



UNIVERSITÀ  
DEGLI STUDI  
FIRENZE

## FLORE

# Repository istituzionale dell'Università degli Studi di Firenze

### **Qualitative and quantitative effects on the morphology of the small intestine after multiple daily fractionation.**

Questa è la Versione finale referata (Post print/Accepted manuscript) della seguente pubblicazione:

*Original Citation:*

Qualitative and quantitative effects on the morphology of the small intestine after multiple daily fractionation / A. BECCIOLINI; D. CREMONINI; D. FABBRICA; M. BALZI. - In: ACTA RADIOLOGICA. ONCOLOGY. - ISSN 0349-652X. - STAMPA. - 23(5):(1984), pp. 353-359.

*Availability:*

The webpage <https://hdl.handle.net/2158/339830> of the repository was last updated on

*Terms of use:*

Open Access

La pubblicazione è resa disponibile sotto le norme e i termini della licenza di deposito, secondo quanto stabilito dalla Policy per l'accesso aperto dell'Università degli Studi di Firenze (<https://www.sba.unifi.it/upload/policy-oa-2016-1.pdf>)

*Publisher copyright claim:*

La data sopra indicata si riferisce all'ultimo aggiornamento della scheda del Repository FloRe - The above-mentioned date refers to the last update of the record in the Institutional Repository FloRe

(Article begins on next page)

## QUALITATIVE AND QUANTITATIVE EFFECTS ON THE MORPHOLOGY OF THE SMALL INTESTINE AFTER MULTIPLE DAILY FRACTIONATION

A. BECCIOLINI, D. CREMONINI, D. FABBRICA and M. BALZI

### Abstract

The behaviour of qualitative and quantitative morphologic parameters in rats exposed on the abdomen only to multiple daily fractionation (MDF) of 3 Gy $\times$ 2 and 3 Gy $\times$ 2 $\times$ 2 with 12 h splits was analyzed. Both doses were sublethal. The number of epithelial cells in the crypts and in the whole formation, the mitotic index, and the labelling index appeared to be markedly reduced at early intervals after irradiation. Repair and recovery quickly led to normal morphology. The effects of an 8 Gy single dose were compared with those from multiple fractionations.

For many years now, new schedules of dose administration have been investigated in animals in order to find out when—given the same total dose—healthy tissues show the highest tolerance (11, 12, 15, 16, 23, 26–28).

The intestinal epithelium is suitable for assessing tolerance of healthy tissues, with high cell renewal, to treatments with chemical and physical agents. Highly proliferative tissues such as the bone marrow, intestine, and epidermis, affect the radiation dose and its modes of administration.

The aim of this experiment was to evaluate the effect produced by 3 Gy fractions given twice or four times every 12 hours.

Qualitative and quantitative morphologic parameters such as the number of crypt and villus cells, labelling, and mitotic indices were determined at different time intervals after the end of treatment.

The time of day when the irradiation was given was kept strictly controlled as the effects have been shown to be dependent on the time when the exposure is carried out (7, 8, 12, 15).

Comparison was made between the effects from single session irradiation with different doses (12, 14–17) and from 6 Gy $\times$ 2 fractionation (11).

### Material and Methods

A series of 132 female Wistar rats, 10 to 12 weeks old and weighing between 180 and 200 g, with the same characteristics as those previously analyzed, were kept at a constant light/darkness cycle (6.30 a.m. to 6.30 p.m.) and with water and food ad libitum. Experimental conditions were the same as those reported previously (13).

Anesthetized animals were exposed on the abdomen only (field 5 cm $\times$ 5 cm) to a  $\gamma$ -ray source (telecobalt unit, 0.8 Gy min<sup>-1</sup> dose rate). The dose was calculated at midline. Irradiation started at noon. Three Gy per fraction were used every 12 h up to a total dose of 6 or 12 Gy. Fifty minutes was the maximum time for irradiation and killing of the different groups.

At 11 days, 4 groups of animals were killed at 2 and 8 a.m. and 2 and 8 p.m. to evaluate variations in

Accepted for publication 23 November 1983.

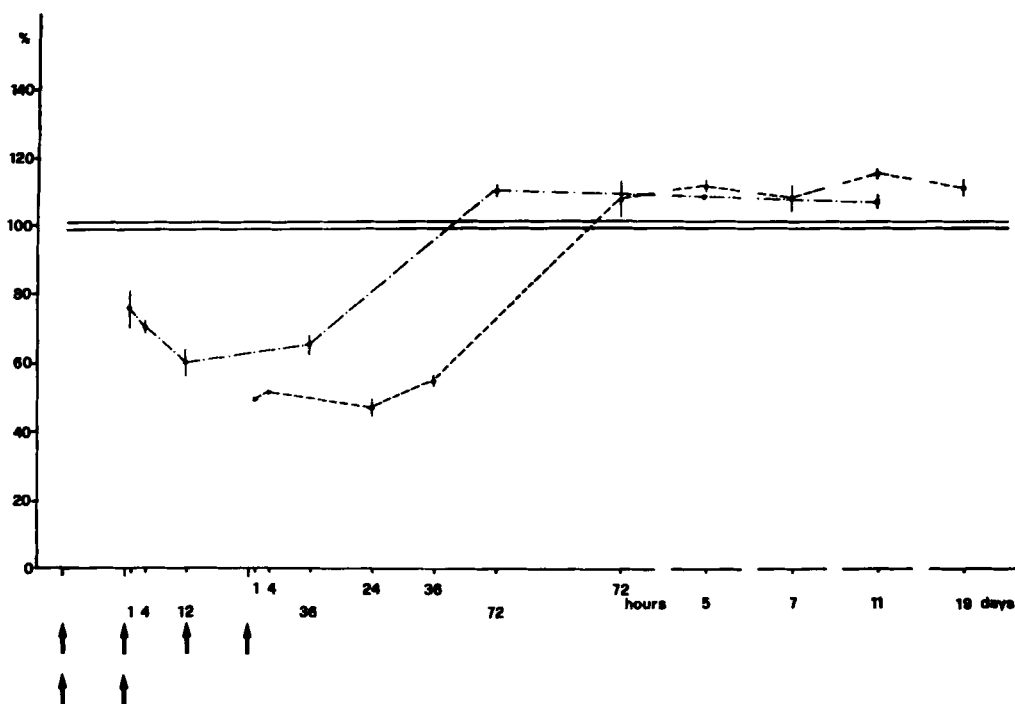


Fig. 1. Number of epithelial cells ( $\pm$ SEM) in the left side of the crypts from groups of animals killed at different intervals after the last fraction of 6 and 12 Gy MDF. The values are calculated

as percentages of control values. Controls (—), 3 Gy $\times$ 2 MDF (---), 3 Gy $\times$ 2 $\times$ 2 MDF (-·-·-).

the parameters analyzed over a 24 h period. Fifty controls were divided into 5 groups of 10 animals each killed at 3 a.m., 8 a.m., 2 p.m., 4 p.m. and 8 p.m. One hour before the killing 6 to 8 controls per group and 3 to 4 irradiated animals were injected intraperitoneally with 3.7 MBq (100  $\mu$ Ci) of  $^3$ H thymidine (specific activity 74 GBq/mmol).

The following parameters were considered in this analysis: morphologic observations, number of crypt cells, number of cells in the whole formation, labelling index and mitotic index.

Histologic methods and those concerning the counting of the sections of the proximal jejunum have been reported previously (7-9, 12, 13).

As already observed (7-9, 12, 13) the number of epithelial cells in the left side of the crypt and in the whole formation presented no statistically significant differences in control animals killed at different times of the day. Therefore the mean value  $\pm$ SEM was taken as 100 per cent and shown in Fig. 1 as a continuous line.

Because of the differences in the mitotic and labelling indices in controls during the L/D cycle, the irradiated animals were compared with controls killed at the same time of the day and the results

were expressed as percentages. In the figures the standard error of control is the maximum value observed in the different groups.

Three Gy $\times$ 2 fractionation was used to evaluate the injury produced in the intestinal epithelium by only two fractions. When analyzing the results, the time that elapsed from the beginning of treatment to the killing should be taken into account. This time was 13 h in 3 Gy $\times$ 2 MDF and 37 h in 3 Gy $\times$ 2 $\times$ 2 MDF.

It should be noted that when the damage was in its acute phase, especially at 36 and 72 h after 12 Gy MDF, counts were carried out in those areas where the epithelium presented a sufficiently regular structure, in other words where the damage was less severe.

## Results

### Qualitative modifications

**3 Gy $\times$ 2 fractionation.** One hour after the end of treatment alterations were present only in the lower half of the crypt, where cells larger than in the controls, with globous nuclei, were observed; numerous Feulgen positive fragments of nuclear material were present in the cytoplasm.

At 4 h, the Feulgen positive fragments decreased; a reduced number of cells with evident nuclear and cytoplasmic alterations was present even in the upper part of the crypts. At 12 h altered cells were observed also in the lower third of the villi. Phlogosis occurred in the stroma.

At 36 h, an increase in proliferative activity was observed even if in some areas misaligned cells with globous nuclei were still present.

Alterations occurred also in the upper part of the villi and in some parts of the epithelium anucleated cells were observed. An increased number of phlogosis cells and dilated blood vessels appeared in the stroma.

The morphology of intestinal epithelium at 72 h was similar to that of the controls: the cells of crypt-villus formations were regular in shape, well aligned, even if the globous nuclei were still present mostly in the villi. Mitotic activity was evident. The cell composition of the stroma appeared to be within normal limits.

At later intervals (5–11 days) a morphology similar to that of the controls was observed.

*3 Gy×2×2 fractionation.* The proliferative compartment and the lower part of the differentiated compartment showed morphologic alterations 1 h after the last fraction. The markedly shortened epithelium presented heavy cellular alterations, and thin crypts with globous, widely spaced nuclei separated by areas with cytoplasm only. No mitoses were present. Rare Feulgen positive fragments were observed in the crypt. Very numerous phlogosis cells were present in the stroma.

At 4 h, the proliferative compartment showed wide areas that had lost their original structure. Altered cells were present up to 2/3 of the villi which, in some cases, had lost their individuality. Mitotic activity was low. Phlogosis cells in the stroma had further increased. Feulgen positive fragments were still present in the crypt.

At 24 to 36 h, alterations in the whole formation indicated a further shortening of the epithelium. A high number of phlogosis cells was present in the stroma together with some dilated vessels; some proliferative activity occurred. At 72 h, the high proliferation led to reduced morphologic injury in the crypts although some altered cells were still present.

Recovery occurred unevenly. In fact, in the same section—next to the regenerating crypts—heavily altered crypts occurred. The cell composition of the

stroma appeared to be nearly normal in the healing area. At 5 days, a few enlarged and slightly stained nuclei were still present in the villi. At 7, 11 and 19 days, the morphology of the intestinal epithelium was similar to that of the controls.

#### *Quantitative results*

In the control animals used in this experiment the values for the number of crypt cells, total cells and mitotic and labelling indices were similar to those obtained in previous experiments:  $38.1 \pm 0.21$  for crypt cells,  $106.1 \pm 1.74$  for total cells. The values for the mitotic index fluctuated between  $1.94 \pm 0.17$  at 8 a.m. and  $2.41 \pm 0.14$  at 2 p.m. The labelling index varied between  $21.66 \pm 0.37$  at 8 a.m. and  $23.39 \pm 0.63$  at 3 a.m.

*Number of epithelial crypt cells.* With 3 Gy×2 fractionation, the number of cells in the left side of the crypt was significantly lower ( $p < 0.001$ ) 1 h after the end of exposure, and the lowest value was observed at 12 h (Fig. 1). No significant increase appeared at 36 h, whereas starting from 72 h the values were significantly above those of the controls ( $p < 0.01$ ).

Three Gy×2×2 fractionation produced a more marked initial reduction in the number of crypt cells due to the higher dose used and to the time elapsing between the beginning of treatment and the first observation. The values remained at about 50 per cent of those in the controls until 24 h.

At 36 h, a modest increase, like that occurring with the 6 Gy total dose, was observed, and at 72 h the number of epithelial cells was higher than in the controls in the areas where counts were possible, but the difference was not statistically significant. At subsequent intervals, and up to the end of the experiment, the number of crypt cells was significantly higher than in the controls ( $p < 0.01$ ).

*Number of total cells.* Misalignment in the upper part of the crypt-villus formation in some of the animals treated with 3 Gy×2 MDF and in some treated with 3 Gy×2×2 did not allow sufficient counts at some of the intervals, and these data are therefore not reported graphically.

The results of MDF with 3 Gy×2 showed reduction already at the early intervals and 4 h after exposure the total number of epithelial cells in the left side of the formation had significantly decreased when compared with the controls. At 3 and 5 days, the values were similar to those of non-irradiated animals.

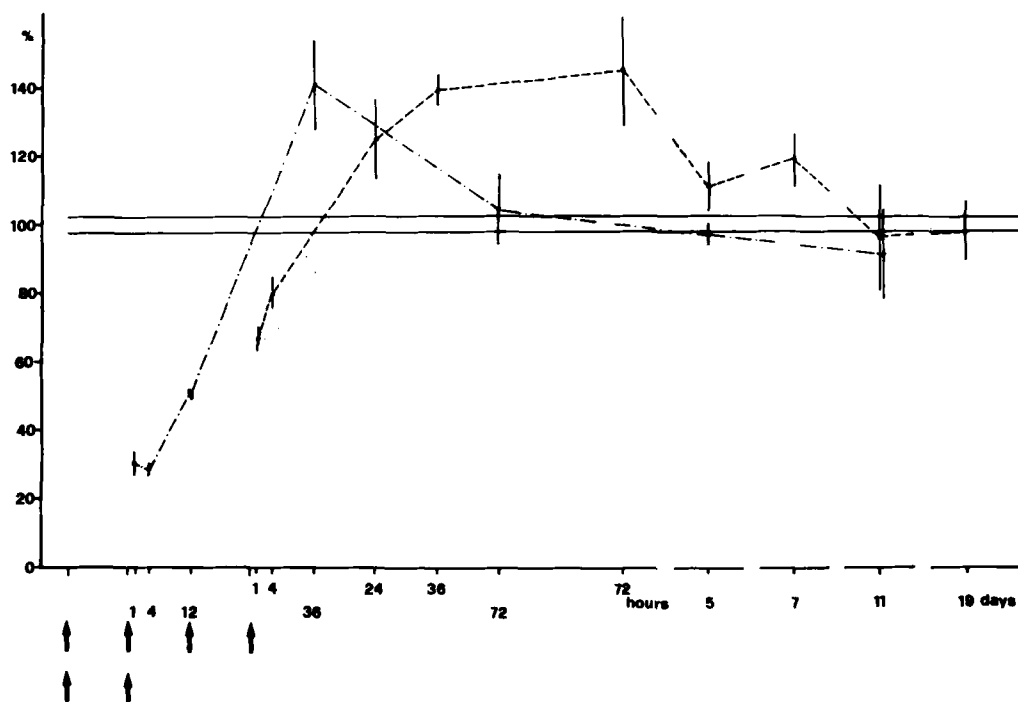


Fig. 2. Labelling index ( $\pm$ SEM) at different intervals after 6 and 12 Gy MDF. The values are expressed as percentages of those of controls killed at the same times of the day (cf. Fig. 1).

With 3 Gy $\times$ 2 $\times$ 2 fractionation a statistically significant reduction in the number of total cells was observed as early as 1 h after the last fraction. The lowest level (about 40% of that in the controls) was reached 24 and 36 h after exposure.

At later intervals the number of cells increased up to nearly control values, and remained higher, although not significantly.

**Labelling index.** With 3 Gy $\times$ 2 fractionation 3H thymidine uptake was blocked at the first two intervals and the labelling index was about 30 per cent of that in the controls (Fig. 2). Twelve h after the last fraction, the increase was progressive and at 36 h it led to values significantly higher than in the controls ( $p < 0.01$ ). At subsequent intervals the labelling index remained at control levels.

One and 4 h after 3 Gy $\times$ 2 $\times$ 2 fractionation, the values were significantly higher than those observed at the same initial intervals with 3 Gy $\times$ 2 fractionation, even if they were markedly lower than in the controls ( $p < 0.01$ ). At 24 h, the values were higher than in the controls ( $p < 0.05$ ) and this maximum increase was observed at 36 h and 72 h ( $p < 0.01$ ). Only at 11 and 19 days was the labelling index similar to that of non-irradiated animals.

Although with some variations, the behaviour of

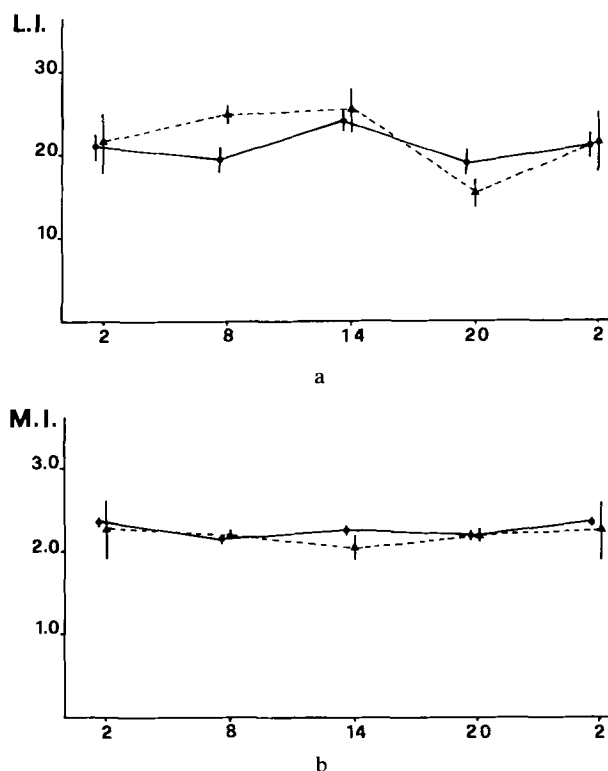


Fig. 3. Behaviour of a) labelling index and b) mitotic index in animals irradiated with 3 Gy $\times$ 2 $\times$ 2 MDF and killed at different times of the day, 11 days after exposure. Controls (—). Irradiated animals (---).

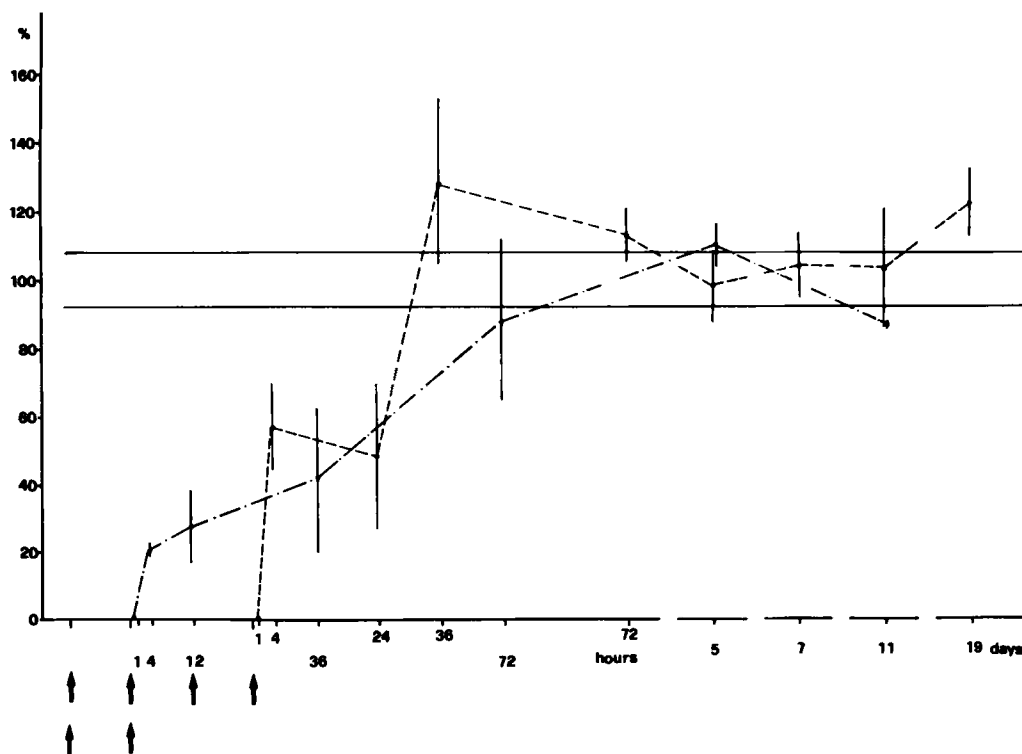


Fig. 4. Mitotic index ( $\pm$ SEM) at different intervals after 6 and 12 Gy MDF. The values are expressed as percentages of those of controls killed at the same times of the day (cf. Fig. 1).

the labelling index in the animals killed at different hours after 11 days did not show significant differences with respect to the controls killed at the same hours (Fig. 3 a).

**Mitotic index.** The block of proliferative activity brought the mitotic index to zero 1 h after the last fraction in the 3 Gy $\times$ 2 MDF (Fig. 4).

Later, and until 36 h, the levels increased to 20 to 40 per cent of those of the controls. Although marked differences were apparent among the animals of the same groups the mitotic index reached control values at 72 h and remained steady until the end of the experiment.

After MDF with 3 Gy $\times$ 2 $\times$ 2 the mitotic index was generally higher than that observed with the other MDF at the same intervals. The mitotic index was zero at the initial intervals and was above control levels at 36 h. Later, the values appeared to be similar to those in non-irradiated animals.

In the animals killed at different hours, the mitotic index 11 days after exposure was similar to that in the controls at 2 a.m. and 2 p.m. whereas it was significantly higher at 8 a.m. ( $p < 0.01$ ) and lower at 8 p.m. ( $p < 0.05$ ; Fig. 3 b).

### Discussion

The use of multiple daily fractionation in clinical practice is a fairly recent method (1, 3, 13, 18–22, 24) and experiments have been performed to evaluate the tolerance of healthy tissues. These investigations have mostly been carried out on the skin and, rarely, on the small intestine (6, 11, 23, 27, 28).

When fractionated doses are used, the onset of injury is obviously influenced by the effect of the different fractions to a varying extent, according to the split between them as well as to the time that has elapsed before killing.

With multiple fractionation of 3 Gy $\times$ 2, morphologic alterations appeared to be comparatively slight, and repair and recovery occurred easily. With the higher total dose, the injury was obviously more severe but in both cases the number of crypt cells was above the control values at 72 h.

Comparison of results obtained with both multiple fractionations demonstrated that the curves followed the same slope when the time that elapsed from the last fraction was equal, and protracted irradiation led to increased damage with a more marked reduction in epithelial cells. With the 12 Gy

total dose also, the injury appeared to be healing rather rapidly, at least in those areas where counts were possible.

At the early intervals, the reduction in the cells of the villi appeared to be smaller than in the cells of the crypts, owing to cell turnover which caused injury in the villi only later. Subsequently, and up to 36 h, reduced proliferative activity caused considerable alterations in the whole formation.

At early intervals after the last fraction the labelling index of 3 Gy $\times$ 2 fractionation showed a marked reduction in tracer uptake, which was more marked than that shown with the higher dose. This result indicated repair and recovery mechanisms which operated in the intervals between the fractions, and demonstrated that the 12 h split is high enough to limit the injury to DNA synthesis.

Moreover, the results show that with the higher total dose the uptake of 3H thymidine remains at significantly higher levels much longer than with the 6 Gy dose.

It is worth noting that with both multiple daily fractionations a comparatively close relation existed between the number of crypt cells and the labelling index; the latter tended to return to normal levels when the number of epithelial cells was equal to or above control values.

The mitotic index also confirmed these data, although it presented much wider fluctuations in the animals from the different groups, particularly at the early intervals.

In the animals killed at different times during the eleventh day the mitotic index showed values similar to those of the controls at 2 a.m. and 2 p.m., while it was higher at 8 a.m. and lower at 8 p.m.

Comparison between the 12 Gy multiple daily fractionation and the 8 Gy single dose showed a reduction in the number of epithelial cells which was similar in both cases up to 72 h from the beginning of treatment.

Morphologic alterations were qualitatively similar whereas, quantitatively, the injury appeared to be markedly less severe with multiple daily fractionation inasmuch as there was a higher number of areas showing sufficiently regular morphology, where counts were possible. Lesser differences were observed when comparing the 96 h single dose specimen with those from multiple daily fractionation 72 h after the end of treatment.

The labelling index 1 h after multiple fractionations with 3 Gy $\times$ 2 $\times$ 2 was similar to those observed

12 h and 36 h respectively after the 8 Gy single dose delivered in the dark period.

After the 6 Gy total dose, the labelling index rapidly exceeded control values and returned to normal levels just as rapidly. With 3 Gy $\times$ 2 $\times$ 2 fractionation the increase of the labelling index lasted longer when considering the time that elapsed from the beginning of exposure.

As regards the mitotic index, the values at the end of the fractionations were lower with respect to the single dose but increased much more rapidly than after 8 Gy. The return to normal values occurred more slowly with respect to the labelling index and no significant increase was observed, when compared with controls, either after 8 Gy or multiple daily fractionation.

If the results of 12 Gy MDF are compared with those of the 12 Gy single session on the abdomen (2, 10), qualitative and quantitative morphologic modifications produced by 12 Gy fractionation were much less marked and were present for a shorter period.

After the 12 Gy single session about 30 per cent (10) of the animals died from an intestinal radiation syndrome, whereas after multiple fractionation no death was observed. When 2 fractions of 6 Gy administered with 6, 12, 24, 48, 72, and 120 h splits were used (4, 11), a much more severe injury occurred than after 12 Gy fractionation. The injury was particularly marked when the intervals between fractions were from 6 h to 48 h.

The data from the present experiment showed that the small intestine had good tolerance for this schedule of multiple daily fractionation, at least in the acute radiation phase of the injury.

## ACKNOWLEDGEMENTS

Grants were received from CNR-PFCCN, contracts 81.01296.96 and 82.00228.96.

## REFERENCES

1. ARCANGELI G., MAURO F., MORELLI D. and NERVI C.: Multiple daily fractionation in radiotherapy Biological rationale and preliminary clinical experiences. *Europ. J. Cancer* 15 (1979), 1077.
2. ARGANINI L., CASTAGNOLI P., DE DOMINICIS R. e BECCIOLINI A.: Aspetti morfologici dell'epitelio del tenue dopo irradiazione in seduta unica nel ratto. (In Italian.) *Radiol. med.* 59 (1973), 739.
3. BÄCKSTRÖM A., JAKOBSSON P. Å., LITTBAND B. and WERSÄLL J.: Fractionation scheme with low individu-

- al doses in irradiation of carcinoma of the mouth. *Acta radiol. Ther. Phys. Biol.* 12 (1973), 401.
4. BECCIOLINI A., ARGANINI L., CARIAGGI P., MAZZI S. e FRANCIOLINI F.: Effetti di dosi frazionate di radiazioni sulla morfologia dell'intestino tenue in relazione alle contemporanee modificazioni delle attività di disaccarasi e dipeptidasi. (In Italian.) *Nuntius Radiol.* 39 (1975), 13.
  5. — — TEDDE G., VANNELLI G. and CARIAGGI P.: Biochemical and morphological changes in the epithelial cells of the small intestine after irradiation. *Int. J. Radiat. Oncol. Biol. Phys.* 1 (1976), 915.
  6. — BALZI M., BENUCCI A. et coll.: Effect of multiple daily fractionation of the dose in the small intestine of rats. Progress Report 1978, *Radiat. Protection Programme, EUR 6263*, p. 589. Harwood Academic Publishers.
  7. — — CREMONINI D., CINOTTI S. and FABBRICA D.: Behaviour of the proliferative compartment of the small intestine at different times of the day. *Acta radiol. Oncology* 22 (1983), 201.
  8. — — — and FABBRICA D.: S-phase cell distribution in the small intestine irradiated at different times of the day. I. Acute irradiation injury. *Acta radiol. Oncology* 22 (1983), 305.
  9. — — — — S-phase cell distribution in the small intestine irradiated at different times of the day. I. Recovery phase. *Acta radiol. Oncology* 22 (1983), 337.
  10. — CARIAGGI P., ARGANINI L., CASTAGNOLI P. and DE GIULI G.: Effect of 200, 650 and 1200 R on the intestinal disaccharases and dipeptidases. *Acta radiol. Ther. Phys. Biol.* 13 (1974), 141.
  11. — CASTAGNOLI P., ARGANINI L. and DE GIULI G.: Post irradiation enzyme activities of rat small intestine. Effects of dose fractionation. *Radiat. Res.* 55 (1973), 291.
  12. — CREMONINI D., BALZI M., FABBRICA D. and CINOTTI S.: Irradiation at different times of the day. Morphology and kinetics of the small intestine. *Acta radiol. Oncology* 21 (1982), 169.
  13. — — FABBRICA D. and BALZI M.: Modifications of S-phase cell distribution in the intestinal crypts after multiple daily fractionation. *Acta radiol. Oncology* 22 (1983), 441.
  14. — GERBER G. B., BURACCHI A. and DEROO J.: Intestinal enzyme distribution after supralethal irradiation. *Strahlentherapie* 153 (1977), 485.
  15. — GIACHÈ V., LANINI A., CREMONINI D. and DRIGHI E.: Modifications of small intestine lysosomal enzymes after irradiation at different times of the day. *Acta radiol. Oncology* 21 (1982), 61.
  16. — LANINI A., GIACHÈ V., BALZI M. and BINI R.: Modifications of the brush border enzymes of the small intestine after irradiation at different times of the day. *Acta radiol. Oncology* 21 (1982), 273.
  17. — RAVINA A., ARGANINI L., CASTAGNOLI P. and DE GIULI G.: Effect of ionizing radiation on the enzymes of the intestinal mucosa of rats at different time intervals after abdominal irradiation. *Radiat. Res.* 49 (1972), 213.
  18. CHOI C. H. and SUIT H. D.: Evaluation of rapid radiation treatment schedules utilizing two treatment session per day. *Radiology* 116 (1975), 703.
  19. DUTREIX J., WAMBERSIE A. and BOUNIK C.: Cellular recovery in human skin reactions. Applications to dose fraction number overall time relationship in radiotherapy. *Europ. J. Cancer* 9 (1973), 153.
  20. ESCHWEGE F., COSSET J. M., DUTREIX J. et WIBAULT P.: L'irradiation hyperfractionnée. *J. Europ. Radiother.* 2 (1981), 269.
  21. GONZALES-GONZALES D., BREUR K. and V.D. SCHUEREN E.: Preliminary results in advanced head and neck cancer with radiotherapy by multiple fractions a day. *Clin. Radiol.* 31 (1980), 417.
  22. HOLSTI L. R., SALMO M. and ELKIND M. M.: Unconventional fractionation in clinical radiotherapy. *Brit. J. Cancer* 37 (1978), 307.
  23. MAISIN J., WAMBERSIE A., LAMBERT-COLLIER M. and GUEULETTE J.: Intestinal proliferation after multiple fractions of gamma-irradiation. *Radiat. Res.* 71 (1977), 338.
  24. SVOBODA V. H. J.: Radiotherapy by several sessions a day. *Brit. J. Radiol.* 18 (1975), 131.
  25. — Further experience with radiotherapy by multiple daily sessions. *Brit. J. Radiol.* 51 (1978), 363.
  26. TURESSON I. and NOTTER G.: The response of pig skin to single and fractionated high dose-rate and continuous low dose-rate <sup>137</sup>Cs irradiation. II. Theoretical consideration of the results. *Int. J. Radiat. Oncol. Biol. Phys.* 5 (1979), 955.
  27. WAMBERSIE A. and DUTREIX J.: Early recovery for intestinal stem cells as a function of dose per fraction evaluated by survival rate after fractionated irradiation of the abdomen in mice. *Radiat. Res.* 58 (1974), 498.
  28. WITHERS H. R., CHU A. M., REID B. O. and HUSSEY D. H.: Response of mouse jejunum to multifraction radiation. *Int. J. Radiat. Oncol. Biol. Phys.* 1 (1975), 41.