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● *Original Contribution*

# THE RADIOSENSITIZING EFFECT OF ORNIDAZOLE IN HYPOXIC MAMMALIAN TISSUE: AN IN VIVO STUDY

SAIT OKKAN, M.D.* AND REHA UZEL, M.D.†

Cerrahpasa Medical Faculty, Department of Radiotherapy, University of Istanbul

**In this study the sensitizing effect of ornidazole is investigated *in vivo*. The selected test system is the acute killing effect of radiation within 4–6 days after abdominal irradiation ranging from 9 to 24 Gy, in groups of $C_{57}$ black mice. Ornidazole is given intraperitoneally in 500 mg/kg, 100 mg/kg, 20 mg/kg doses prior to irradiation of animals breathing air, oxygen or nitrogen. A decrease of $LD_{50}$ dose is observed from 24.39 ± 5.66 to 16.38 ± 1.86 and 18.04 ± 2.48 Gy, respectively, in nitrogen breathing animals. No sensitizing effect was observed in doses of 20 mg/kg. Enhancement Ratio (ER) was found to be 1.48 ± 0.25 and 1.35 ± 0.27; relative sensitizing efficiency (RSE) was 40% and 29% respectively. No sensitizing effect was observed in animals irradiated in oxic conditions. These results showed that ornidazole (Ro-7-0207) has a sensitizing effect on hypoxic cells *in vivo*. It is worthwhile to try this drug in a clinical study.**

**Radiosensitization, Ornidazole, Hypoxic cell sensitizer.**

## INTRODUCTION

Ornidazole (Ro-7-0207), one of the 5-nitroimidazoles, sensitizes anoxic bacteria almost as efficiently as 2-nitro derivatives.[3] In contrast, *in vitro* mammalian cell studies show that the 2-nitroimidazoles are more efficient than 5-nitro compounds,[3,32] and also according to pulse radiolysis results, it has higher electron affinities than 5 nitro derivatives.[32]

Preliminary results of a limited testing of ornidazole using KHT tumor as an *in vivo* screen showed no sensitizing effect. Reson for this could not be clearly explained.[24]

In this study, the sensitizing effect of ornidazole is investigated *in vivo*. The selected test system is the acute killing effect of irradiation, 4–6 days after abdominal irradiation of mice. Death at this time is primarily a result of damage of the stem cells of the intestinal epithelium.[17,23] On the other hand, irradiation given while the animals are breathing nitrogen is less effective than when they are breathing oxygen, with an OER about 2.6–2.7.[2,18] Therefore, this test system is suitable for examining the effects of ornidazole on both oxic and hypoxic cells *in vivo*.

## METHODS AND MATERIALS

Ornidazole* is δ-(Chloromethyl)-2-methyl-5-nitroimidazole-1-ethanol.[16] It has a marked antiprotozoal activity *in vitro* and *in vivo*.[8] It has been demonstrated that ornidazole is active against anaerobic gram negative bacteria and anaerobic infections.[5,9,11]

For this study three different solutions of ornidazole are prepared by diluting in sterile distilled water. (i.e. 1 mg/ml, 10 mg/ml and 20 mg/ml). Three to four month-old male and females $C_{57}$ black mice weighing 20 to 25 grams were used as experimental animals. Ornidazole is given intraperitoneally in doses of 20 mg/kg, 100 mg/kg, 500 mg/kg and 1000 mg/kg to groups of mice, each group containing 10 animals, in order to determine the drug toxicity. A dose of 1000 mg/kg killed all the mice within 24 hours. No lethal effect is seen with a dose of 500 mg/kg, but all the mice remained heavily somnolent for 6–10 hours. All animals survived doses of 100 mg/kg and 20 mg/kg. According to these findings it is decided to use 500 mg/kg, 100 mg/kg and 20 mg/kg doses in irradiation studies.

After ether anesthesia, ornidazole is given intraperitoneally in the above dilutions, 10–15 minutes prior to irradiation. Animals are kept in 2 lt volume polyethylene bags. Oxygen and nitrogen flowed through the bag at a rate of 6 lt/min for 10–32 seconds before and during irradiation. The total breathing time is 60 seconds for nitrogen and 50 seconds for oxygen. The animals breathing nitrogen are saved from death from anoxia by changing to oxygen flowing at the rate of 12 lt/min after irradiation. Another group of mice are left to breath air freely.

Animals are divided into 9 groups for experimental irradiation.

1. Oxic group (breathing oxygen)

*Associate Professor.
†Professor and Director.
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*Ro 7-0207 (Tiberal®, F. Hoffman-La Roche and Co. Ltd., Basel, Switzerland). Biteral® is kindly supplied by Roche Co. of Turkey.

Table 1. Survival of mice irradiated while breathing nitrogen

| Dose (Gy) | $N_2$ | | $N_2$ + ornidazole (500 mg/kg) | | $N_2$ + ornidazole (100 mg/kg) | | $N_2$ + ornidazole (20 mg/kg) | | $N_2$ + ornidazole after irradiation | |
|---|---|---|---|---|---|---|---|---|---|---|
| | Number of mice irradiated | Survivors at 6 days | Number of mice irradiated | Survivors at 6 days | Number of mice irradiated | Survivors at 6 days | Number of mice irradiated | Survivors at 6 days | Number of mice irradiated | Survivors at 6 days |
| 11 | — | — | 10 | 10 | 10 | 10 | — | — | — | — |
| 13 | — | — | 10 | 6 | 10 | 7 | 10 | 10 | — | — |
| 14 | 10 | 10 | — | — | — | — | — | — | — | — |
| 15 | 10 | 9 | 10 | 6 | 10 | 8 | 10 | 8 | 10 | 9 |
| 17 | 10 | 7 | 10 | 5 | 10 | 6 | 10 | 9 | 10 | 8 |
| 19 | 10 | 6 | 10 | 3 | 12 | 5 | 11 | 10 | 10 | 6 |
| 21 | 10 | 7 | — | — | — | — | 11 | 7 | 10 | 7 |
| 24 | 10 | 6 | — | — | — | — | — | — | 10 | 5 |
| LD 50/6 with 95% confidence limits | 24.39 ± 5.66 Gy | | 16.38 ± 1.86 Gy | | 18.04 ± 2.48 Gy | | 25.36 ± 4.78 Gy | | 23.77 ± 5.21 Gy | |

2. Air group (breathing air)
3. 500 mg/kg ornidazole is given before the irradiation of oxygen breathing animals.
4. 500 mg/kg ornidazole is given before the irradiation of air breathing animals.
5. Hypoxic group (breathing nitrogen)
6. 500 mg/kg ornidazole is given before the irradiation of nitrogen breathing animals.
7. 100 mg/kg ornidazole is given before the irradiation of nitrogen breathing animals.
8. 20 mg/kg ornidazole is given before the irradiation of nitrogen breathing animals.
9. 500 mg/kg ornidazole is given after the irradiation while breathing nitrogen.

Each group is subdivided so that irradiation could be given in varying doses ranging from 9–24 Gy. For this reason irradiation time also varies from 17 to 50 seconds. Details of these groups and subgroups are given in Tables 1 and 2. Animals are given total abdominal irradiation through a single circular dorsal field of 5 cm diameter, at 5 cm FSD using a dermopan unit (50 KV, 0.75 mm Al HVL). Doses are expressed as surface doses. The exposure rate is 29.06 Gy/min.

Death occurs within 4–5 days in mice receiving whole body irradiation.[16,17,23] This is attributed to the loss of the proliferative capacity of the stem cells in the crypts of Lieberkühn of the small intestine.[23] In our study animals died on the fourth, fifth and sixth days. This difference may be a result of the use of abdominal irradiation instead of total body irradiation. In this study, therefore, the number of survivors are determined at the end of the sixth day.

The LD 50/6 values are obtained by probit analysis

Table 2. Survival of mice irradiated while breathing oxygen and air

| Dose (Gy) | $O_2$ | | $O_2$ + ornidazole (500 mg/kg) | | Air | | Air + ornidazole (500 mg/kg) | |
|---|---|---|---|---|---|---|---|---|
| | Number of mice irradiated | Survivors at 6 days | Number of mice irradiated | Survivors at 6 days | Number of mice irradiated | Survivors at 6 days | Number of mice irradiated | Survivors at 6 days |
| 9 | 10 | 10 | 10 | 8 | — | — | — | — |
| 10 | 10 | 6 | — | — | — | — | — | — |
| 11 | 10 | 4 | 10 | 5 | 10 | 10 | 10 | 9 |
| 12 | 10 | 4 | 10 | 5 | 11 | 10 | — | — |
| 13 | 10 | 1 | 10 | 1 | 10 | 7 | 11 | 9 |
| 14 | — | — | — | — | 10 | 7 | 10 | 8 |
| 15 | 10 | 0 | — | — | — | — | — | — |
| 16 | — | — | — | — | 10 | 3 | 11 | 4 |
| 18 | — | — | — | — | 12 | 0 | 10 | 0 |
| LD 50/6 with 95% confidence limits | 11.01 ± 0.63 Gy | | 11.11 ± 1.01 Gy | | 14.30 ± 0.91 Gy | | 15.06 ± 0.80 Gy | |

†Siemens.

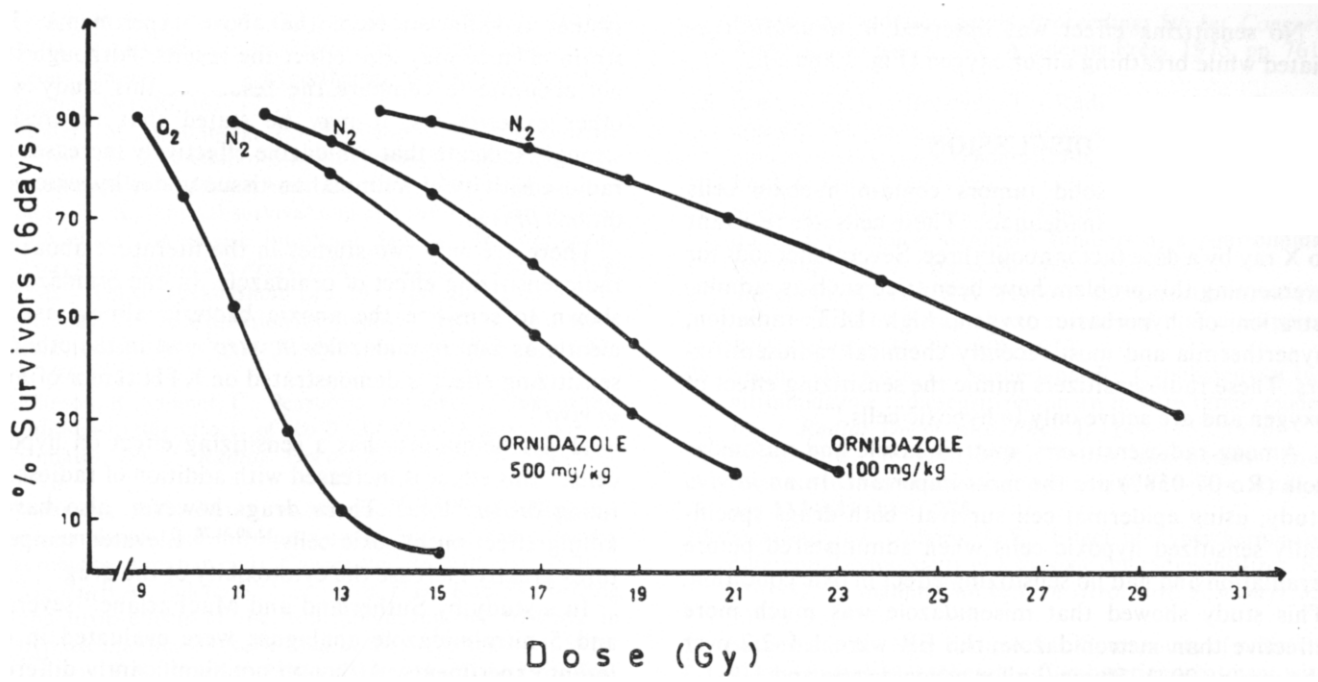


Fig. 1. Survival at 6 days of mice given abdominal irradiation while breathing nitrogen with or without ornidazole.

with 95% confidence limits.[22] ER and OER are found at the level of LD 50/6; according to these values RSE (Relative sensitizing efficiency) was calculated from formula of

$$RSE = (ER - 1)/(OER - 1) \times 100\%.^{6}$$

## RESULTS

The number of mice given abdominal irradiation while breathing $N_2$ or $O_2$ and the numbers of survivors at the sixth day after irradiation are shown in Table 1 and 2. LD 50/6 was 24.39 ± 5.66 Gy in hypoxic and 11.01 ± 0.63 Gy in oxic condition. Thus OER was found to be 2.21 ± 0.23.

In the hypoxic condition, application of ornidazole in doses of 100 mg/kg and 500 mg/kg decreased LD 50/6 to 18.04 ± 2.48 Gy and 16.38 ± 1.86 Gy (Fig. 1.). Thus ER is found to be 1.35 ± 0.27 and 1.48 ± 0.25, respectively. RSE was 29% for low and 40% for high doses. No sensitizing effect was observed in doses of 20 mg/kg. Animals given ornidazole after irradiation while breathing $N_2$ (group 9) showed the same sensitivity as animals without ornidazole.

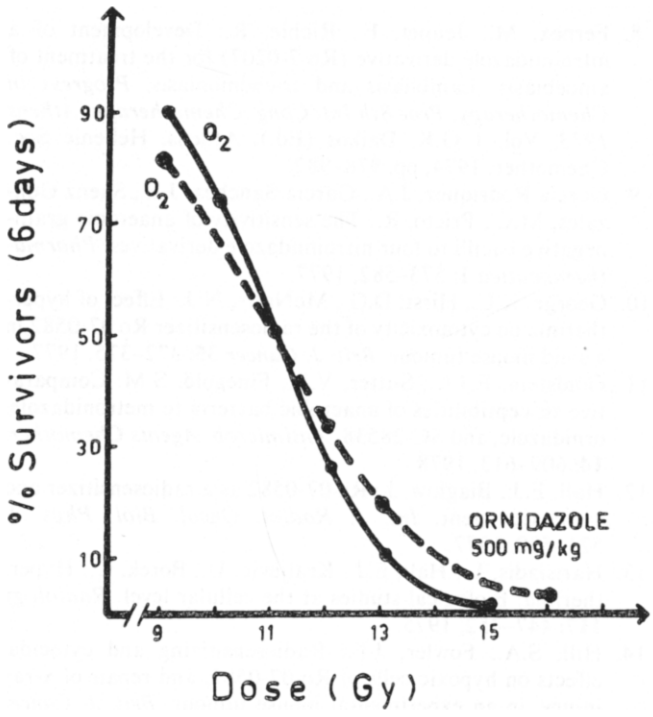


Fig. 2. Survival at 6 days of mice given abdominal irradiation while breathing oxygen with or without ornidazole.

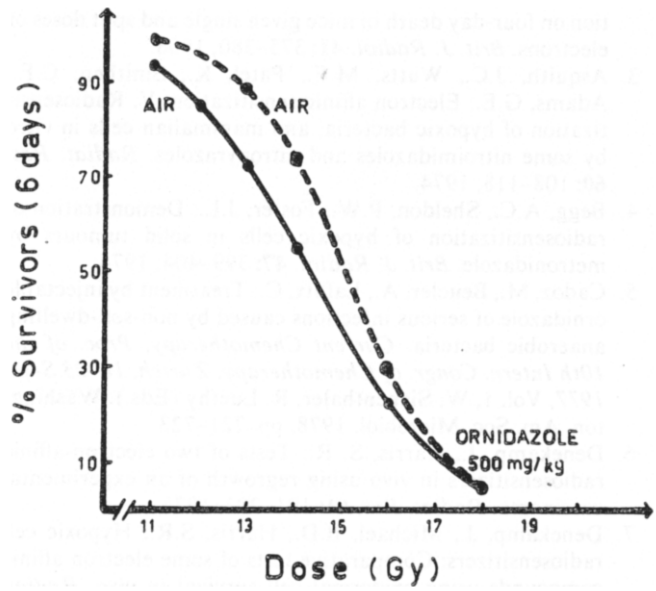


Fig. 3. Survival at 6 days of mice given abdominal irradiation while breathing air with or without ornidazole.

No sensitizing effect was observed in animals irradiated while breathing air or oxygen (Fig. 2 and 3).

## DISCUSSION

Many types of solid tumors contain hypoxic cells because of vascular inadequacy. These cells are resistant to X ray by a dose factor about three. Several methods for overcoming this problem have been tried such as, administration of hyperbaric oxygen, high LET radiation, hyperthermia and most recently chemical radiosensitizers. These radiosensitizers mimic the sensitizing effect of oxygen and are active only to hypoxic cells.[1]

Among radiosensitizers, metronidazole and misonidazole (Ro-07-0582) are the most important. In an *in vivo* study, using epidermal cell survival[7] both drugs specifically sensitized hypoxic cells when administered before irradiation and had no sensitizing effect in oxic condition. This study showed that misonidazole was much more effective than metronidazole; the ER were 1.4–2.2 over the range 100–1250 mg/kg for misonidazole and 1.1–1.3 over the same range for metronidazole.

In mouse tumor systems, misonidazole, when given *in vivo*, showed a greater radiosensitizing effect than metronidazole. While metronidazole has an ER of 1.9 *in vitro*, it does not exceed 1.3 with clinically tolerable doses *in vivo*[3,4,7,27,29] whereas misonidazole has an ER of 2.5 *in vitro* and 1.1–1.9 *in vivo*.[6,7,14,19,24,25,26,27]

In our study ER is 1.48 ± 0.25 and 1.35 ± 0.27 in doses of 500 mg/kg and 100 mg/kg respectively. These results show that the ER for ornidazole is higher than metronidazole, but is lower than misonidazole. However our test system is different from the above experiments. The strain of mice may also effect the results. Although it is not accurate to compare the results of this study with other experiments, it may be stated that the results strongly indicate that ornidazole effectively increases the radiosensitivity of mammalian tissue under hypoxic conditions *in vivo*.

There are only two studies in the literature about the radiosensitizing effect of ornidazole. In one ornidazole is shown to sensitize the anoxic bacteria almost as efficiently as 2-nitroimidazoles *in vitro*[3] and in the other no sensitizing effect is demonstrated on KTH tumor of mice *in vivo*.[24]

Hyperthemia also has a sensitizing effect on hypoxic cells;[13] this effect is increased with addition of radiosensitizing drugs.[10,12,15,30] These drugs however, also have a killing effect on hypoxic cells.[12,20,21,28] Elevated temperatures greatly increase the cytotoxicity of the drug.

In a study by Sutherland and MacFarlane[31] several 2 and 5 nitroimidazole analogues were evaluated in different experiments. Although not significantly different, it appears that two of the 5-nitroimidazoles may be more effective than two of the nitroimidazoles when combined with hyperthemia. In this study about twice as many cells were killed by ornidazole than by misonidazole.[31] It is thus shown that ornidazole by itself is more effective than misonidazole in killing hypoxic cells.

Two of these studies and our results show that ornidazole increases cell-kill ratio when combined with hyperthermia or irradiation in hypoxic cells. Therefore it is worthwhile to try this drug in clinical radiosensitization studies.

## REFERENCES


1. Adams, G.E., Dische, S., Fowler, J.F., Thomlinson, R.H.: Hypoxic cell sensitizers in radiotherapy. *Lancet* **1:** 186–188, 1976.
2. Alper, T., Hornsey, S.: The effect of hypoxia during irradiation on four-day death in mice given single and split doses of electrons. *Brit. J. Radiol.* **41:** 375–380, 1968.
3. Asquith, J.C., Watts, M.E., Patel, K., Smithen, C.E., Adams, G.E.: Electron affinic sensitization, V. Radiosensitization of hypoxic bacteria, and mammalian cells in vitro by some nitroimidazoles and nitropyrazoles. *Radiat. Res.* **60:** 108–118, 1974.
4. Begg, A.C., Sheldon, P.W., Foster, J.L.: Demonstration of radiosensitization of hypoxic cells in solid tumours by metronidazole. *Brit. J. Radiol.* **47:** 399–404, 1974.
5. Cadoz, M., Beucler, A., Lafaix, C.: Treatment by injectable ornidazole of serious infections caused by non-soil-dwelling anaerobic bacteria. *Current Chemotherapy, Proc. of the 10th Intern. Congr. of Chemotherapy, Zurich, 18–23 Sept. 1977*, Vol. 1, W. Siegenthaler, R. Luethy (Eds.). Washington, Am. Soc. Microbiol. 1978, pp. 721–723.
6. Denekamp, J., Harris, S. R.: Tests of two electron-affinic radiosensitizers in vivo using regrowth of an experimental carcinoma. *Radiat. Res.* **61:** 191–203, 1975.
7. Denekamp, J., Michael, B.D., Harris, S.R.: Hypoxic cell radiosensitizers: Comparative tests of some electron affinic compounds using epidermal cell survival *in vivo*. *Radiat. Res.* **60:** 119–132, 1974.
8. Fernex, M., Jeunet, F., Richle, R.: Development of a nitroimidazole derivative (Ro 7-0207) for the treatment of amoebiasis, Lambliasis and trichomoniasis. *Progress in Chemotherapy, Proc 8th Int. Cong. Chemotherapy, Athens 1973*, Vol. 1 G.K. Daikos (Ed.). Athens, Hellenic Soc. Chemother. 1974, pp. 978–982.
9. Garcia Rodriquez, J.A., Garcia Sanchez, J.E., Saenz Gonzales, M.C., Prieto, R.: The sensitivity of anaerobic gramnegative bacilli to four nitroimidazole derivatives. *Pharmatherapeutica* **1:** 573–582, 1977.
10. George, K.C., Hirst, D.G., McNally, N.J.: Effect of hyperthermia on cytotoxicity of the radiosensitizer Ro 07-0582 in a solid mouse tumour. *Brit. J. Cancer* **35:** 372–375, 1977.
11. Goldstein, E.J.C., Sutter, V.L., Finegold, S.M: Comparative susceptibilities of anaerobic bacteria to metronidazole, ornidazole, and SC-28538. *Antimicrob. Agents Chemother.* **14:** 609–613, 1978.
12. Hall, E.J., Biaglow, J.: Ro 07-0582 as a radiosensitizer and cytotoxic agent. *Int. J. Radiat. Oncol. Biol. Phys.* **2:** 521–530, 1977.
13. Harisiadis, L., Hall, E.J., Kraljevic, U., Borek, C.: Hyperthermia: Biological studies at the cellular level. *Radiology* **117:** 447–452, 1975.
14. Hill, S.A., Fowler, J.F.: Radiosensitizing and cytocidal effects on hypoxic cells of Ro 07-0582, and repair of x-ray injury, in an experimental mouse tumour. *Brit. J. Cancer* **35:** 461–469, 1977.

15. Hofer, K.G., Hofer, M.G., Leracitano, J., McLaughlin, W.H.: Radiosensitization of hypoxic tumor cells by simultaneous administration of hyperthermia and nitroimidazoles. *Radiat. Res.* **70:** 362–377, 1977.
16. Hoffer, M., Grunberg, E.: Synthesis and antiprotozoal activity of 1-(3-Chloro-2-hydroxypropyl)-Substituted nitroimidazoles. *J. Med. Chem.* **17:** 1019–1020, 1974.
17. Hornsey, S.: Animal survival and cell survival seen in dose rate effects on mouse intestinal damage. In *Biological Aspects of Radiation Protection* T. Sugahara and O. Hug (Eds.). Tokyo, Igaku Shoin Ltd. 1971, pp. 207–212.
18. Hornsey, S.: Sensitization of hypoxic cells in vivo with triacetoneamine N-oxyl (TAN). *Int. J. Radiat. Biol.* **22:** 91–94, 1972.
19. Johnson, R., Gomer, C., Pearce, J.: An investigation of the radiosensitizing effects of Ro 07–0582 on hypoxic skin in primates. *Int. J. Radiat. Oncol. Biol. Phys.* **1:** 593–599, 1976.
20. Mohindra, J.K., Rauth, A.M.: Increased cell killing by metronidazole and nitrofurazone of hypoxic compared to aerobic mammalian cells. *Cancer Res.* **36:** 930–936, 1976.
21. Moore, B.A., Palcic, B., Skarsgard, L.D.: Radiosensitizing and toxic effects of the 2-nitroimidazole Ro 07-0582 in hypoxic mammalian cells. *Radiat. Res.* **67:** 459–473, 1976.
22. Pearson, E.S., Hartley, H.O.: *Biometrika Tables for Statisticians*, Vol. 1. Cambridge, The Syndics of the Cambridge Univ. Press. 1962, pp. 4–9, 114–121.
23. Quastler, H.: The nature of intestinal radiation death. *Radiat. Res.* **4:** 303–320, 1956.
24. Rauth, A.M., Kaufman, K., Thomas J. E.: *In vivo* testing of hypoxic cell radiosensitizers. *Proceedings 5th Int. Congress Radiation Research.* NY, Academic Press. 1975, pp. 761–772.
25. Rofstad, E.K., Brustad, T.: Radiosensitizing effect of misonidazole in acute and fractionated irradiation of a human osteosarcoma xenograft. *Int. J. Radiat. Oncol. Biol. Phys.* **6:** 1163–1167, 1980.
26. Sheldon, P.W., Foster, J.L., Fowler, J.F.: Radiosensitization of C3H mouse mammory tumours by a 2-nitroimidazole drug. *Brit. J. Cancer* **30:** 560–565, 1974.
27. Sheldon, P.W., Hill, S.A.: Hypoxic cell radiosensitizers and local control by X-ray of a transplanted tumour in mice. *Brit. J. Cancer* **35:** 795–808, 1977.
28. Sridhar, R., Koch, C., Sutherland, R.: Cytotoxicity of two nitroimidazole radiosensitizers in an in vitro tumor model. *Int. J. Radiat. Oncol. Biol. Phys.* **1:** 1149–1157, 1976.
29. Stone, H.B., Withers, H.R.: Tumor and normal tissue response to metronidazole and irradiation in mice. *Radiology* **113:** 441–444, 1974.
30. Stratford, I.J., Adams, G.E.: Effect of hyperthermia on differential cytotoxicity of a hypoxic cell radiosensitizer, Ro 07-0582, on mammalian cells *in vitro*. *Brit. J. Cancer* **35:** 307–313, 1977.
31. Sutherland, R., Mac Farlane, W.: Cytotoxicity of radiosensitizers in multicell spheroids: Combination treatment with hyperthermia. *Brit. J. Cancer* **37:** 168–172, 1978.
32. Whillans, D.W., Adams, G.E., Neta, P.: Electron affinic sensitization, VI. A pulse radiolysis and ESR comparison of some 2- and 5-nitroimidazoles. *Radiat. Res.* **62:** 407–421, 1975.