CHAPTER II

REVIEW OF LITERATURE
Radiation:

As early as 1906 the French radiobiologists Bergonie and Tribondeau recognized that different types of mammalian cells differ greatly in their radiosensitivity. They proposed a law which stated that cells are radiosensitive if they have a high mitotic rate, a long mitotic future and are undifferentiated.

The intestinal epithelium, because of its high proliferative capacity and its significance in the gastrointestinal syndrome, has received much attention in the field of radiobiology. Krause and Ziegler (1906) were the first to report changes in the mouse intestinal mucosa following irradiation. They observed degenerative changes in the intestinal crypts and villi. Since then, there have been several reports on the various aspects of GI lesions caused by ionizing radiations (Regaud et al. 1912, Quastler 1956, Ellinger 1957, Kurtsin 1963, Lesher 1967, Withers and Elkind 1970, Maisin et al. 1971, Uma Devi et al. 1978, Cheeseman et al. 1985, Griffin and Hornsey 1986, Murray and Meyn 1987, Mason et al. 1989, Böhm et al. 1990).

Regaud et al. (1912) in their study on the X-ray damage of GI tract of dogs, irradiated on the abdomen, observed destruction of mucosa, ulcer and perforation. They concluded that the basal cells of the crypt of intestine were more susceptible to radiation damage than the villous epithelial cells. Extensive studies on radiation damage in rodent intestine were made by a number of investigators (Devik 1971, Tsubouchi and Matsuzawa 1973, Cairnie 1973, Hendry and Potten 1974, 1982, Becciolini et al. 1982, Falk 1984, Dewit et al. 1983d, Chwalinski and Potten 1986, Rao and Fritz-Niggli 1988, Overgaard and Matsui 1990).
Pierce (1948) studied the effects of 800 rads of X-rays on the GI tract of rabbits, reporting destructive changes as early as 30 minutes after the exposure. Damage was greater in the small intestine than in the stomach, colon or rectum and was greatest in the crypts. Crypt cells showed extensive damage consisting of nuclear pyknosis, fragmentation and karyolysis. Other changes reported in the irradiated crypts by later workers include karyorrhexis, necrosis, nuclear and cytoplasmic swelling, abnormal mitoses, cytoplasmic vacuolation, enlarged nuclei and hypertrophy (Devik 1968, Potten 1977, Uma Devi et al. 1979, Ijiri and Potten 1984).

Different parameters have been used to assess the small intestinal response to cytotoxic agents. Among these, the crypt survival as measured by the microcolony assay, first developed by Withers and Elkind (1970), is very useful to assess the acute radiation effects and/or drugs on the intestinal tract epithelium. The assay has merit not only as a method for measuring the cellular response of intestinal mucosa, but also as a model for other tissues. The technique involved in this assay is fairly simple and the results are reproducible.

Withers and Elkind (1970), using the microcolony assay, reported a significant reduction in the number of jejunal crypts of mouse after 3.5 days of exposure to radiations. Since then the assay has been used by several investigators to study the changes in the proliferative cells of mouse small intestine following irradiation (Hendry and Potten 1974, 1982, Potten and Hendry 1975, Ito et al. 1986, Potten et al. 1988). Reynaud and Travis (1984) assessed the late radiation damage
in the mouse jejunum after priming doses (9.0 or 11.5 Gy) and re-
treatment with single doses of X-rays, using this method. It was
observed that jejunal crypt cells of previously irradiated mice were
more resistant to a second dose of irradiation.

Since the beginning of research into the effects of ionizing radia-
tion on living cells it has been known that one of the first effects
in the actively dividing cells is a temporary reduction in the number
of cells undergoing division and this effect has been called 'mitotic
delay' or 'division delay' (Elkind and Whitmore 1967). Lesher (1967,
1968) reported the inability of intestinal crypt cells to progress
through cell cycle following irradiation of mouse with 3.0 Gy and
a ten fold reduction in the number of mitotic figures at 1 h after
0.75 Gy. Similar mitotic inhibition was observed by many workers
after different doses of radiation (Devik 1973, Tsubouchi and Matsuzawa
which lasted as long as 3 h and 8 h for 3.0 Gy and 8.0 Gy respectively.
Devik (1971) reported that following mitotic depression there was
an increase in the mitotic counts, most mitoses being abnormal. Similar
findings were reported by Uma Devi et al. (1979) following exposure
of mice to 1000 R of gamma radiation.

Gupta and Uma Devi (1979) studied the effect of different doses
of gamma radiation (5.0, 7.5, 10.0 Gy) on the mitotic cells of intestinal
crypts and reported a dose-dependent increase in the number of abnormal
mitoses. Uma Devi and Mathur (1981) observed mitotic suppression,
necrosis and pyknosis in mouse ileum at early intervals after irradia-
tion. At recovery when mitotic counts were normal some of the mitoses
exhibited abnormalities.
Chwalinski and Potten (1986), in their study on the sensitivity of mouse small intestinal crypt cells to irradiation, reported that the duration of the mitotic delay was strongly dependent on the radiation dose and the cell position. The cells at the top of the crypt were inhibited for 1 h per gray and the duration was shorter for mature proliferating cells and longest for the stem cells.

The epithelial cells in a crypt of the mouse small intestine may be regarded as forming a well defined and delimited cell population which is well suited for quantitative investigation following radiation damage (Devik 1971). Devik (1971, 1973) estimated the number of epithelial cells in the mouse small intestinal crypt following X-irradiation with different doses in the range of 200-2400 rads and reported a significant decrease in the cell number which was more pronounced and lasted longer at higher doses. Similar reduction in the crypt cells of mouse intestine following irradiation has been reported by several investigators (Tsubouchi and Matsuzawa 1974, Potten and Hendry 1975).

Following exposure to high doses of radiation very few surviving cells remained in the intestinal crypts (Potten et al. 1978). Exposure to 5.0, 7.5 and 10.0 Gy of gamma rays resulted in marked degeneration and reduction in the crypt cells (Uma Devi et al. 1978, 1979, Gupta and Uma Devi 1984, Mathur 1980, Uma Devi and Mathur 1981). Potten et al. (1988) estimated the number of clonogenic cells in the intestine of mouse exposed to a single dose (8.0 Gy) of gamma rays and observed an initial reduction in the cell number. Following this there was an exponential increase in cellularity.
Several sensitive assay systems have been used to detect radiation-induced damage in animal experiments, one such system being cell death in mouse intestine. Radiation-induced cell death has been reported by several workers in the mouse small intestinal crypt (Quastler 1956, Devik 1971, 1973, Potten et al. 1978, Uma Devi et al. 1978, 1979, Uma Devi and Mathur, 1981, Gupta and Uma Devi 1984, Ijiri and Potten 1990). Devik (1971) studied the number of radiation-induced dead cells in the mouse jejunum after different time intervals from one hour to seven days following exposure to X-irradiation in the range of 200-2400 rads. Dead cells with pyknotic and fragmented nuclei appeared suddenly at about two hours reaching a peak at three hours after irradiation. Thereafter the number gradually returned to normal. Similarly, Tsubouchi and Matsuzawa (1974) reported immediate appearance of pyknotic nuclei in the mouse intestinal epithelium after 1000 rads of X-irradiation. Uma Devi et al. (1979) observed maximum number of pyknotic nuclei and necrotic cells at 3 h after 1000 R of gamma irradiation in the mouse jejunal crypt cells. Hendry and Potten (1982) and Ijiri and Potten (1984) demonstrated that the incidence of dead cells increased with time at three to six hours after irradiation. At doses higher than 1 Gy the high incidence persisted for longer.

Cell death can be recognized either by the loss of the reproductive capacity of the cells or by a morphological appearance of a cell undergoing apoptosis. Apoptosis is a pattern of cell death involving nuclear pyknosis, cytoplasmic condensation and karyorrhexis (Kerr et al. 1972, Wyllie 1981). The incidence of radiation-induced apoptosis depended on the cell position and the dose applied (Hendry et al. 1982). Ijiri and Potten (1987) studied the spatial distribution of
apoptotic cells in the mouse intestinal epithelium following exposure to gamma rays, β rays and tritiated thymidine and displayed peak position of apoptosis near the base of the crypt. Ijiri (1989) studied the frequency of apoptosis after treatment with continuous irradiation in the crypts of five different portions of mouse bowel. He concluded that the apoptotic incidence was higher in the small intestine than in any part of the large bowel.

The radiosensitivity of intestinal crypt cells differ depending on the time of irradiation. A diurnal variation in the number of progenitor cells of irradiated small intestine was indicated in mouse (Hendry 1975, Ijiri and Potten 1988) and rat (Becciolini et al. 1982). A definite circadian rhythm has also been reported for the radiation-induced apoptosis in the mouse small intestine (Duncan et al. 1983, Ijiri and Potten 1988).

The cells of villi exhibited a lesser degree of radiosensitivity characterized by nuclear swelling, vacuolated cytoplasm and clumping of goblet cells (Quastler 1956, Quastler and Hampton 1962, Tsubouchi and Matsuzawa 1973, Becciolini et al. 1976). Exposure of mouse to lethal doses (10.0 and 12.0 Gy) resulted in a significant reduction in the height of villi which became broad with blunt surface. The tips of villi were ruptured and the lumen of the intestine was filled with exudate which contained a large amount of denuded epithelium (Uma Devi 1977, Uma Devi et al. 1978, Uma Devi and Mathur 1981). Similarly, Becciolini et al. (1982), in their study of the effect of irradiation at different times of the day, reported a reduction in
Radiation-induced functional alterations in the mouse intestinal villi were studied by several investigators. Rydberg and Johanson (1975) compared the DNA strand breaks in the crypt and villous cells after irradiation and concluded that the yield of breaks induced per rad and the kinetics of repair were similar in both the cell populations. However, Murray and Meyn (1987) found that proliferating cells of crypt repaired DNA strand breaks more rapidly than the differentiated cells of villus.

Several studies on cell migration through villi have indicated that it continues at a near normal rate after sublethal or lethal (Potten et al. 1982) and even after supralethal doses (Tsubouchi et al. 1983).

There are a few reports available on the radiation-induced changes in other cell populations of mouse small intestine. Quastler (1956) observed severe oedema in the connective tissue following irradiation. Goblet cells disappeared when the number of crypt cells reduced significantly after exposure to gamma rays (Devik 1971). Uma Devi (1977) reported an apparent increase in the goblet cell number and reduced submucosal connective tissue after treatment with high doses of radiation.
The mouse intestinal response to ionizing radiation varies depending on the mode of irradiation. Lesher and Lesher (1974) studied the effects of partial-body X-irradiation on the epithelial cell kinetics and reported that changes in the cells were less severe than that observed with whole-body irradiation. Also, the recovery took place more rapidly after partial-body irradiation. Similarly, Hamilton (1977) observed an enhancement of colon crypt survival of about 27% by local as opposed to whole-body irradiation. Dewit et al. (1985d) reported a significantly higher $LD_{50/15}$ after partial-body irradiation (16.3 Gy) than after total-body irradiation (14.3 Gy) for acute intestinal syndrome. However, Mason et al. (1989) in a similar kind of study, did not find any significant difference in stem cell survival in pathogen-free mice.

It is well known that the intestinal mucosa possesses a marked capacity for recovery after irradiation. Following exposure of the animals to irradiation, radiation-induced cell loss through mitotic delay, apoptosis and migration of crypt cells to villus lead to cell loss. Previously reported experiments showed that after single whole-body exposure, damage, repair and recovery of the rapidly dividing crypt cells of the intestinal epithelium follow a well defined pattern. During recovery the surviving cells resume mitosis and with each cycle severely damaged cells are eliminated. The extent of damage and the degree of compensatory reaction increase with dose (Lesher and Bauman 1969).

Following irradiation with high doses a temporary and compensatory 'overshoot' in mitosis, repopulation of crypt with the surviving
cryptogenic cells and reduction in the number of dead cells indicated recovery (Quastler 1956, Devik 1971, Lesher and Lesher 1970, Tsubouchi and Matsuzawa 1973, Potten and Hendry 1975, Uma Devi et al. 1979, Potten et al. 1984, 1988). Two mechanisms, namely shortening of the mitotic cycle and increase in the size of the proliferative compartment contributed to an increase in the cell production in order to compensate for the cell destruction by irradiation. In addition, the cell loss brought about the recruitment of dormant (G₀) cells in the crypt base into cycle (Potten et al. 1984, Tsubouchi and Potten 1985). Repair and recovery by shortening of the cell cycle and an increase in the crypt cell proliferation also took place after continuous irradiation as well as dose-fractionation (Wimber and Lamerton 1966, Fry et al. 1963, Lesher et al. 1966, Maisin et al. 1977). Recovery of villi following irradiation was demonstrated by Uma Devi (1977), Uma Devi et al. (1978) and Becciolini et al. (1982).

Nitroimidazoles:

The nitroimidazoles, metronidazole (Metro) and misonidazole (MISO), have been considered very effective sensitizers of hypoxic cells to ionizing radiations in vitro and in vivo and have been under clinical trials. A large number of reports on their radiosensitization potential has accumulated in literature.

Denekamp and Michael (1972) using skin clone assay found sensitization factors of 1.25 and 1.35 after intraperitoneal (i.p.) injection of 0.5 and 1.0 mg/g body weight (b.wt.) respectively of Metro in mouse skin made artificially hypoxic. Rauth (1973), studying the effect of Metro at 1.5 and 2 mg/g.b.wt. reported sensitization
factors in the range of 1.4 - 1.6 in KHT mouse tumor. Similar sensitization was observed by Denekamp et al. (1974) who obtained an enhancement ratio (ER) of 1.5 for mouse epidermal cell survival. Begg et al. (1974), based on $^{\text{125}}$I-UdR release assay, demonstrated positive radiosensitization in a mouse tumor by Flagyl. Similarly, Denekamp and Harris (1975) obtained an ER of 1.6 for mammary carcinoma in mice. The drug sensitized tumor cells both in air-breathing animals and in clamped tumors.

Brown (1975a) studied the effect of Metro on the radiosensitivity of the EMT6 tumor and MDAH/M C4 mammary carcinoma in mouse. Maximum sensitization was observed at 30 minutes (min) after an intraperitoneal (i.p.) injection of the drug, with a dose modifying factor (DMF) of 2.5. However, Inch et al. (1977) could not find any effect of the drug at 1.2 mg/g.b.wt. on tumor bearing mice.

More recently, the radiosensitizing effect of Metro was tested on the changes in bone marrow cellularity after whole-body irradiation of rats protected with abdomen compression. Metro administered to the unprotected rats had no effect, but in abdomen compressed rats it led to a pronounced reduction of radioprotection. Dose enhancement ratio (DER) for lower-body compressed animals corresponded to 1.96 which decreased to 1.52 by Metro application (Vodička et al. 1989).

Denekamp et al. (1974) first demonstrated sensitization of mouse epidermal cells by MISO using the skin clone assay. Mice were temporarily made hypoxic and MISO was administered at different
concentrations before irradiation. Sensitization was evident with enhancement ratios of 2.2, 1.36 and 1.12 at drug doses of 1.25, 0.1 and 0.02 mg/g.b.wt. respectively.

Most of the studies with MISO demonstrated significant sensitization of a wide variety of mouse tumor systems. For drug doses of 1 mg/g.b.wt. most of the tumor systems gave sensitization ratios of the order 2. Even for the lower drug doses of 0.2 - 0.3 mg/g.b.wt., ratios as high as 1.7 - 1.9 could be obtained (Sheldon et al. 1974, Denekamp and Harris 1975, Adams and Fowler 1976, Bleehen et al. 1977). Enhancement ratios in the range 1.0 - 1.3 have been reported for MISO given after irradiation in various tumors including CBA carcinoma 'NT' (Denekamp and Harris 1975) and WHT squamous carcinoma (Hill and Fowler 1977).

Rofstad and Brustad (1978, 1979), on the basis of their studies on a human malignant melanoma grown in athymic nude mice, concluded that MISO was a more efficient hypoxic cell radiosensitizer than Metro when given along with fractionated gamma exposure (daily 3.75 Gy over four consecutive days).

Grdina et al. (1984) using lung colony assay reported that when 1 mg/g.b.wt. of MISO was combined with radiation an ER of 1.9 was observed for cell survival from murine fibrosarcoma. More recently, Hofer et al. (1987) studied the kinetics and extent of cell death in hypoxic BP-8 murine sarcoma cells using \(^{125}\)I UdR - pre-labelling assay. They reported that tumor cell death was a function of drug concentration, the survival was 100% below 1 mg/ml, 73% at 2 mg/ml, 28% at 4 mg/ml and 9% at 6 mg/ml. When MISO was administered before 10.0 Gy of gamma rays, it caused significant radiosensitization.
Survival values of 44% for the 10.0 Gy control group and 10% for the MISO group were observed.

Cytotoxic and radiosensitizing actions of MISO depends on the duration of drug exposure. Hypoxic BP-cells received 0.5 mg/ml MISO followed by 15.0 Gy at various intervals after the addition of the drug. MISO exposure for 1 min reduced the cell survival to 6% and the survival was 2.1% and 1.8% for exposures of 30 and 60 min respectively (Hofer et al. 1987).

There are increasing number of observations which indicate that part of the radiosensitization by nitroimidazoles results from cytotoxicity of these drugs. A selective hypoxic cytotoxicity has been observed in multicellular spheroid systems by Metro (Sutherland 1974a, Mohindra and Rauth 1976) and by MISO (Adams et al. 1976, Hall and Roizin-Towie 1975, Sridhar et al. 1976, Korbelik et al. 1981). Metro-induced cytotoxicity has also been demonstrated for hypoxic Ehrlich ascites cells (Foster et al. 1976) and for HeLa and human bone-marrow cells (Mohindra and Rauth 1976).

Several reports indicated the cytotoxicity of Metro as well as MISO in various murine tumors. Begg et al. (1974) reported an inhibition of tumor growth with Metro alone either as a single dose (1.5 mg/g) or as repeated injections of 0.3 mg/g. A similar decrease in the rate of growth has been found with 'BA' mouse mammary adenocarcinoma (Inch and McCredie 1975). Foster et al. (1976) also showed cytotoxic effect of Metro on the 'NT' tumor of CBA/CA mice.
Denekamp and Harris (1975) have reported an additional delay in the regrowth of 'Ca NT' tumors in mice injected with Ro-07-0582 (1 mg/g) when the injection was given after irradiation. At the same concentration MISO killed about 90% of tumor cells in the EMT6 tumor in BALB/c mice and the MDAH/M Ca_{4} tumor in C_{3}H mice (Brown 1975b). Conroy et al. (1980) using clonogenic assay reported MISO-induced toxicity in EMT6 tumors, displaying maximum cytotoxicity at 18 - 24 h after a single i.p. dose of 1 mg/g.b.wt.

Preliminary clinical trials of nitroimidazoles as radiosensitizers were initiated because of their wide distribution in tissues and particularly their long metabolic life. Urtasun et al. (1976) showed distinct advantage of Metro in glioblastoma patients treated with 6 g/m^{2} Metro 4 h before irradiation. They also demonstrated the superiority of the drug combined with radiation in prolonging the survival of patients (Urtasun et al. 1977a). Metronidazole was also used in advanced tumors (Karim 1978) and in Crohn's disease (Mitelman et al. 1976).

Large scale trials with MISO showed that the drug may improve the radiotherapeutic management of a wide range of tumors. Thomlinson et al. (1976) reported sensitization in patients with multiple subcutaneous nodules from a carcinoma of cervix and bladder cancer. The drug has also been tested in the treatment of patients with carcinoma of breast, prostate, pulmonary metastases, osteosarcoma (Ash et al. 1979), and head and neck cancers (Paterson et al. 1981, MRC working party 1984) with some beneficial effect.

The hypoxic cytotoxicity of nitroimidazoles may have potential as a chemotherapeutic tool targeted at resistant hypoxic cells. On
the other hand such cytotoxicity could be detrimental to partially hypoxic normal cells (Korbelik et al. 1981). If radiation exposure also enhances the drug toxicity of aerobic tissues, this could be a deciding factor in the clinical application of these drugs for tumor therapy.

Results in human patients indicated certain side-effects of nitroimidazoles. Urtasun et al. (1975) observed immediate toxic effects like nausea and vomiting in patients who received oral doses of Metro at 8 - 15 g over 3 weeks. A dose of the order of 200 mg/kg of Metro produced GI symptoms (Dische et al. 1976) and the drug was reported to produce chromosome aberrations in patients (Mitelmen et al. 1976). Similarly, GI symptoms limited the dose of MISO to below 140 mg/kg.b.wt. (Gray et al. 1976). It was suggested that the total dose of MISO that can be administered cannot exceed 25 g, because high doses resulted in peripheral neuropathy (Urtasun et al. 1978, Dische et al. 1977).

In clinical testing of new chemicals or treatment modalities the response of normal tissues must be given equal care as tumor control. Hence several toxicological studies of nitroimidazoles have been carried out both in animals and in man. In a toxic study in dogs, Schärer (1972) observed that daily doses of MISO resulted in ataxia, convulsions and death. Some toxicity was also observed for aerobic cells but only after larger exposure times (Brown 1975a, Stratford et al. 1978, Adams 1979).

Nitroimidazoles have also been found to increase the sensitivity of some normal tissues to radiation. Various concentrations (0.1 - 1.0 mg/g)
of MISO were reported to give dose modifying factors (DMF) in the range 1.0 - 1.3 for normal mouse skin (Brown 1974, Denekamp et al. 1974, Stewart et al. 1982). Stone and Withers (1974) reported similar sensitization of mouse skin by metronidazole. Yuhas et al. (1977) in their study on MISO-sensitization of normal mouse tissues observed DMF in the range 0.9 - 1.2 for thigh and foot skin, hair loss and LD_{50/30}. Gonzales and Breur (1978) demonstrated positive sensitization of tibial cartilage to irradiation by MISO. Similarly, Hendry (1978a) obtained DMFs of 1.1 - 1.5 for tail necrosis.

Suzuki et al. (1977) studied the radiosensitization of mouse testis by MISO (1 mg/g) injected 30 min before exposure to 1300 rads. $D_0$ value for irradiation alone was 181, which decreased to 140 for irradiation after treatment with MISO, indicating significant sensitization of spermatogenic stem cells.

However, Stone and Withers (1974) and Hendry (1978b) did not find any sensitization in mouse jejunum either by Metro or by MISO. Similarly, Uma Devi and Bisht (1987) could not observe any difference in the frequency of micronuclei in the irradiated mouse bone-marrow cells after treatment with 0.5 mg/g of MISO.

No sensitization of human skin was observed in patients treated with MISO (Dische et al. 1976, 1979). The only clinical trial in which enhanced normal tissue reactions have been reported is the Italian study of Arcangeli and Nervi (1980) for irradiations of oropharyngeal sites.
Pharmacokinetic studies indicated that after an oral dose of radio-labelled misonidazole to mice, rats, baboons and human volunteers, peak plasma concentrations of radioactivity were rapidly reached within less than 2 h after administration with a half-life ($t_1/2$) of 12.5 h for man (oral, 13 mg/kg) and 1.0 - 1.5 h for mouse (i.p. 100 mg/kg) (Flockhart et al. 1978). A comparison between the mouse plasma levels of misonidazole given intravenously (i.v.) and i.p. showed that after both modes of drug administration the drug decreased logarithmically, with a plasma half-life ($t_1/2$) of approximately 1.5 h (Chin and Rauth 1981).

Numerous hypotheses have been advanced to explain the mechanisms responsible for the cytocidal and radiosensitizing effects of nitroimidazoles. However, the precise mechanism of action is not yet well understood. There is evidence that metabolic reduction of the nitro group is responsible for the toxic effects of MISO (Varghese et al. 1976, Taylor and Rauth 1978, Josephy et al. 1981, Brown 1982). Several investigators maintain that a close correlation exists between cytotoxicity and DNA damage in MISO treated cells (Palcic and Skarsgard 1978, Olive et al. 1979, Skov et al. 1979).

DNA is damaged by hypoxic exposure of mammalian cells to Ro-07-0582 (Skov et al. 1979, Olive et al. 1979) and it has been proposed that the failure of cells to repair accumulated DNA damage may be responsible for cell death (Palcic and Skarsgard, 1978). Chin and Rauth (1981) studied the drug levels and kinetics of MISO in mouse
normal tissues using radio-labelled misonidazole and concluded that the prime sites for nitro reduction in the whole animal are liver and GI tract. Brown (1982) suggested that cytotoxicity of nitro-imidazoles depends on their reduction potential. Upon entry into the cell the metabolic nitroreduction of imidazoles lead to the formation of reactive intermediates like nitroso, hydroxylamine and free radicals. These products bind to cellular macromolecules, viz. DNA and glutathione. This binding results in H-abstraction from DNA leading to single strand breaks and cell injury. The binding of reactive metabolites to glutathione depletes intracellular glutathione level, thus sensitizing the cells to the drug.

The hypoxic cytotoxicity of MISO is a function of concentration of the drug (Hall and Roizin-Towle 1975, Moore et al. 1976). Direct drug-induced cell death also increased with the length of exposure, temperature of incubation and the degree of hypoxia (Stratford and Adams 1977, Hofer 1978, Wong et al. 1978). According to Brown (1977) MISO-cytotoxicity is not limited to the radiobiologically hypoxic cells. He proposed that hypoxia is needed to trigger the cytotoxic effects, but that the killing is not confined to the hypoxic cells.

Hofer et al. (1987) in their investigation on the mechanism of radiosensitizing and cytocidal effects of MISO, concluded that these two effects are two distinct phenomena mediated by different cellular mechanisms. Radiosensitization by MISO represents a two-component effect composed of true modification and dose additive damage interactions, but these additive effects occur at a site different from the cellular structure responsible for direct drug-induced cell death.
Bleomycin:

Bleomycin (BLM) is an antibiotic and antineoplastic glycopeptide. Umezawa et al. (1967) first demonstrated the toxic and antitumor effect of BLM in Escherichia Coli at a concentration of 2 μg/ml. They reported that the chemical also inhibited DNA synthesis in HeLa cells and the growth of Yoshida sarcoma cells. The antitumor effect of BLM was also evident against Ehrlich carcinoma, Rous sarcoma and ascitic hepatoma in mouse (Umezawa et al. 1967). They pointed out that the daily injections of BLM resulted in an additive toxic reaction.

Matsuzawa et al. (1972) reported a synergistic effect on mouse mammalian carcinoma cells following treatment with BLM and radiation. Similarly, Jørgensen (1972) presented experimental evidence that mouse squamous carcinoma might be controlled more successfully when BLM is added to radiotherapy. He tested three different schedules of BLM in the treatment of a chemically-induced carcinoma in C3H mice and concluded that BLM treatment resulted in a significant reduction of tumor growth.

Bienkowska et al. (1973) demonstrated a subadditive effect in HeLa cells when BLM was administered at 150 μg/ml before or after irradiation. Similarly, Bleehen et al. (1974) studied the effect of combination of BLM and radiation in bacterial and mammalian cell lines and reported marked sensitization of radiation resistant bacteria. In contrast, Sakamoto and Sakka (1974) and Kimber (1979) were unable to demonstrate any interaction of the two modalities in mouse squamous carcinoma and in Chinese hamster ovary (CHO) cells. However, Terasima et al. (1975) reported a supra-additive effect in mouse L5...
HeLa S<sub>3</sub> cells when BLM (10 µg/ml) was used 30 min before and during 4.0 Gy of radiation. Similar results were also reported by Roizin-Towle (1979) in CH-V-79 cells.

In combined modality therapy of radiation and BLM the interaction of BLM and radiation depends on the time interval between and the sequence of administration of the two agents and vary according to the tumor and tissue in question. Von der Maase (1985) evaluated the response of mouse mammary carcinoma to BLM alone and BLM combined with radiation. The drug was administered as single dose either 15 minutes before or 4 h after graded single doses of irradiation. It was observed that BLM alone increased tumor growth delay and doubling time, but it had no effect on the radiation response of the tumor.

The interaction between BLM and radiation also depends on the drug exposure time. Wu et al. (1985) measured, by cell survival and DNA damage, the response of CHO cells and human oat cell carcinoma cell lines to combination treatment of BLM and radiation at different time intervals. Cytotoxicity from BLM and radiation was additive for 1 or 4 h exposure and was supra-additive when the exposure time was extended to 24 h.

It has been observed that cells exhibit a differential sensitivity to BLM. Östling and Johanson (1987) treated CHO cells with BLM (75 or 300 µg/ml) or irradiated with 60<sup>Co</sup> gamma rays of 200 and 400 cGy. DNA strand breaks in single cells were analyzed using microelectrophoretic technique. BLM was reported to act in a selective
manner so that in some cells the DNA was heavily degraded while in others there was only moderate or measurable damage. In contrast, a uniform response was found after gamma irradiation.

The cytotoxicity of bleomycin is dependent on the temperature and pH of the treatment. This was demonstrated by Urano et al. (1988) who investigated the effect of BLM at elevated temperatures in murine tumor (FSa-11) cells. The surviving fraction was found to reduce to 0.1 within 20 min of treatment at pH 7.4 in the temperature range of 39 - 43.5°C and within 10 min at pH 6.7. The data indicated that at elevated temperatures BLM-induced toxicity was enhanced.

Prompted by the encouraging results obtained in experimental animals, large scale trials were carried out in patients. In a preliminary clinical study, Ichikawa et al. (1964) found a therapeutic effect of BLM on skin cancer and observed skin inflammation and hair-loss as the side effects. They also showed the drug to be effective in the treatment of carcinoma of the penis and scrotum (Ichikawa et al. 1969).

The clinical administration of BLM had been recommended twice daily or weekly. In most of the Japanese clinical studies the same schedule was followed. In these studies the antitumor effect of BLM against squamous cell carcinoma of the head and neck, mouth and cervix was evaluated yielding a varied rate of response (Ichikawa et al. 1969). Similar antitumor effect of BLM in head and neck cancer was also reported by Livingston and Carter (1970).

A large number of subsequent studies have been reviewed by
Blum et al. (1973). BLM, administered at 15 mg/m² twice weekly, was found to produce varied response rates in the head and neck, mouth, nasopharynx and skin tumors. The drug was uniformly active against all types of lymphomas but in malignant melanoma patients the chemical failed to show any antitumor effect.

BLM given in combination with irradiation in squamous cell and oesophageal carcinoma seemed to improve the results (Kolaric et al. 1976). Among patients with advanced malignant diseases Krakoff et al. (1979) reported the largest number of responses to BLM in patients with cervical carcinoma. Patients exhibited drug-induced toxicities like skin reaction, alopecia and pulmonary toxicity. BLM showed significant antitumor activity against oral cancer as demonstrated by Shantha and Krishnamurthy (1980). The main toxic features were acute mucositis, pneumonia, and dermatitis.

BLM was demonstrated to be toxic not only to cancer cells but also to normal tissues. The drug showed the first toxic sign in the skin where it is highly concentrated (Umezawa et al. 1967). Other clinical toxicities included skin pigmentation, fever, pulmonary fibrosis, mucositis, anorexia, vomiting and nausea (Ichikawa et al. 1969, Blum et al. 1973).

An interaction between radiation and bleomycin has been reported in normal experimental animal tissues. Phillips et al. (1975) in their study on various mouse normal tissues observed that BLM administration at 3 mg/kg.b.wt. before irradiation (600-2000 rads) increased the radiation injury to the oesophagus, with a dose effect factor (DEF) of 1.14, and reduced non-significantly the intestinal crypts, with a DEF of 1.10.
BLM was reported to enhance the radiation damage in mouse skin (Leith et al. 1975, Guigon et al. 1978, Molin et al. 1981). von der Maase (1984a) studied the importance of time interval between BLM and radiation administration in the mouse skin. BLM was injected 15 min, 1 or 24 h before to 24 h after irradiation with 26.0 Gy. The results indicated that the drug enhanced the damage to the skin, the damage occurred earlier and it was maximum when BLM was injected 24 h before and 4 h after irradiation.

Fu et al. (1984) reported the chemical to enhance the radiation response in jejunal crypt cells when continuously infused in combination with low dose rate irradiation. von der Maase (1984b) studied the effect of BLM alone and the interaction of radiation and BLM in jejunal crypt cells using the microcolony survival assay. BLM was given from 14 d before to 72 h after 7.0 Gy of radiation. BLM alone at 100 mg/kg reduced significantly the intestinal crypt number. The combined effect of radiation and BLM was extremely dependent on the sequence and interval between drug administration and irradiation. The most pronounced effect was observed when BLM was given 24 h before irradiation, the DEF being 2.40.

Steel et al. (1979) demonstrated the potentiating effect of high dose of BLM given before irradiation of the mouse thorax. Mice were exposed to doses in the range 12.0 to 18.9 Gy with or without BLM (75 or 100 mg/kg), administered 14 day beforehand. Using mortality as end point for lung damage the authors concluded that the drug shortened the lifespan of the animals.

von der Maase et al. (1986) based on the ventilation rate and lethality reported that BLM enhanced radiation-induced lung damage
in mouse. Single drug dose (100 mg/kg) was administered at various time intervals before and after irradiation with doses in the range 6.0 to 20.0 Gy. BLM administered 15 min before irradiation enhanced the radiation response with a DEF of 1.56.

Feng et al. (1986) studied the effect of single doses of irradiation (12.0 - 18.0 Gy) in combination with BLM, using schedules with different time intervals, sequence and drug doses (5 - 100 mg/kg) on mouse lip mucosa. The results indicated that BLM at doses of 40 and 80 mg/kg significantly increased the radiation damage to lip mucosa with dose modifying factors (DMF) of 1.28 and 1.50 respectively. The highest reaction was scored with the injection 2 h before irradiation (DMF - 1.28). However, Vanuytsel et al. (1988), investigating the effect of simultaneous administration of BLM on the acute skin reactions of mice exposed to gamma radiation, did not find any influence of the drug on radiation-induced reactions.

In contrast, Uma Devi et al. (1990) reported the potentiating effect of BLM on irradiated mouse bone-marrow and testis. They studied the effect of BLM (20 mg/kg) on irradiated (1.0 - 7.0 Gy) mouse bone-marrow and testis on day 1 and 14 by observing the incidence of micronuclei. They concluded that BLM by itself was toxic to bone-marrow but did not have any effect on testis. When combined with radiation the drug significantly increased the frequency of micronuclei in both the tissues.

Umezawa et al. (1967) studied the distribution of BLM administered subcutaneously (s.c.) or i.p. at a dose of 50 mg/kg in tumor bearing
mice. Three hours after the injection high concentrations of BLM were observed in the lung, skin and tumors. The persistence of BLM in the serum after intravenous (i.v.) injection was very brief, the half-life being less than 2 h (Fugita 1970).

The mechanism of action of BLM is not yet well understood. Jørgensen (1972) suggested that BLM may affect tumor cells by inhibiting DNA synthesis and by injury to DNA while Matsuzawa et al. (1972) proposed inhibition of the radiation damage repair by BLM. Barranco et al. (1973) presented evidence that low doses of BLM was cell-cycle specific, inhibiting cell progression at the S-G2 boundary and in the mitotic period. Cells in the other stages were less affected. Haidle et al. (1972) argued that at low concentration BLM could cause elimination of thymine bases leading to DNA strand breakage and inhibition of cell growth. It is suggested that BLM-induced strand breakages and cell death are similar to those induced by ionizing radiation (Kunimoto et al. 1967).

von der Maase (1984b) in his study on mouse intestinal crypt cells proposed that BLM induced damage in the crypt cells which were fully repaired under normal conditions. However, administration of radiation doses within a few hours might interfere with repair mechanisms resulting in irreversible damage.

**Chlorpromazine:**

Chlorpromazine (CPZ), a common tranquilizer belonging to the phenothiazine group, is currently used clinically as an anti-psychotic and antiemetic agent. In addition, the compound demonstrated significant radiosensitization of hypoxic cells *in vitro* and *in vivo* (Shenoy
et al. 1975, George et al. 1980). CPZ sensitized hypoxic E. coli to gamma rays at concentrations varying from 25 to 100 \( \mu \text{M} \) with enhancement ratios varying from 1.8 to 5.0. The drug also sensitized mammalian cells including rat thymocytes and Yoshida ascites-tumor cells to radiation under hypoxic condition (Shenoy et al. 1975).

In addition to being a hypoxic cell radiosensitizer, CPZ has also been shown to be cytotoxic to hypoxic cells. Shenoy and Singh (1978) reported preferential cytotoxicity of CPZ towards hypoxic E. coli at a concentration of 0.1 mM. The magnitude of toxicity under hypoxia was three times greater than in euoxic conditions.

The radiosensitizing and cytotoxic effects of CPZ have been reported for mouse fibrosarcoma. George et al. (1980) used varying concentrations of CPZ (20, 40 and 100 mg/kg.b.wt.) to study the cytotoxicity and 20 or 40 mg/kg of CPZ 5 min before local irradiation with 20.0 GY to study the radiosensitizing effect. The drug alone, at all doses, exhibited a definite cytotoxic effect in the tumor and the combined effect of CPZ and radiation was synergistic. Similarly, Shenoy and Singh (1980) demonstrated CPZ—induced cytotoxicity in mouse Sarcoma-180A tumor when administered once or twice a week at doses of 10, 15, 20 or 40 mg/kg. In addition, significant sensitization was observed when the drug was applied before irradiation with 10.0 Gy.

Shenoy et al. (1982) studied the chemotherapeutic effect of CPZ on lymphosarcoma and on a spontaneously occurring mammary adenocarcinoma in CBA mice. CPZ, administered i.p. at a dose of 20 mg/kg.b.wt.
three times a week, did not show any effect on lymphosarcoma and ascites tumor. However, the drug had a carcinostatic effect on mammary adenocarcinoma, where it increased the longevity of the tumor-bearing mice.

Darkin et al. (1984) reported CPZ to be toxic to and to induce reversible DNA breakage in mouse mastocytoma cells. In another study, it was demonstrated that CPZ was cytotoxic to both aerated and hypoxic CHO cells and that the cytotoxicity was only slightly more towards hypoxic cells (Lehnert 1987).

The radiosensitizing and cytotoxic effects of chlorpromazine depend on the temperature of treatment. Shenoy and Singh (1978) reported that 0.1 mM of CPZ was toxic to E. coli at 37°C in air. At 0.075 mM the drug was non-toxic under oxic as well as hypoxic conditions up to 30°C; however, hypoxic cytotoxicity steadily increased with temperature beyond 30°C. Similarly, temperature dependent enhancement of hypoxic cytocidal action and radiosensitizing effect of CPZ in E. coli has been demonstrated by the same authors (Shenoy and Singh 1985), where they prolonged the hypoxic incubation from 10 min to 45 min and increased the irradiation temperature from 20°C to 37°C (DMF : 2.0 - 9.4).

CPZ is also a highly photoactive compound. Photoreactivation of CPZ produced oxidizing CPZ radicals that are known to have cytotoxic effects on biological systems (Fujita et al. 1981, Ciulla et al. 1986). Peak et al. (1989) assessed the effect of CPZ on hairless mice exposed to UV-radiation and concluded that CPZ treatment diminished tumor yields by approximately 80%.
Shenoy et al. (1982) investigated the distribution of CPZ in plasma and tumor tissues of mice. It was observed that the peak level in plasma was reached at 30 min following a single i.p. injection of the drug. Most significantly, the peak level in tumor tissues was seen at 90 min following injection and was 2.5 times greater than the peak attained in the plasma.

The radiosensitization of E. coli to gamma rays by CPZ has been shown to be partly due to some transients of the drug formed as a result of its reaction with hydroxyl radicals generated during irradiation (Shenoy et al. 1975). The drug inhibited markedly the protein and DNA synthesis in irradiated cells. Chlorpromazine has also been shown to affect the permeability and stability of biological membrane and to interfere with co-enzymes involved in oxidative metabolism (Lee et al. 1976).

Mechanisms involved in the cytotoxic effects are not clear. However, in vitro studies indicated that the presence of the drug results in the inhibition of energy metabolism, especially when the cells have to derive energy from anaerobic glycolysis (Shenoy and Singh 1979). Various studies demonstrated that the treatment of the cells with chlorpromazine resulted in marked depletion of both non-protein sulfhydryl compounds (NPSH) (Shenoy et al. 1982) as well as protein sulfhydryls (Schorn et al. 1983). Furthermore, CPZ also caused a blockage of cell progression at G1/S and G2 + M and inhibited repair of X-ray-induced potential lethal damage. Shenoy and Singh (1985) observed the inhibition of the rejoining of DNA single strand breaks by CPZ.
As far as the author is aware, there is no published report on the effect of CPZ either on normal animal tissues or on man.

**Cis-diaminedichloroplatinum:**

Cis-diaminedichloroplatinum (c-DDP) or Cisplatin is a cytotoxic chemical first recognized as an antitumor agent by Rosenberg et al. (1969). Cisplatin has been demonstrated to have significant radiosensitizing activity against prokaryotes. Richmond and Powers (1976) and Richmond et al. (1977) observed a significant increase in the radiation-induced lethality in bacterial spores and E. coli and the effect was larger under hypoxic conditions than in air. Richmond (1984) reported hypoxic cell sensitization in Salmonella by c-DDP which was temperature-dependent. He suggested that sensitization might involve temperature sensitive processes such as free radical and free electron scavenging.

Several investigators found a true radiosensitizing effect of Cisplatin in cultured mammalian cells. Cisplatin in the dose range of 8 - 100 μg/ml when administered 1 or 2 h before to after irradiation enhanced the radiation damage to CHO cells (Szumiel and Nias 1976, Nias et al. 1979, Murthy et al. 1979). Similarly, the chemical had an additive effect on hypoxic CH-V-79 cells when administered at 10 and 2.5 μg/ml either before or after irradiation (Douple and Richmond 1978, Towle et al. 1980).

Yuhas et al. (1979) in their study on human MDA-361 and murine Mca-11 cell lines reported that the combined treatment of c-DDP and
radiation had subadditive effect on the former and more than additive effect on the latter. Similarly, Carde and Laval (1981) reported a DMF of 1.25 for hypoxic rat hepatoma cells treated with c-DDP preceded by irradiation. Recently Basham et al. (1990) demonstrated independent cell killing and additive toxic effects in euoxic human foetal lung fibroblast treated with Cisplatin and radiation.

Studies on cultured mammalian cells suggested that the degree of enhancement of the radiation effect by the drug was not distinctly different in tumor cells and normal cells in vitro (Dewit 1987). The first evidence for interaction of Cisplatin with radiation in vivo came from Zak and Drobnik (1971) who observed a change in the mouse survival by the drug when combined with whole body irradiation. It was suggested that an interaction between Cisplatin and radiation lead to a greater-than-additive cell killing effect. Cisplatin has been demonstrated to have antitumor activity in a number of animal tumor systems including mouse leukemia L-1210 and Sarcoma-180 A (Rosenberg et al. 1969, Rosenberg and Van Camp 1970). Other tumors reported to be susceptible to Cisplatin include mouse mammary carcinoma and rat brain tumor in which an enhanced radiation damage was observed (Douple et al. 1977). Similarly, c-DDP was shown to have synergistic effect on mouse carcinoma when administered at a dose of 3 mg/kg before irradiation (Weldon et al. 1979).

In the combination treatments the tumor response depends upon the timing as well as sequence of treatments and the time interval between administration of the two agents may be crucial in determining the tumor behaviour. Douple and Richmond (1982) performed a study to test the efficiency of treatment sequencing of c-DDP and radiation
on a mouse mammary carcinoma. Cisplatin at a dose of 5 mg/kg was administered 15 min and 1 h before or immediately after irradiation with 58.0 Gy. The combination resulted in an ER of 1.3 and the greatest potentiation occurred when the chemical was given 1 h before irradiation.

The optimal time interval and the best sequence of administration of c-DDP and radiation are still not understood. Many investigators found that the drug caused greater-than-additive effect when given a short time before irradiation (Douple et al. 1977, Kovacs et al. 1979, Douple and Richmond 1979a, 1982, Lelieveld et al. 1985). In some cases the largest effects were found when the time interval was increased to 6 to 24 h. (Douple 1977, Douple and Richmond 1978, Kovacs et al. 1979).

The first convincing evidence for supra-additive effect of Cisplatin and radiation on murine tumors came from the work of Kovacs et al. (1979) who used Cisplatin 24 h before irradiation under hypoxic condition and found significant reduction in the cell survival, the effect being more than additive. Greater-than-additive effects by the combination treatments of radiation and Cisplatin have subsequently been demonstrated also in unclamped tumors (Soloway et al. 1979, Bartelink et al. 1986).

The enhancement of radiation effect by Cisplatin demonstrated in various experimental models has generated clinical interest and a substantial number of pilot studies have been reported. In 1972, Hill et al. reported the first group of patients with distinct tumor response after Cisplatin treatment. Later the antitumor potential of the drug was studied in testicular tumors and breast and thyroid
cancers using c-DDP at a dose of 20 mg/m² for 5 days. The drug was found to have a long lasting effect on the tumors. Similarly, encouraging tumor responses were reported for carcinoma of urothelial tract following treatment with varied doses of Cisplatin combined with radiation (Rosoff et al. 1977, Soloway 1978, Marrin 1978, Yagoda et al. 1978). Cisplatin was also claimed to be active against cancers of head and neck (Creagon et al. 1981, Schmitt et al. 1983, Coughlin and Richmond 1985), melanomas (Reimer et al. 1981, Dewit et al. 1985a), metastatic carcinoma and vaginal cancer (Higi et al. 1982), bladder cancer (Jakse et al. 1983, Hemstreet et al. 1984), glioblastoma (Feun et al. 1983) and lung and oesophageal tumors (Keizer et al. 1984, Green et al. 1985).

In spite of the usefulness of Cisplatin as an anticancer drug its clinical application is limited by the toxicity induced in critical normal tissues. The drug-induced clinical toxicities included nausea, vomiting, anorexia and diarrhoea (Higby et al. 1974). Increased acute damage in irradiated oral mucosa (Reimer et al. 1981, Haselow et al. 1982) and gut mucosa (Creagon et al. 1981, Higi et al. 1982, Green et al. 1985, Dewit et al. 1985a) has been reported in patients receiving high doses of Cisplatin. Hazardous effects on the kidney and bone-marrow have also been reported after high drug doses (Leipzig 1983, Leipzig et al. 1985, Haselow et al. 1982, Keizer et al. 1984).

Enhanced radiation damage to certain normal tissues by Cisplatin has been observed in experimental studies. Allan and Smyth (1986), compared the toxicity of Cisplatin with its analogues, Cis-diammine-1,1-cyclobutanedicarboxylate platinum (III) (CBDCA) and Cis-dichloro-transdihydroxyisopropylamine (IV) (CHIP), on the morphology, functional
activity and cell production rate of mouse ileal crypt cells. They observed that all the drugs had profound effects on crypt cell production rate, villous height and mucosal function. While CBDCA showed the least toxicity, Cisplatin and CHIP were equitoxic to ileal crypts.

Cisplatin-induced toxicity was also observed in HeLa, CHO and HaK cells. The cytotoxicity of the three cell lines varied considerably, HeLa and HaK cells being more sensitive to Cisplatin than CHO cells which had a large capacity to absorb damage (Eichholtz-Wirth and Hietal 1986).

Burholt et al. (1979) studied the interaction of c-DDP and radiation in mouse gastric, jejunal and colon epithelial cells and reported that the drug enhanced radiation damage to all three parts of the GI tract, jejunum being the most sensitive. Luk et al. (1979) conducted a study on intestinal response to different doses of Cisplatin (3-13 mg/kg.b.wt.) administered before or after irradiation and concluded that the drug significantly enhanced the damage with a DEF of 1.3. Similarly, Schenken et al. (1979) demonstrated a drug-dose dependent suppression, delay of recovery and reduced proliferation rate of surviving mouse jejunal crypt cells when c-DDP was injected at doses of 2, 4 or 8 mg/kg before or after 10.0 Gy of irradiation. An enhancement in the radiation damage to intestinal crypt cells was also reported by Phillips (1979) who used 9 or 13 mg/kg.b.wt. of the drug before graded doses of radiation.

Recently, von der Maase (1984b) reported a true sensitizing effect of Cisplatin for mouse jejunal crypt cells when the drug was used at 6 mg/kg before single doses of radiation (DEF - 1.22). However,
Fu et al. (1984) found no effect on the radiation response of intestine when the drug was administered in continuous infusion with low dose irradiation. Similarly, Bartelink et al. (1986) observed only a small enhancement of radiation effect in mouse duodenal crypt cells treated with Cisplatin. Maurer-Schultz et al. (1989) reported an increase in the percentage of necrotic cells and a steep decrease in the number of mitotic figures in the mouse jejunal crypt epithelium following treatment with Cisplatin (4 or 8 mg/kg) and whole-body irradiation (4.2 Gy). However, both parameters recovered rapidly to normal values after 36 h of treatment.

Cisplatin significantly enhanced radiation reactions in rat skin under both normal and hypoxic conditions (Douple et al. 1979). An increased damage for mouse skin was also reported by von der Maase (1984a) but not by others (Baker et al. 1984, Lelieveld et al. 1985, Bartelink et al. 1986). There are also reports on Cisplatin-induced enhancement of radiation damage in other tissues including oesophagus (Phillips 1979), haematopoietic organ (von der Maase 1985), kidney (Stewart et al. 1986) and rectum (Dewit et al. 1987). However, the chemical had no influence on the radiation response of mouse lung (Peckham and Collis 1981, von der Maase 1986). Recently, Adler and Tarras (1990) reported c-DDP to increase the radiation-induced aberrant cells in meiotic prophase and to enhance the radiation-induced weight reduction in mouse testis.

Hoeschele and van Camp (1972) monitored the radioactivity of labelled cisplatin after an i.p. injection in mice. The drug was rapidly distributed in the extracellular fluid of the body, with high concentration
in the kidney, liver and intestine. The excretion of the drug followed
a biphasic pattern with an initial half-life of 1.5 h and second half-
life of approximately 20 h.

Very little is known about the mechanisms of action of Cisplatin
and radiation in biological systems. Possible mechanisms of the
enhanced tumor cell killing activity of c-DDP are hypoxic cell sensitiza-
tion, inhibition of cellular repair processes, depletion of endogenous
radioprotectors, intra-and inter-strand cross linking and cell cycle
perturbation (Richmond and Simic 1978, Biaglow and Varnes 1983, Douple
and Richmond 1980). Pulse radiolysis experiments have shown that
Cisplatin reacts with free aqueous radical electrons resulting in Pt (1)
intermediates, thereby fixing the damage. This process is a possible
way of hypoxic cell sensitization (Adams and Cooke 1969, Richmond
and Simic 1978, Butler et al. 1985). In the presence of oxygen (O₂)
the Pt (1) intermediates also acted with O₂ to form peroxy complexes.
This mechanism might account for aerobic cell radiosensitization
(Richmond and Simic 1978). Depletion of non-protein bound thiols
increased the radiation sensitivity of both hypoxic as well as aerobic
cells (Biaglow and Varnes 1983). Formation of intra- and inter-strand
cross-linking of DNA by platinum complexes was proposed by Roberts
and Pera (1983) to be the possible model for c-DDP - radiation interac-
tion.

Recently, in split dose experiments in vivo, Fu et al. (1985)
showed a diminished recovery in tumor cells when c-DDP was injected
1 h before the first X-ray dose. Inhibition of repair of sublethal
radiation damage (SLD) by c-DDP has been suggested in mouse intestinal
crypt cells when the drug was injected before X-rays (Burholt
et al. 1979, Luk et al. 1979, Bartelink et al. 1983, Dewit et al. 1985b). Thus, the inhibition of SLD repair may play a significant role in the interaction between c-DDP and X-ray in tumor cells and rapidly proliferating normal cells. If c-DDP was administered shortly before X-rays it inhibited SLD repair and if the two modalities were separated by 24 h or more they acted independently (van der Maase 1984b, Dewit et al. 1985b, Tanabe et al. 1987).

**Radiation dose fractionation:**

Radiotherapists have learned by experience that the use of a series of small daily fractions against single large doses of radiation is the best way to eradicate tumor without excessive damage to the normal tissues. The split dose studies of Elkind and Sutton (1959), the first to point towards the rapid recovery process in mammalian cells, demonstrated that the radiation dose in two fractions required for a specified level of cell killing was higher than the biologically equivalent single dose. Later it was demonstrated that LD$_{50}$ in mice for GI death increased considerably when the radiation was delivered in two or three fractions in comparison with single dose exposure (Hornsey and Vatistas 1963, Withers and Elkind 1969, Wambersie et al. 1974).

Withers and Elkind (1969) demonstrated a great capacity for intracellular repair of sublethal damage of mouse gut epithelial cells following irradiation with two doses of X-rays at varying time intervals. Similarly, Lesher et al. (1975) reported that the mouse intestinal
epithelium can accumulate large total doses of radiation by maintaining its recovery potential over a large number of fractions.

The sparing of normal tissue damage by dose fractionation was attributed to the intracellular repair of sublethal injury taking place between dose fractions. Hence, interfractional interval is an important factor to be considered during fractionation. Sufficient time has to be allowed after each dose for the complete repair of normal tissue damage. It was suggested that the interval between fractions should not be shorter than 3 h and should, if possible, be greater than 4 h (Thames 1984, Fowler 1984a). Hageman et al. (1971) measured the intestinal crypt survival and total cells per crypt following irradiation of mice with split doses (1000 + 400 rads) at varying time intervals (4 and 14 h). The results indicated that after an initial exposure, the crypt cells increased rapidly when the time interval was 4 h and the crypt cellularity was about 2.5 times the control value for 14 h interval. Similarly, Lesher and Lesher (1973) in their experiment on split dose effects found that at short interval the S-phase and dividing cells in the mouse duodenal crypt cells decreased considerably and at longer intervals there was an increase in the number of both types of cells.

Becciolini et al. (1973) examined the changes in morphology and enzyme activities of the small intestinal epithelium of rats exposed to 1200 rads given in two equal exposures. Following irradiation, there was an initial increase in the enzyme activity followed by a reduction and then return to normal level. Crypts showed morphologically changed epithelial cells and a reduction in the number of mitoses.
There was loss of cellular organization and cells were flat and enucleate with large cytoplasmic vacuoles. However, at 5 and 11 days after the second irradiation the epithelium appeared normal, both from the morphological and enzymatic points of view.

It is well established in radiotherapy as well as radiobiology that the total dose required to produce a given biological effect increases both with increasing number of fractions and overall treatment time. Withers et al. (1974) carried out a multifraction experiment in mice by exposing them to a number of dose fractions varying from 1 to 20 at an interval of 3 h. It was obvious that the total dose required for an iso-effect increased with increasing fractionation which could be attributed to the repair of sublethal injury between fractions. However, Wambersie et al. (1974) concluded that the total dose to a given effect does not depend very much on the number of fractions provided that the overall treatment time is kept constant and the dose per fraction is not very high.

Phenomena other than repair of sublethal damage also contribute to the sparing effect of dose fractionation. One of these is regeneration of surviving cells during the intervals between dose fractions, especially when the interval is extended beyond 3 h. To investigate the role of regeneration in the fractionation response of colonic mucosa Withers and Mason (1974) used fractionation regimens in which the interfractional interval was lengthened to 12 or 24 h. There was obvious displacement of survival curves from that of single dose-curve towards higher doses; the biggest displacement resulted with 24 h interval exposure. This
reflects the added contribution of regeneration to the recovery of colonic mucosa.

Rao and Fritz-Niggli (1983) studied the effects of fractionated doses of pions and X-rays on the mouse jejunal crypt cells by determining the relative biological effectiveness (RBE) values. For single fractions the pion/X-ray RBE was 1.26 and it increased to about 1.31 when two fractions were applied.

Jensen et al. (1986) assessed the influence of varying time intervals between dose fractions on the development of late radiation-enteropathy after split dose irradiation of rat small intestine. There was a significant reduction in the radiation damage as the interval between the fractions was increased. However, Dewit and Oussoren (1987) reported late radiation injury in the mouse intestinal epithelium, mucosa, crypt and villi following a daily fractionated irradiation schedule of 3 Gy for 14 days.

Few experimental reports are available on the influence of dose fractionation on other normal tissues. Constant repair capacity and reduction in the mouse skin damage following multifractionation was reported by Denekamp (1973), Denekamp et al. (1976), Joiner and Denekamp (1986) and Würschmidt et al. (1988). There was also considerable sparing of radiation damage to rodent thyroid (Malone et al. 1974), tail (Hendry et al. 1976), kidney and liver (Maisin et al. 1977), spinal cord (White and Hornsey 1978), lungs (Parkins et al. 1983, van Rongen et al. 1990) and hair follicles (Vegesna et al. 1989).
Altered fractionation regimens:

In recent years, there has been an increasing tendency to favour non-conventional or non-standard fractionation schedules in place of the conventional fractionation regimen (Thames et al. 1983, Fowler 1984b, Peters et al. 1988). Normally, the aim of these procedures is to reduce the damage to normal tissues. Some of the altered dose fractionation schedules under clinical trials include hyperfractionation, accelerated fractionation and hypofractionation.

With accelerated fractionation or multiple daily fractionation (MDF) schedule, a conventional number of dose fractions is delivered in a significantly shortened overall treatment time in order to reduce the opportunity for tumor cell regeneration during treatment. With hyperfractionation, on the other hand, a large number of significantly reduced dose fractions is used to give a greater total dose in a conventional overall treatment time. The rationale for this strategy is the increased opportunity for tumor cell redistribution and reoxygenation between dose fractions (Withers et al. 1982, Thames et al. 1983). In hypofractionation, larger and fewer dose fractions than conventional schedules are administered in the same overall time (Cox 1985). High-dose fractions may result in considerable cell killing and greater reoxygenation of tumor tissues during the longer intervals between fractions (Eichorn 1981).

However all the three altered fractionation schedules may be limited by acute normal tissue reaction. There are several experimental data on the response of rodent small intestine and other normal tissues
to altered dose fractionation. Becciolini et al. (1983b) studied the changes in distribution of S-phase cells along mouse small intestine after exposure to multiple daily fractionation (MDF) of total doses of 6.0 and 12.0 Gy. Irradiation was given as 3.0 Gy fractions twice or four times every 12 h. They concluded that the small intestine tolerated this treatment well and after the acute damage the epithelium quickly recovered and returned to normal conditions. They made similar observations in rat small intestine exposed to MDF and reported repair and recovery within 72 h after exposure (Becciolini et al. 1986).

Becciolini et al. (1985) also compared the effects of a single dose (8.0 Gy) and MDF of 6.0 and 12.0 Gy with different doses per fractions (2.0 and 3.0 Gy) and time intervals (4 or 12 h) on the goblet cells of rat small intestine. Both regimens caused an initial increase of the goblet cells followed by a decrease and finally return to near normal levels. After MDF the increase was more marked and the return to normal level occurred earlier than after single dose.

Jensen et al. (1986) examined the late radiation-enteropathy in rat small intestine exposed to 23.0 Gy of radiation administered as a single dose or in two equal fractions separated by different intervals (4 - 92 h). It was observed that an increased fractionation interval led to a reduced degree of radiation injury and that the cells involved in the late radiation damage had a large capacity for sublethal radiation damage repair. Jensen et al. (1988) further studied the effect of the overall treatment time on acute and early radiation-
enteropathy of rat small intestine after exposure to 3 different fractionation schedules. A total dose of 56.0 Gy was given either as conventional fractionation in 26 days or accelerated fractionation in 12 or 7 days. Histological observation indicated a significant increase in radiation injury as a result of shortening the overall time. Irradiation with one fraction per day exhibited moderate pathological changes, two fractions per day resulted in definite pathological mucosal architecture and three fractions per day caused complete denudation and ulceration of mucosa.

Ang et al. (1985) assessed the rate of repair in mouse lip mucosa exposed to single dose and 2, 4 or 10 fractions in 3 days. The results indicated that cellular repair in lip mucosa starts immediately after irradiation and the rate of repair is dependent on the size of the fraction, being faster after a smaller dose. Similarly, sparing effect of altered dose fractionation, high repair capacity and tolerance to tissues to non-standard fractionation have also been demonstrated for mouse lungs (Giri et al. 1985, Parkins et al. 1988), kidney (van Rongen et al. 1988), spinal cord (Van der Schueren 1988) and foot skin (Abe and Urano 1990).

Pobert et al. (1974) compared the response of normal mouse leg skin after treatment with three fractions per week (3F/week) and five fractions per week (5F/week) schedules. It was observed that a greater total dose was necessary to produce the same skin reaction with standard fractionation (5F/week) schedule than with hypofractionation (3F/week).
Altered fractionation radiotherapy in clinical practice is a fairly recent method. Hence a number of clinical trials have been carried out to assess the advantage of these schedules over the conventional fractionation regimen. The first reported study of accelerated fractionation was for the treatment of glioblastoma (Simpson and Platts 1976). Norin and Onyango (1977) obtained improved clinical results with three times daily treatment for Burkitt's lymphoma. Similarly good tumor response with tolerable acute reactions was reported for head and neck carcinoma (Svoboda 1975, 1978, Gonzales et al. 1980, Horiot et al. 1983). Significant tumor control was observed for lung and neck node metastases (Arcangeli et al. 1979, 1984), skin and lung cancer (Lipsett et al. 1984). Similar results have been reported for brain metastases (Choi et al. 1985) and bronchial and oesophageal carcinoma (Saunders et al. 1988) also.

However, all studies do not demonstrate the superiority of MDF schedules compared to conventional fractionation. The European Organization for Radiotherapy of Cancer (EORTC) group study (1985) reported no survival benefit for glioma patients treated with MDF over the conventional arm. Similarly Rezvani (1989) and Wiernik (1990) found similar and acceptable degree of normal tissue damage and equally good tumor cure rates in patients with larynx-pharynx carcinoma treated with accelerated and conventional fractionation.

Clinical experience with predominantly hyperfractionated regimens has been limited. Littbrand et al. (1975) and Edsmyr et al. (1985) reported an improvement in clinical results of radiotherapy of bladder carcinoma patients treated with thrice-a-day schedule. Encouraging tumor responses were also obtained in patients with head and neck
cancer (Shukovski et al. 1976, Meoz et al. 1984, Horiot et al. 1985),
skin cancer (Morgan et al. 1987) and carcinoma of larynx (Wendt et al. 1989). However, Parsons et al. (1984) reported that the results of hyperfractionated treatment of head and neck cancer were as good as those obtained with conventional fractionation. Similarly, Herrman et al. (1987) could not prove a higher efficiency for hyperfractionation in the treatment of carcinoma of glottis relative to conventional schedule. In fact, severe early reactions were more for hyperfractionation.

The review of hypofractionation showed that large dose fractionation was disadvantageous in most cases with regard to either effects on normal tissues, control of the tumor, or both (Ilanda et al. 1980, Cox 1985). Fowler et al. (1972) estimated the therapeutic effect by comparing the degree of skin reaction with local tumor control by treating mice with radiation given as single dose, five or nine fractions or three fractions per week. The results indicated that X-ray given as five fractions were the most effective scheme tested.

Clinically, Byhardt et al. (1977) analyzed the results for carcinoma of oral cavity and oropharynx by comparing three fractions with five fractions per week and reported 99% local control in conventional regime as against 12% with hypofractionation. Similarly, Handa et al. (1980) noted that two fractions per week schedule was disadvantageous in the control of oral carcinoma.

Many investigators found no significant differences in survival, local tumor control or therapeutic gain among patients treated with hypofractionation or conventional fractionation for a wide variety
of tumors of different sites (Henk and James 1978, Wiernik et al. 1982, Asby and Harmen 1986). Similarly, Schumacher (1976), Salazar et al. (1983) and Slawson et al. (1988) obtained results with hypofractionation which were not inferior to those obtained with common fractionation.

There are a few exceptions to the rule of higher tumor responsiveness to more, as opposed to fewer, treatments with smaller dose fractions. Moulder and Fisher (1976), in their study on acute skin reactions in rats exposed to three or five fractions per week, observed that the skin was more sensitive to the regimen with smaller, more numerous fractions. Similar findings were reported clinically for liposarcoma (Reitan and Kaalhaus 1980, Thames and Suit 1986) and melanomas (Overgaard 1986).

It is likely that some tumors overcome their own hypoxia by a natural process of reoxygenation that occurs during a course of multiple doses of radiation (Kallman 1972). However, some tumors may reoxygenate poorly. In such cases the problem of hypoxia can be overcome by combining chemical radiosensitizers with fractionated doses of radiation (Adams and Fowler 1976). Experimental data concerning this aspect are as yet sparse. Sheldon et al. (1976) examined the effects of fractionated doses of MISO (0.67 mg/g per fraction) and X-rays (three or five fractions per week) on mouse skin and mammary tumor. A significant enhancement of tumor control occurred in MISO treated group with an ER in the range 1.11 - 1.22 and there was no enhancement of radiation effect on the skin. Similar enhancement of radiation damage was reported by Denekamp and Harris (1976) for five
and two fractions of X-rays combined with MISO in mouse carcinoma (ER - 1.3 and 1.6). Stone (1983) reported significant sensitization of a mouse mammary tumor following treatment with different fractionated schedules of radiation and drug. However, Abe et al. (1979) observed that the effects of fractionated doses of MISO and radiation was almost the same as that obtained in single doses of radiation.

Clinical reports on the effect of fractionated radiotherapy combined with MISO are very few and conflicting. Arcangeli and Nervi (1980) examined the drug toxicity in head and neck cancer patients exposed to fractionated doses of radiation and MISO and found that some patients developed mucositis. Paterson et al. (1981) demonstrated complete tumor regression and local tumor control rate of 60% in head and neck cancer patients who received fractionated doses of MISO 4 h before hypofractionation. Similarly, Metro in fractionated doses prior to high dose fractionation significantly increased the clearance rate of cervix cancer (Balmukhanov et al. 1987). However, no difference in the tumor regression was observed between MISO and control group of patients with oesophageal cancer (Morita 1983), lung cancer (Abe 1983), astrocytoma (Shin et al. 1985) or cancer of the uterine cervix (Girinski et al. 1985, Overgaard et al. 1989). Similarly, there was no beneficial effect of MISO with multifractionation radiotherapy of head and neck cancer (Panis et al. 1984, Fulton et al. 1984, Lee et al. 1989) and cancer of larynx and pharynx (Overgaard et al. 1989). There are no experimental reports regarding the normal tissue response to fractionated doses of MISO and radiation.

Few data are available on the effects of fractionated doses
of other therapeutic drugs and radiation. Vanuytsel et al. (1986) investigated the effect on the mouse lip mucosa of different irradiation fractions (1, 2, 4, 10 and 20) with or without simultaneous continuous bleomycin infusion. They found that lowering the fraction size resulted in an increase of DMF from 1.19 for single dose to 1.86 for twenty fractions. Parvinen et al. (1985) obtained a favourable effect on tumor clearance in patients with head and neck cancer who received fractionated radiotherapy combined with bleomycin. However, there was no significant difference in the toxicity, local recurrence or survival rate between the combined and control treatment groups. Maiche (1989) also reported complete clearance of primary tumor of the penis in patients treated with fractionated doses of BLM and irradiation.

Weldon et al. (1979) evaluated c-DDP (2.5 mg/kg) combined with high-dose (400-600 rads per day) fractionation in a murine carcinoma and observed no difference in tumor response between the drug treated and control groups. However, a supra-additive effect of fractionated doses of c-DDP and irradiation was reported for RIF-1 tumor (Lelieveld et al. 1985) and a DEF of 1.82 for squamous cell carcinoma (Tanabe et al. 1987) in mouse. Landuyt et al. (1986) studied the tolerance of mouse lip mucosa to different fractionated irradiation and c-DDP schedules. Their data did not show an increase of the radiosensitivity nor an inhibition of repair. On the contrary, they indicated a minor protection dependent on the fractionation schedule used. In contrast, Tanabe et al. (1987) reported supra-additive effects on lung and duodenal crypt cells of mice when c-DDP was administered daily before each of the 5 daily X-ray doses or single dose of drug before the first of the 5 daily fractions.
From this review it is clear that the information available on the effect of single and fractionated doses of chemotherapeutic drugs with radiation on the small intestine is meagre. Such information is essential for the safe administration of the two modalities in cancer treatment. Hence, the present study was undertaken in order to evaluate the response of the mouse jejunal mucosa to chemical sensitizers and radiation, taking crypt survival and crypt and villous cell damage and recovery as end points.