, but prevented it. The time sequence of appearance of morphological damage to the cells of the germinal centers of the lymphoid tissue was about the same as that with the crypt cells. Also, like the epithelial cells, these cells showed considerable recovery, such that within 16 hr the Peyers' patches showed at most only minimal atrophy of the germinal centers. The peripheral lymphocytes about the centers were intact and not remarkable at this time.

The findings with HN2 or X-irradiation were somewhat different and more complex in that these treatments did not induce a single cycle of cell death followed by recovery, but rather a progressive sequence of cell damage lasting many hours. The epithelial changes during the first few hours were virtually identical with those observed with ara-C or hydroxyurea, both in respect to time sequence and morphology. Light microscopic evidence of cell damage was evident at about 2 hr after HN2 or X-irradiation and was fully developed by 3 hr. However, no recovery was seen at 8, 16, or 24 hr. Instead, there was evidence of continuing cell death with progressive loss of crypt cells, so that the crypts at 24 hr were much shorter than in control animals (Fig. 4). In addition, the remaining crypt cells at this time still showed signs of acute cell damage, as well as other changes, such as cytoplasmic vacuolization and large irregular nuclei. Thus, the acute effects of these treatments, as is well established by many previous studies (e.g., Ref. 28), last for at least 24 hr. It is likely that this is due to repeated and consecutive cycles of cell damage with progressive depletion of the cell population. The lymphoid tissue in the intestine showed acute cell damage in the germinal centers similar to that seen with ara-C or hydroxyurea. However, no evident recovery occurred within 24 hr, the tissue showing progressive atrophy with striking loss of the lymphocytes about the germinal centers.

The protection conferred by 1 dose of cycloheximide lasted for roughly 6 to 8 hr, at which time evidence of cell death began to appear. At 24 hr the crypt epithelium showed quite marked stigmata of cell death, roughly equivalent to the appearance at 3 to 3.5 hr after treatment with HN2 or X-ray alone. However, a 2nd dose of cycloheximide at 8 hr conferred a remarkable degree of protection of the intestinal epithelium at 24 hr (Fig. 5). The thickness of the epithelium is similar to that of control animals and only an occasional dead cell can be seen. In contrast, the lymphoid tissue showed no evident protection at any time (Figs. 10 and 11). At 24 hr the degree of lymphoid tissue destruction was as severe with cycloheximide as without it.

The findings in experiments lasting longer than 24 hr would be most interesting. Unfortunately, experiments of this duration have not been undertaken because of the problem of devising a dosage schedule for cycloheximide.
which would produce a more or less continual inhibition of protein synthesis of the appropriate degree (see below) for periods longer than 24 hr. One of the many critical questions to be answered is whether cycloheximide is only delaying the onset of cell death with HN2 or X-irradiation or is conferring complete protection.

**Other Inhibitors of Protein Synthesis.** A consideration basic to the understanding of the mechanism of action of cycloheximide in this system is whether the protective effect is truly due to inhibition of protein synthesis or is the result of some other property of this compound. This led to the study of another inhibitor of protein synthesis, tenuazonic acid (α-acetyl-2-sec-butyltetramic acid) (18, 37, 40). Another well-known inhibitor of protein synthesis, puromycin, could not be used since it is very damaging to cells of the gastrointestinal tract as well as of the pancreas (11, 24), presumably due to the large number of peptide intermediates released into the cell by the interruption of the synthesis of proteins (24). Emetine, a compound reported to have effects on protein synthesis similar to those of cycloheximide (16), was also unsuitable because of its damaging action on the intestinal mucosa.

The structures of tenuazonic acid (I) and cycloheximide (II) are shown in Chart 2. Tenuazonic acid has been reported to inhibit protein synthesis in several organs in rats and mice (37) and we have confirmed this finding. It has been suggested that this agent may interfere with the release of nascent protein from the ribosome (37).

![Tenuazonic Acid and Cycloheximide](image)

**Chart 2.** Chemical structures of tenuazonic acid (I) and cycloheximide (II).

Tenuazonic acid has protective effects against cell death induced by ara-C and HN2 similar to those of cycloheximide. The effect is selective in that intestinal crypt epithelial cells are protected but lymphoid tissue is not. It protects when administered either before or up to 30 to 45 min after the necrogenic stimulus. Like cycloheximide (44), tenuazonic acid inhibits leucine incorporation into protein and thymidine incorporation into DNA (Chart 1) in the intestine, but does not inhibit uridine incorporation into RNA. Thus, although the 2 compounds are different chemically, their effects on macromolecule synthesis in the intestine and in the damaging action of ara-C or HN2 upon crypt epithelium are very similar. Therefore, it may be tentatively concluded that both protect by virtue of a common effect on protein or DNA synthesis. Since ara-C and hydroxyurea inhibit DNA but not protein synthesis and have actions on crypt cells quite different than do the 2 protective compounds, it appears most likely that it is the effects on protein metabolism and not DNA metabolism that are responsible for the protective action of the 2 compounds.

**Relationship between Levels of Inhibition of Protein Synthesis and Protective Effects.** Not only is there a qualitative similarity among the protective compounds, but there is also a quantitative one. With both inhibitors, a similar relationship between the degree of inhibition of protein synthesis and the protective effect with different doses exists (Chart 3). As is evident from Chart 3, the line of separation between protective and nonprotective doses of each of the compounds falls between 70 and 80% inhibition of protein synthesis. Amounts of each inhibitor producing less than 70% inhibition are without effect on cell death induced by ara-C or HN2. Amounts inducing an 80% or more inhibition of protein synthesis are maximally effective, while doses producing an intermediate degree of inhibition are quite variable. Wistar rats were utilized, in the main, in experiments with cycloheximide, while Fischer rats were used with tenuazonic acid. The Fischer animals are genetically quite homogeneous, while the Wistar rats are not. Although both strains showed the same qualitative effects with both compounds, the quantitative reproducibility with tenuazonic acid was satisfactory only with the Fischer animals. This is particularly important with this inhibitor of protein synthesis, since the difference between the effective and highly toxic doses is quite narrow. Doses of tenuazonic acid above the range of 80 to 100 mg/kg body weight are themselves damaging to the crypt epithelial cells and thus are not useful in the analysis of the relationship between protein metabolism and cell death. With cycloheximide, virtually no irreversible cell damage is seen unless a dose of 1.5 mg/kg or larger is repeated several times at hourly intervals.

![Inhibition of Incorporation of Leucine-14C into Total Protein](image)

**Chart 3.** Inhibition of incorporation of leucine-14C into total protein of the first 15 cm of the small intestine by cycloheximide (○—○) or tenuazonic acid (○—○) as a function of administered dose. The period of incorporation was 10 min.
Protective Effect in Mice. The selective protective effect of cycloheximide against crypt epithelial damage induced by HN2 is seen also in mice. Although our experience with mice is quite limited, the results with cycloheximide and HN2 indicate that this species shows the same protective phenomenon as does the rat. However, the mouse is very much less sensitive to cycloheximide, such that the effective dose range for protective and inhibitory effects on protein synthesis is 40 to 50 times greater than in the rat (11).

Specificity of Protective Effects of Cycloheximide against Cell Death. The protective effect of cycloheximide against cell death is not a general phenomenon with necrogenic agents. Not only is the lymphoid tissue unprotected against cell damage induced by ara-C, HN2, or X-rays, but there is also no protection of the renal cortical epithelium during ischemia. Treatment of rats with cycloheximide had no discernible effect upon necrosis of the proximal convoluted tubule induced by temporary ligation of the renal pedicle (47), although cycloheximide has a marked inhibitory effect on protein synthesis in this organ (43). In addition, pretreatment of rats with cycloheximide accentuated rather than protected the intestinal crypt epithelium against damage induced by large doses of tenuazonic acid and often had little or no effect upon crypt cell necrosis induced by hydroxyurea (see below). Thus, inhibition of protein synthesis is not protective against cell death in general, but only with certain types of cell damage.

Many experiments were done in this study with hydroxyurea as the necrogenic agent. As indicated above, the general characteristics of crypt cell damage induced with this agent (30) resembled closely those seen with ara-C (H. S. Schwartz, personal communication). The protective effect of cycloheximide or tenuazonic acid was quite variable. Some experiments showed striking protection, while others showed little. The reason for this lack of reproducibility is not evident. However, there have been suggestions that hydroxyurea may have metabolic effects (2, 4, 39, 50) in addition to inhibition of DNA synthesis via interference with the reduction of ribonucleotides to deoxyribonucleotides (1, 14, 22, 49). Further analysis of this hopefully may clarify the possible relationship between hydroxyurea, protein synthesis, and cell death.

DISCUSSION

It is tentatively concluded from the results of the present study that protein synthesis is somehow important in the lethal response of some continuously proliferating cells in vivo, such as the intestinal epithelial crypt cells, to 3 different necrogenic stimuli: ara-C, HN2, or X-irradiation. This involvement of protein synthesis in this form of cell death appears to be reasonably specific, since ischemic cell death in the kidney and crypt cell damage by hydroxyurea or by large doses of tenuazonic acid are not regularly influenced in any obvious way by an inhibitor of protein synthesis.

ara-C probably inhibits DNA synthesis either by acting directly upon DNA polymerase (15) or by incorporation into the DNA strand with subsequent premature chain interrup-tion (26). Chromosome breaks are seen in some eukaryotic cells with this inhibitor (e.g., Ref. 27).

Induction of the nature of the primary biochemical lesions induced by nitrogen mustard or other alkylating agents is still poorly understood (see Refs. 32, 48). However, there is increasing evidence that alkylation of DNA is an early effect. As observed in this study there is often a moderate degree of inhibition of DNA synthesis without any apparent effect on protein synthesis. In addition, chromosomal damage by alkylating agents has been repeatedly documented.

The situation with X-irradiation and other forms of ionizing radiation is similarly somewhat unclear. However, an early and possibly primary effect on DNA is believed to be important in the cytotoxic action (see Ref. 23 for recent review). Chromosomal damage is often seen.

It appears that cell damage induced in the intestinal crypt epithelium by these 3 agents is an active, rather than passive, process and is due to a metabolic reaction of the cells to some preceding biochemical lesion, and not to the biochemical lesion per se. The reaction would appear to be dependent upon new protein formation and is perhaps akin to enzyme induction. Since inhibition of DNA synthesis and/or the direct alteration of DNA appear to be early and important biochemical lesions induced by the 3 necrogenic agents studied, it is suggested that a special more or less direct metabolic link occurs between some phase of DNA metabolism and enzyme induction in some types of continuously proliferating cells. Conceivably, the enzymes induced could be active in various forms of DNA repair. The rapid overproduction of 1 or more of these may cause irreparable damage to vital macromolecules in the cell.

That some enzymes related to DNA metabolism may be induced by inhibitors of DNA synthesis is well documented. For example, thymidine kinase has been found to show a relatively large increase in activity in several types of eukaryotic cells after treatment with ara-C, mitomycin C (20, 21), or amethopterin (41), dCMP deaminase (20) and dihydrofolate reductase (13) after ara-C, and an acid and an alkaline deoxyribonuclease after mitomycin C (42). In at least some of these instances (20, 41, 42), the increase in enzyme activities appeared to be dependent upon new protein synthesis. Kit et al. (20) suggested that the accumulation of extra cells in the late G1 phase of the cell cycle as a result of inhibition of DNA synthesis may be one possible basis for the increase in thymidine kinase following ara-C or mitomycin C. This enzyme shows an increase in activity at this time in the cell cycle in some cells (3, 17). However, the similarity in the overall pattern observed in our study between ara-C and HN2, despite the large differences in the degree of inhibition of DNA synthesis (Chart 1), makes this hypothesis less attractive. In the same vein, it should be emphasized that ara-C or HN2, like hydroxyurea, are highly selective with respect to their necrogenic action on proliferating cells. For example, regenerating liver cells, during their predominantly 1-cycle phase of proliferation, show no stigmata of cell death with ara-C or HN2, although liver DNA synthesis was essentially completely inhibited by the ara-C and the intestinal crypt cells are severely damaged in the same animal (12). Thus, cell death in a proliferating cell
is not simply the direct consequence of a biochemical lesion related to DNA metabolism, such as interference with replication, but depends also upon the type of organizational pattern of the target cell. A similar difference in response to hydroxyurea between cells programmed for continual proliferation and those engaged in a single cycle of proliferation has been observed (12).

The behavior pattern of the epithelial cells of the intestinal crypts in the present study shows a resemblance to the phenomenon of "unbalanced growth" and thymineless death first described by Cohen and Barner (6, 7) in Escherichia coli and subsequently by many authors in other microorganisms. An apparently similar behavior is also seen in some mammalian cell cultures in vitro in the presence of amethopterin, 5-fluorodeoxyuridine, or ara-C (8, 10, 19, 35). This is characterized by an interference with DNA metabolism and a cessation of cell division in the presence of continuing RNA and protein synthesis. The cells become much larger with net increases in RNA and protein content. In addition, the loss of viability in several microorganisms and in eukaryotic cells is often prevented or delayed by inhibition of protein synthesis and in some cases also by RNA synthesis (9, 33, 35). An apparently similar protective effect of cycloheximide and acetoxycycloheximide against loss of cell viability has also been reported for irradiated cells (9, 23, 31).

Despite the many overall similarities, there are some obvious differences between the cell damage seen in vivo and the unbalanced growth phenomenon in vitro. One such difference related to the time sequence. Unbalanced growth appears to require many hours (often 12 to 24 hr) for loss of viability to become evident, while the cell damage in the crypt cells in vivo occurs rapidly (within 2 to 3 hr). Another difference is the absence of any obvious increase in cell size in vivo, a feature very prominent in many of the reports on unbalanced growth. Yet the evident similarities demand a considerable reservation in judgment until the mechanisms of the cell changes in each of the systems have been unravelled.

Unfortunately, a major complication in interpretation in this field concerns the criteria for cell damage and cell death. In vitro, several quantitative indices are available: dye uptake, cell size, and cell counts. Such criteria are not yet available for most in vivo studies, especially for epithelial cells like intestinal crypt cells. Here, major reliance must be placed on overall cell appearance, as determined by light and electron microscopy. It must be emphasized that these are readily observable and easy to identify when present.

An important facet of the present in vivo study is the obvious difference in behavior between different cells, even in the same organ. The crypt epithelial cells of the intestinal mucosa show a pattern of cell damage by interference with DNA metabolism and protection by inhibition of protein synthesis. In contrast, the lymphoid tissue, even in the contiguous portion of the intestinal wall, shows severe cell damage on interference with either DNA and/or protein metabolism. A 3rd type of proliferating cell is present in the regenerating liver and in the salivary gland or renal epithelium stimulated to proliferate (12). These cells show no evidence of cell damage by interference with either DNA or protein metabolism. These differences highlight the importance of cell type and/or the environment in which the cells find themselves. Pfeiffer and Tolmach (29) have emphasized this in studies on the response pattern of mammalian cell in vitro. For example, while Chinese hamster cells are killed by hydroxyurea (38) even during a period of exposure as short as 1 hr during their S phase, HeLa cells were able to withstand up to 19 hr of exposure without significant toxic effects. Both cell lines showed a similar degree of inhibition of DNA synthesis (29, 38). Rosenkranz and Carr (34) also reported that the conditions of growth of E. coli were critical in determining whether or not hydroxyurea had a lethal effect. These considerations emphasize the need to relate in vitro findings to an in vivo environment in order to understand the cellular mechanisms operating in many pathological processes.

Finally, an obvious practical implication of the present study is the theoretical possibility of inducing selective damage to lymphoid tissue by radiation or by some cytotoxic drugs, while protecting the equally susceptible crypt cells (and possibly other epithelial cells engaged in continual proliferation) from concomitant damage. Conceivably, it might be possible by the use of selective protective agents to increase manyfold the administered dosage of radiation or of a cytotoxic agent without harmful effects on the patient as a whole with a disease of the lymphatic system such as a lymphoma as a target. The feasibility of this theoretical possibility depends very much upon the behavior of the cells of the bone marrow to inhibitors of protein synthesis. Although an attempt was made in this study to observe the response pattern of the bone marrow, the complexity of the cellular components of the bone marrow made interpretation impossible. The monitoring of the peripheral blood or of isolated clones of bone marrow cells in the spleen may be feasible alternative approaches. However, this approach will probably require an experimental design of several days duration and will depend upon the ability to maintain a fairly constant inhibition of protein synthesis at a level above 70 to 75% with the available inhibitors.

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Protein Synthesis and Cell Death


Fig. 1. Mucosa of the small intestine 3.5 hr after the i.p. administration of HN2 (2 mg/kg). Note the severe cellular damage to the epithelial cells in the crypts. Compare with Fig. 2. X 300.

Fig. 2. Mucosa of the small intestine 3.5 hr after the i.p. administration of HN2 (2 mg/kg) to animals which had received cycloheximide (1.5 mg/kg) 30 min previously. Note the virtual absence of cell necrosis in the epithelium. Compare with Fig. 1. X 300.

Fig. 3. The mucosa of the small intestine 24 hr after the i.p. administration of ara-C (200 mg/kg). Note the normal appearance of the crypts, including the presence of mitotic figures. X 200.

Fig. 4. Mucosa of the small intestine 24 hr after the i.p. administration of HN2 (2 mg/kg). Note the distortion of the crypts and the presence of many vacuoles in the crypt epithelial cells. Many of the cells in this location showed nuclear aberrations. Compare with Fig. 5. X 225.

Fig. 5. Mucosa of the small intestine 24 hr after the i.p. administration of HN2 (2 mg/kg) to an animal which had received cycloheximide (1 mg/kg) 8 hr after the HN2. Note that the crypts are of normal length and almost normal appearance. Only an occasional cell showing acute cell damage is present. The nuclear alterations observed with HN2 (Fig. 4) are seen only rarely. Compare with Fig. 4. X 225.

Fig. 6. Mucosa of the small intestine 3.5 hr after X-irradiation of the whole organism at 1000 rads over a period of 17 min. Note the severe cellular damage to epithelial cells and crypts. Compare with Fig. 7. X 200.

Fig. 7. Mucosa of the small intestine 3.5 hr after X-irradiation at 1000 rads over a period of 17 min of animals which had received cycloheximide (1.5 mg/kg) 30 min previously. Note the virtual absence of cell necrosis in the epithelium. Compare with Fig. 6. X 225.

Fig. 8. Lymphoid tissue (Peyer’s patch) 3.5 hr after i.p. administration of HN2 (2 mg/kg) to animals which had received cycloheximide (1.5 mg/kg) 30 min previously. Note the extensive necrosis of the cells in the germinal center (G.C.) and the intact periphery of small lymphocytes (S.L.). X 200.

Fig. 9. Lymphoid tissue of the small intestine (Peyer’s patch) 3.5 hr after X-irradiation (1000 rads) of an animal which had received cycloheximide (1.5 mg/kg) 30 min previously. Note the extensive necrosis of cells in the germinal center (G.C.) and the intact peripheral lymphocytes (S.L.). X 225.

Fig. 10. Lymphoid tissue of the small intestine (Peyer’s patch) 24 hr after the i.p. administration of cycloheximide (1.5 mg/kg). Note the intact germinal center (G.C.) and peripheral lymphocytes (S.L.). Compare with Fig. 11. X 225.

Fig. 11. Lymphoid tissue of the small intestine (Peyer’s patch) 24 hr after the i.p. administration of HN2 (2 mg/kg) to animals which had received cycloheximide (1.5 mg/kg) 30 min previously and 8 hr later. Note the extensive loss of cells in the germinal center (G.C.) and some persistence of peripheral lymphocytes (S.L.). Compare with Fig. 10. X 225.
A Probable Role for Protein Synthesis in Intestinal Epithelial Cell Damage Induced in Vivo by Cytosine Arabinoside, Nitrogen Mustard, or X-irradiation
