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MODIFICATIONS IN THE BRUSH BORDER ENZYMES OF THE SMALL INTESTINE AFTER IRRADIATION AT DIFFERENT TIMES OF THE DAY

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The function of the small bowel after irradiation was previously investigated in humans by an absorption test (DALLA PALMA 1968, CIONINI et coll. 1971, BECCIOLINI et coll. 1979 b). A reduced absorption of carbohydrates was demonstrated in rats with the gastro-intestinal radiation syndrome. The membrane digestion process was examined assaying the activity of the brush border enzymes and by means of in vivo absorption tests (BECCIOLINI & RAVINA 1970, BECCIOLINI et coll. 1972, 1973, 1976, 1977 a).

The brush border enzyme activity showed a circadian oscillation depending on the light–darkness cycle, feeding–fast, or—according to the latest hypothesis—on the interaction of different synchronizing agents (SAITO et coll. 1975, STEVENSON et coll. 1975, BECCIOLINI et coll. 1977 b, NISHIDA et coll. 1978). The brush border enzyme activity reached the highest level at night whereas the lowest values were found in the afternoon. The differences were statistically significant. Circadian oscillations in enzyme activity mean that the functional activity of the epithelial cells of the small intestine differs over the 24 h cycle. The aim of the present experiments was to investigate whether exposure to the same sublethal dose at specific times of the day would induce different modifications in the activity of the enzymes involved in the membrane digestion process. The animals were killed every 6 h, from 120 h up to 150 h after irradiation to prove whether during the recovery phase a return to normal circadian rhythms of activity occurred.

Lysosomal enzyme activity in the same animals was reported previously (BECCIOLINI et coll. 1982 a).

Materials and Methods

Ten to twelve week old female Wistar rats weighing 180 to 200 g were used. They were kept at a L/D cycle 6.30 a.m. to 6.30 p.m. with water and food ad libitum.

Control groups of 8 animals each were killed at 0, 4, 8, 12 a.m. and 4, 8 p.m. The other animals were divided into 4 groups (A, B, C, D) irradiated respectively at 0, 6, 12 a.m. and 6 p.m. with 8 Gy of γ-rays from a ^{60}Co source over an abdominal area of 5 cm $\times$ 5 cm including the entire small intestine. The dose was calculated at midline. The animals were killed in groups of 5 at 10 intervals between 6 hours and 29 days after irradiation.

The irradiation and killing of the animals of each group did not take more than 50 min. Immediately thereafter the small bowel was removed, longitudinally opened, washed in cold saline (0.9% NaCl) and cut into 5 equal parts. Two small pieces, cut at the end of the first and third segments, were used for microscopic examination. The 5 segments were homogenized in distilled water (10% w/v) with a glass homogenizator and then centrifuged at 900 $\times$ g. The supernatant was used for the determination of the brush border enzyme activity. Threalase, lactase and alkaline phosphatase were assayed according to the techniques previously reported (BESSEY et coll. 1946, DAHLQUIST 1964). The protein content of the homogenate was also determined (EGGSTEIN & KREUTZ 1955). All assays were made in duplicate, the determinations differing less than 5 per cent; mean values were used. The enzyme activity was

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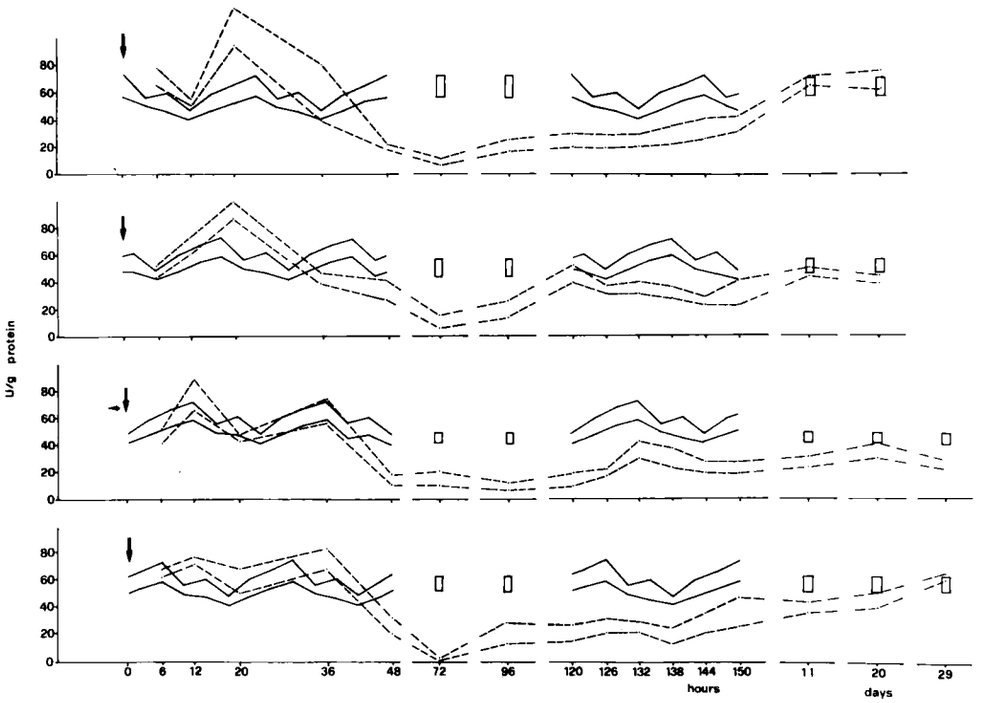

Fig. 1. Threalase specific activity in the first tract of the small intestine in the 4 groups irradiated at different times of the day (from above: A, B, C, D). Time of irradiation (→). The continuous line represents mean values ± SEM of controls and the broken line values from irradiated animals.

expressed as U/g of protein where one unit is the activity that hydrolyses one μmol of substrate per min under standard conditions.

The total activity was calculated considering the weight of the entire small intestine. Student's t-test was used for statistical assessment.

The distribution of disaccharase and alkaline phosphatase differs along the small intestine. The post-irradiation behaviour appeared similar in the 5 tracts, but higher differences between control and irradiated animals were observed in the tracts in which the activity was higher. In order to reduce the number of illustrated curves, only the specific activity of the segment where the activity was highest is reported. The time of exposure is indicated in the illustration with an arrow over the curve of controls killed at the same time.

Results

Threalase activity distribution along the small intestine differs from the other disaccharases and dipeptidases. The activity is high in the initial two tracts, then gradually decreases and appears very low in the terminal ileum. The circadian oscillations are statistically significant in the three initial tracts. The threalase activity in the first tract appears in Fig. 1. In the other segments the activity curves were similar.

In group A, which was irradiated when in the controls the activity was highest, the curve levels were similar to the controls 6 and 12 h after irradiation although at 20 h the increase was statistically significant ($p<0.01$). After 36 h the levels were similar to controls and at 48 and 72 h a statistically significant decrease ($p<0.01$) was observed. From 96 h the activity increased but was lower than in controls as late as 150 h after irradiation. On day 11, threalase reached normal values.

In group B, irradiated when circadian values were decreasing, the curve was at the same level as in the controls until 12 h, then the activity increased and reached the highest level at 20 h ($p<0.02$). Thirty-six hours after exposure the activity was lower than in controls and decreased progressively until 72 h. At 120 and 126 h the values were similar to those in the controls, but later they were significantly reduced ($p<0.05$). Control values were reached on day 11.

In group C, exposed when the circadian oscilla-

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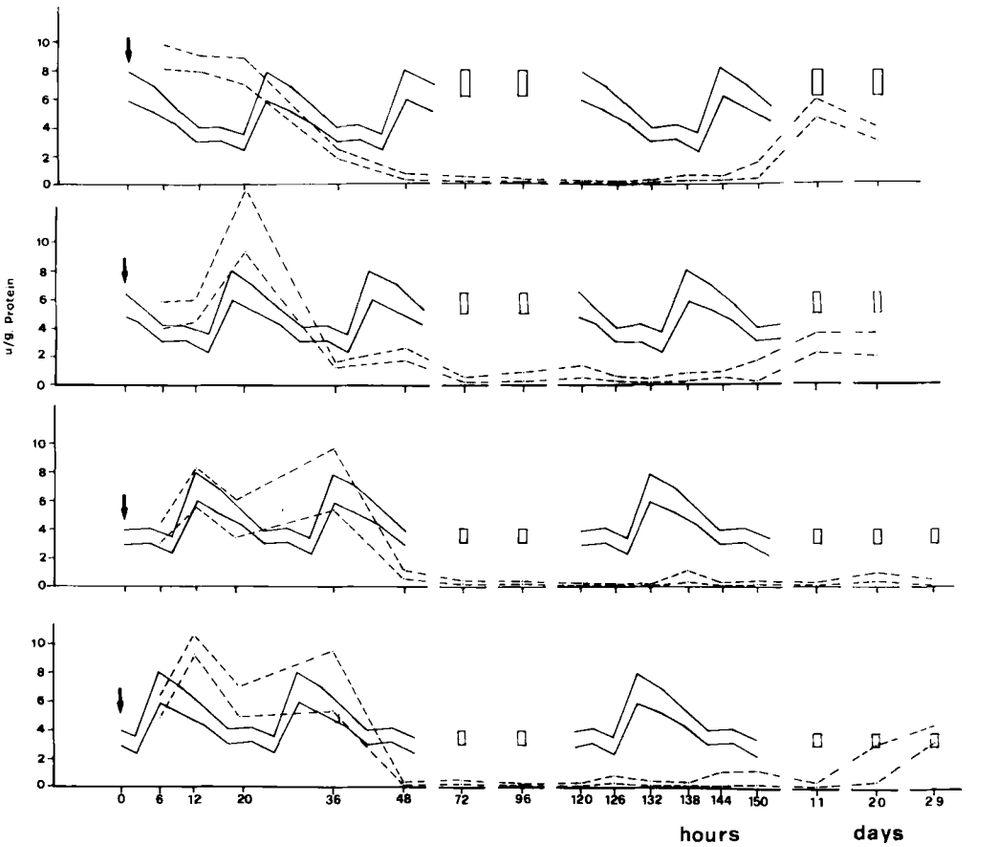

Fig. 2. Lactase specific activity in the second tract of the small intestine (cf. Fig. 1).

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tion was at its lowest, the curve of irradiated rats was at the same level as in controls until 36 h. A very low activity was recorded between 48 and 120 h, then it increased, but significantly reduced levels were present also later ($p<0.02$).

When irradiation was performed during the increase of the activity (group D) the circadian oscillations disappeared and levels significantly higher than in controls were observed 12 ($p<0.02$) and 36 h ($p<0.05$) after exposure. The minimum at 72 h was close to zero; later the activity increased but not above 50 per cent of the control values. Normal values were not found before 20 days after irradiation.

Lactase activity in the second tract appears in Fig. 2. The highest lactase value in the small intestine was found in the middle part and very low values in the duodenum and terminal ileum.

Group A showed a significant increase in lactase activity from 6 to 20 h ($p<0.01$) after irradiation, then it decreased and reached values close to zero until 150 h. Later the activity increased but remained significantly lower than in controls ($p<0.05$).

In group B the activity rose above the normal circadian curve and was at its highest 20 h after exposure ($p<0.02$). From 36 h the lactase levels were significantly lower than in controls ($p<0.01$) and a partial increase was observed at 11 and 20 days.

At initial intervals the lactase activity in group C was at the same levels as with threalase; then, starting from 48 h the activity was close to zero and its values were low as late as 29 days.

Group D showed a significant increase at 12 and 20 h ($p<0.02$); from 48 h after irradiation the values were very low until 20 days after exposure.

Alkaline phosphatase is distributed in the small intestine similarly to threalase. The activity in the second segment, which is more homogeneous than the first segment, appears in Fig. 3. The results showed a different behaviour for alkaline phosphatase activity after irradiation in the 5 tracts of the small bowel; assays with specific substrate showed

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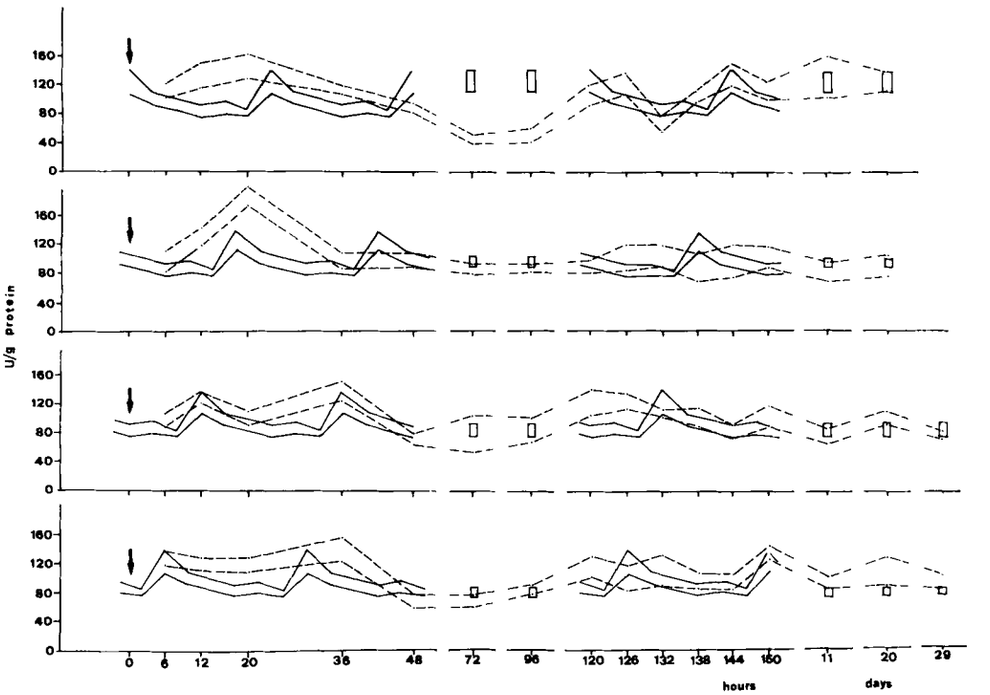

Fig. 3. Alkaline phosphatase specific activity in the second tract of the small intestine (cf. Fig. 1).

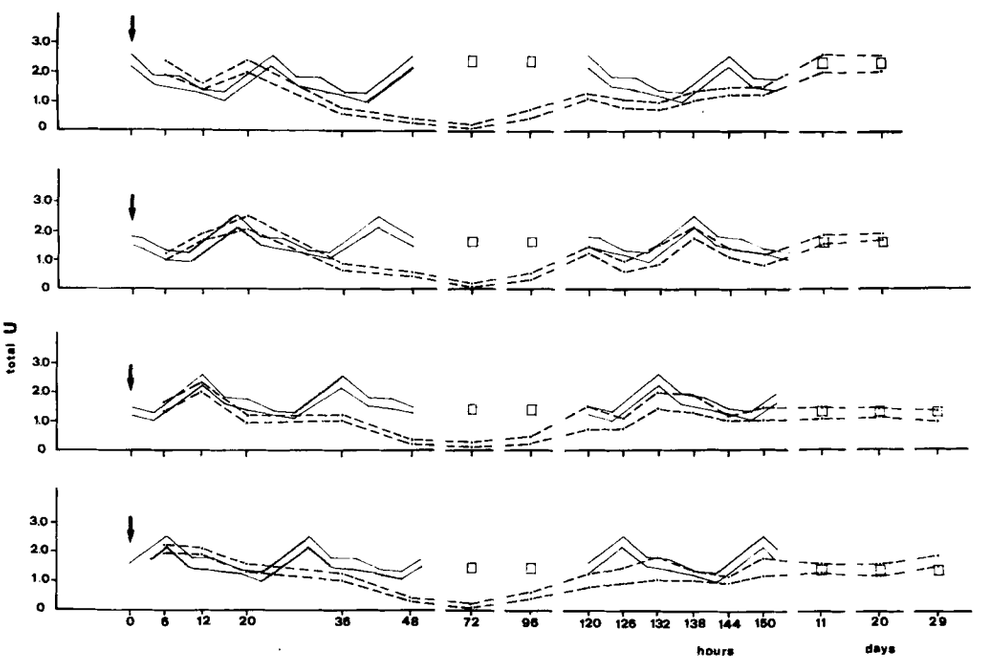

Fig. 4. Total maltase activity in the whole small intestine (cf. Fig. 1).

that the brush border component was high only in the initial segments. The increase phase was rather similar to that of disaccharase. In groups A and B the activity was significantly higher at 12 and 20 h ($p<0.02$); then it decreased but was still higher than in controls at 36 h. In group C the activity was the same as in irradiated animals than in the controls. In group D the increase was statistically significant

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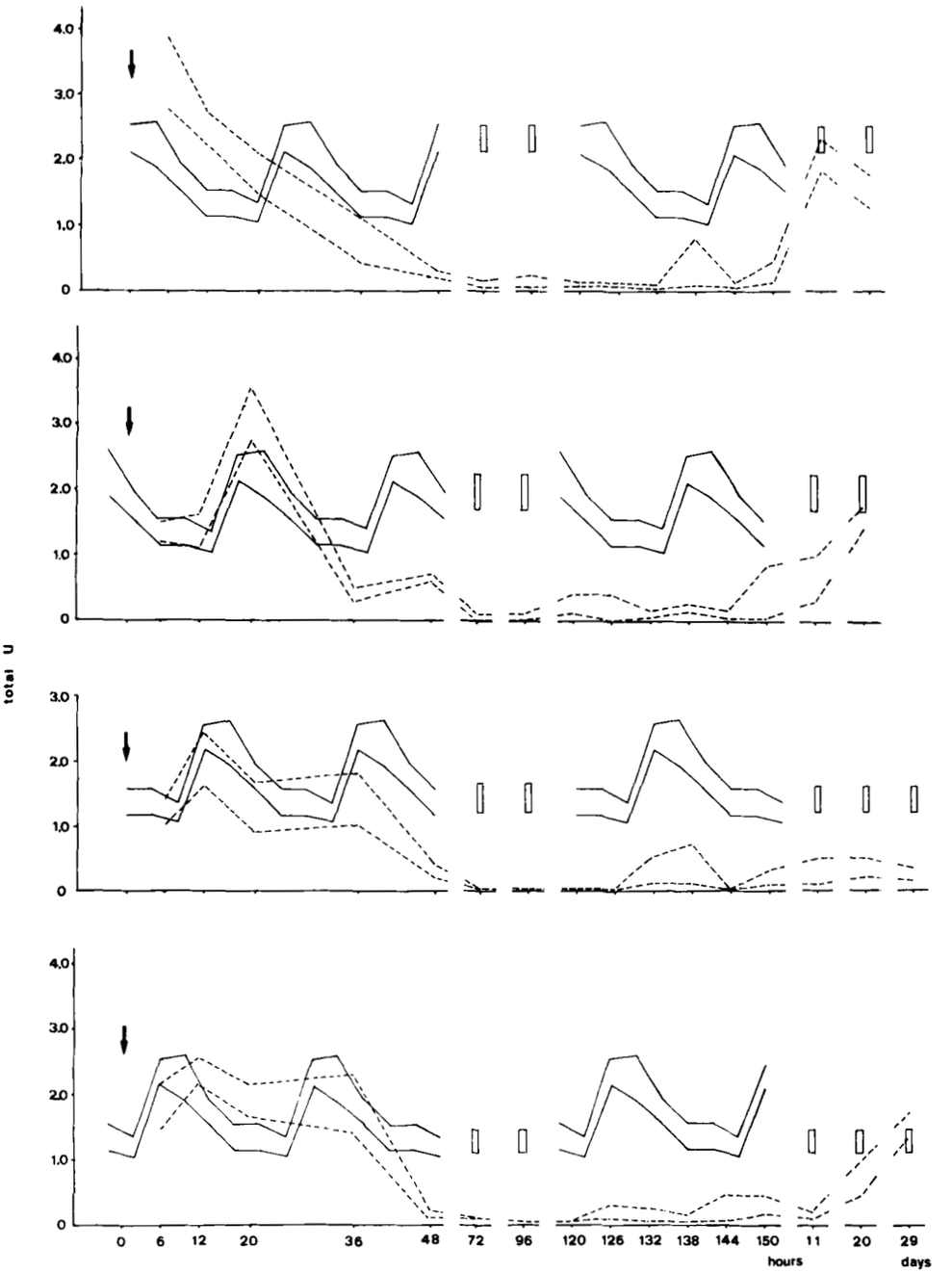

Fig. 5. Total lactase activity in the whole small intestine (cf. Fig. 1).

($p<0.05$) until 36 h after exposure. The differences in decrease in relation to the other brush border enzymes were evident; a statistically significant decrease ($p<0.01$) occurred only in group A. Later, no significant differences were evident in controls or groups A, B, and D; in group C an increase was observed at 120 and 126 h.

Total activity. The weight of the small intestine decreased after irradiation and reached the lowest values (about 30% lower than in controls) at 36, 48 and 72 h. The weight increased up to 140 per cent of that in controls at 120 h, later it tended to return to normal levels. The behaviour in all irradiated groups was rather similar although in group D the reduction was less evident.

The maltase activity in the entire small intestine in

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the 4 irradiated groups is illustrated in Fig. 4. At early intervals the activity was similar to that in controls in all groups. At 36 h the values were significantly lower and were close to zero after 72 h. During the recovery values similar to controls were found only in groups B and C.

The threalase activity was similar to that of maltase in all groups although in group C significantly lower levels occurred as early as at 20 h. In group B the values were similar to controls during the 120 to 150 h period, but at slightly lower levels than in group C.

The total lactase activity (Fig. 5) in groups A and B increased at the beginning, but still during the 120 to 150 h period normal values for the activity had not been obtained in all groups. On day 11 the activity tended to return to normal levels in groups A and B.

The total activity values for alkaline phosphatase were similar to those in controls until 36 h. During the injury phase, the total activity was significantly reduced with a minimum at 72 h in all groups. From 120 h the values were higher than in controls until the small bowel weight was higher than in the controls. The activity tended to return to normal levels in groups A and B on day 11.

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Discussion

The aim of the present experiments was to elucidate the effect of ionizing radiation on the brush border enzyme activity, and thus indirectly on the cell differentiation process during acute injury and recovery. In control animals brush border enzymes are synthesized in the upper third of the crypt by epithelial cells, which have lost the ability to proliferate. The synthesis of these enzymes can be considered characteristic of the differentiation process. Thus the determination of brush border enzyme activity during acute injury and especially during recovery indicates the behaviour of the differentiation process.

Three phases in the post-irradiation behaviour of brush border enzymes were demonstrated previously: initial increase, reduction, and tendency to return to normal (BECCIOLINI et coll. 1972, 1974, 1976). The present results confirm this general behaviour but some differences appeared when the irradiation was performed at different times of the day.

The time of irradiation must be defined and the differing functional activity of the cells during the day must be considered. The analysis of the first part of the curves during the increasing phase of the activity shows that exposure during the highest circadian levels or during the decrease of the activity causes an increase reaching its maximum at 20 h. Higher levels last longer in the group irradiated when the enzyme activity is increasing. When the exposure is performed during the lowest circadian level the disaccharase activity is similar to the corresponding control values.

These results relate the increase of the enzyme activity to the level of activity present at irradiation.

Lactase and alkaline phosphatase behave like threalase except in group A where the initial values are high and no circadian oscillation was observed.

During the acute injury phase, when morphologic alterations of the epithelium occur, the activity of brush border enzymes was very low, and similar in all irradiated groups.

During the recovery phase, α-glycosidase differed from β-galactosidase. The activity level of the latter was close to zero at the 120 to 150 h period and over, whereas the α-glycosidase tended to lie at normal levels.

In group B, values closer to those in controls were found during this phase. In the animals irradiated in the middle of the light period no increase of the enzyme activity was found and normal values were not found before 29 days after exposure.

Since brush border enzyme activities are built up during the cell differentiation, the results confirm that the return to the functional capacity during the recovery phase is incomplete. On the other hand the morphology of intestinal epithelium of the same animals appears normal even if the crypts are made up of more cells than in controls (BECCIOLINI et coll. 1982 b).

The data obtained from animals killed between 120 and 150 h after irradiation confirm this hypothesis: the α-glycosidase activity shows some oscillations different from those in the controls.

The alkaline phosphatase activity corresponded only in part to a brush border enzyme activity, specially in the lower part of the small intestine. During the injury phase only a slight reduction was observed, and during recovery the activity values were similar to those in controls.

SUMMARY

The behaviour of the brush border enzyme activity of the intestinal epithelium after the same sublethal radiation

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dose to the abdomen at different times of the day was investigated. Three previously observed post-irradiation phases (initial increase of activity, reduction, and return to control values) were confirmed, although with some differences. A later return to normal of lactase was also confirmed. The same dose produced different behaviour of the enzyme activities both during the initial and the recovery phase, depending on the time of the day when irradiation was performed, i.e. on the functional condition of the epithelial cells.

ACKNOWLEDGEMENTS

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