S-phase cell distribution in the small intestine irradiated at different times of the day. II. Recovery phase.

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S-PHASE CELL DISTRIBUTION IN THE SMALL INTESTINE IRRADIATED AT DIFFERENT TIMES OF THE DAY

II. Recovery phase

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The aim of the present research has been to find methods for administering irradiation which would ensure the highest possible tolerance in healthy tissues after equal doses of ionizing radiation. Modifications in the small intestine have been under investigation for some years, and they have been evaluated by analyzing biochemical morphologic and cell kinetic parameters (CAIRNIE et coll. 1965, BECCIOLINI et coll. 1972, 1976, 1979, AL-DEWACHI et coll. 1979).

In these experiments the animals were exposed to the same sublethal dose of ionizing radiation administered at different times of the day. As already reported, differences have been observed in the behaviour of the enzyme activity which characterizes the function of the differentiated cells and in some cell kinetic parameters (BECCIOLINI 1981, BECCIOLINI et coll. 1982 b, 1983 a). Although these differences were not such as to affect the animal's survival they were marked enough to be recordable.

The conditions in the proliferative compartment during recovery have been investigated by evaluating the S-phase cell distribution along the Lieberkühn crypts. The activity of invertase—a brush border enzyme first synthetized by differentiating cells —was also evaluated to ascertain the functional capacity of the intestinal epithelium at different times after irradiation.

Material and Methods

The experimental conditions and the methods followed in this part of the investigation have been described elsewhere (BECCIOLINI, BECCIOLINI et coll. 1982 b, 1983 a). Wistar rats were anesthetized with ether and irradiated on the abdomen only (field 5 cm ×5 cm) with 8 Gy of γ-rays from a telecobalt unit at midnight, 6 a.m., noon and 6 p.m., respectively (groups A, B, C, D). They were killed at 96, 120, 126, 132, 138, 144, 150 h, and 11, 20, and, in groups C and D only, 29 days after irradiation.

Groups of control animals were killed at corresponding times of the day. All animals were injected 3.7 MBq (100 μCi) of 3H Thymidine, specific activity 74 GBq/mmol (2 Ci/mmol) 1 h before killing.

After autoradiography 40 left sides of Lieberkühn crypts aligned according to the villus axis were counted for each animal. The positions of the labelled cells in the crypts were processed in Fortran language and a computer supplied the distribution in a normalized theoretic crypt according to the method described by BECCIOLINI et coll. (1983 a, b).

In the Figures, the mean values, and not the SE limit values, were reported in the group where it

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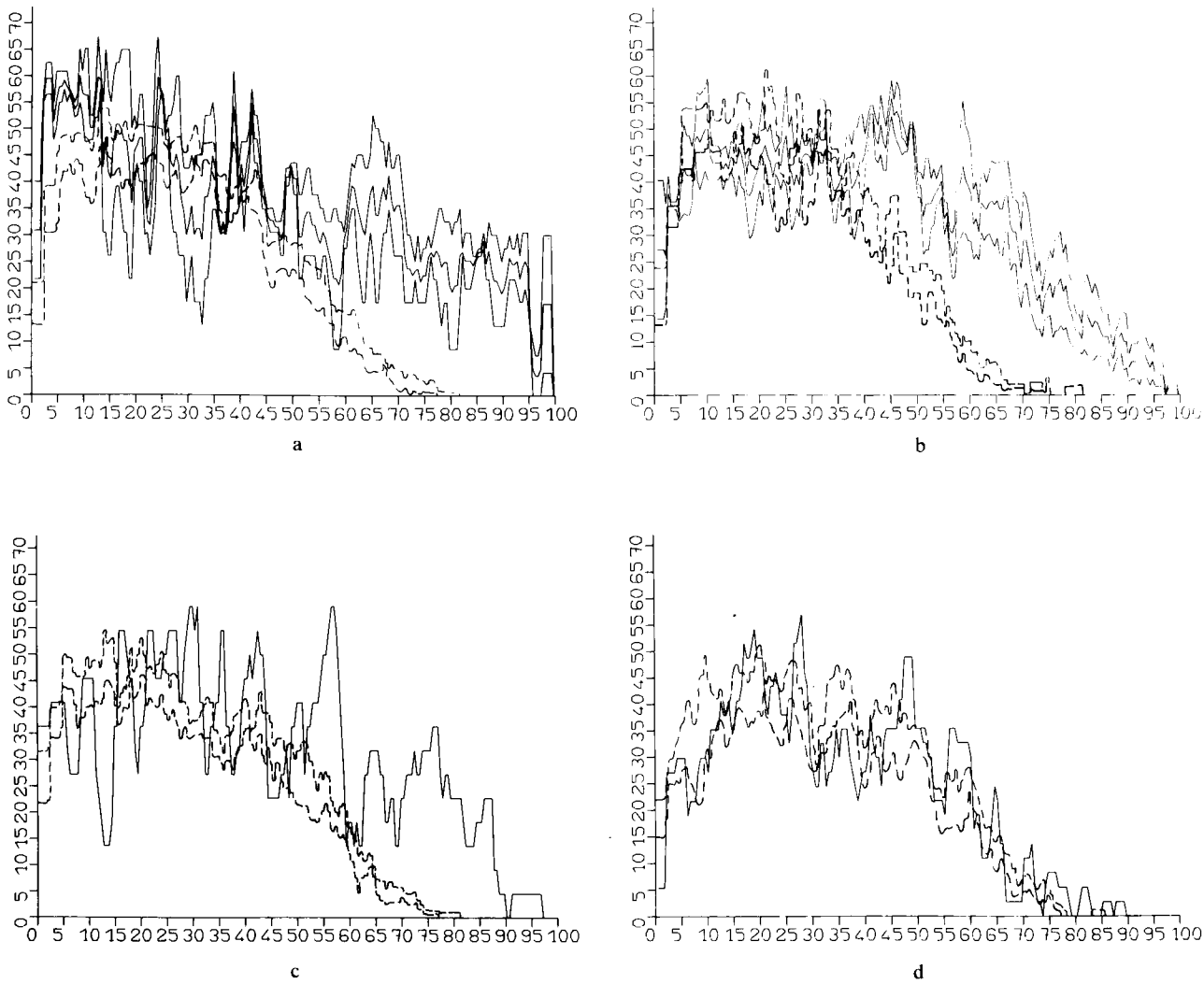

Fig. 1. Labelled cell frequency as per cent between the highest and lowest limits of the standard error of the mean, in the theoretic crypt of animals irradiated at a) midnight, b) 6.00 a.m., c) noon and d) 6.00 p.m., killed after 96 h. Broken lines indicate the distribution of controls killed at the same times of the day. The mean number of epithelial cells in the left side of the crypt was 44, 40, 38 and 45, respectively.

was possible to count completely 1 or 2 animals only; the results have demonstrated, however, that this curve is generally representative of the whole group.

Invertase activity was determined according to the technique described by DAHLQUIST (1964). It was expressed as U/g of protein where 1 U is the activity that hydrolyzes 1 μmol/ml of substrate per min in standard condition. The statistical significance of the differences was evaluated by Student's t-test.

Results

The histologic specimen from animals killed 96 h after exposure demonstrated highly inhomogeneous recovery. Areas where cell disorganization was still marked adjoined areas where recovery was the prevailing feature. In the altered areas the epithelium showed crypts formed by few cells widely separated by stroma containing dilated vessels whereas the villi were short and conglutinated. In the recovering areas the crypts were formed by many cells with nuclei which were still globous and not perfectly aligned, but high mitotic activity was present.

At 120 h the epithelium looked almost normal although some globous nuclei could still be found at the top of the villi. Not all the animals demonstrated complete recovery; repair appeared homogeneous only in group B. At the intervals between 126 and 150 h the morphology was closer to normal, and

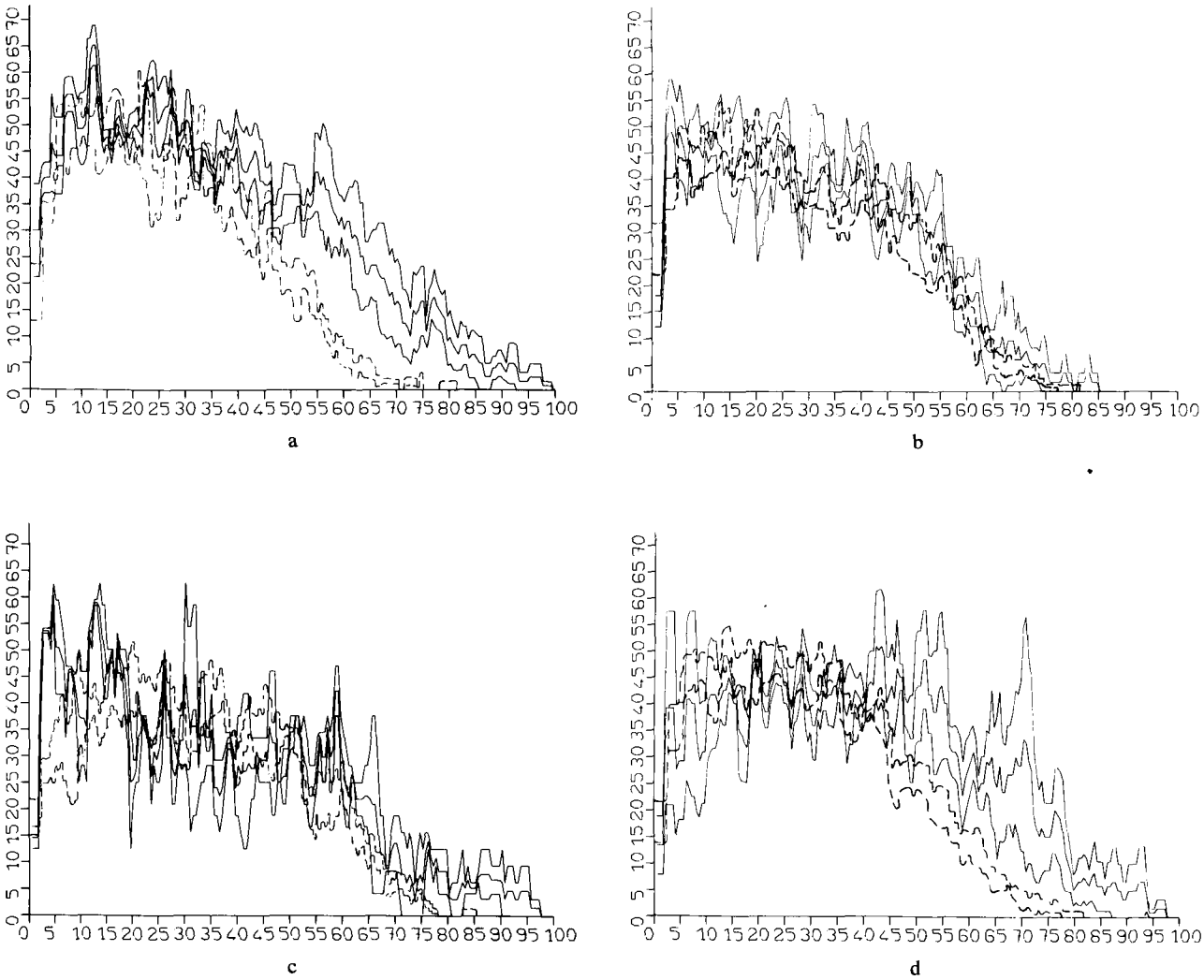

Fig. 2. The frequency, as per cent of labelled cells between the limits of the SEM, in the animals killed at 126 h (cf. Fig. 1). The mean number of epithelial cells in the left side of the crypt was 46, 39, 43 and 46, respectively.

very few epithelial areas where recovery was not complete were distinguished.

During this period counting refers to the less damaged areas.

Despite the increase in the number of epithelial cells and the return to a morphology within normal limits, labelled cells still presented anomalous distribution at 96 h. In the lower third of the crypt, frequency curves were similar to the controls whereas in the rest of the formation, values were sharply higher than in the controls (Fig. 1). Cells capable of incorporating 3H Thymidine were present also in the upper part of the crypts limiting the extension of the differentiating compartment.

Comparison among animals irradiated at different hours demonstrated that the proliferative compartment was still extending rapidly in group A while it was decreasing in the other groups.

In animals killed at 120 h the frequency curve for labelled cells still demonstrated the differences observed at the previous interval, although much reduced.

The distribution in group B was similar to controls killed at the same hours. The other groups demonstrated a marked reduction in the lower half of the crypts while labelled cells were present up to the villus junction in the upper half.

At 126 and 150 h labelled cell frequency in the lower part of the crypts was fairly similar to that in the corresponding controls, although it appeared inhomogeneous among individual animals. At 126 h (Fig. 2) labelled cells were still present in the whole

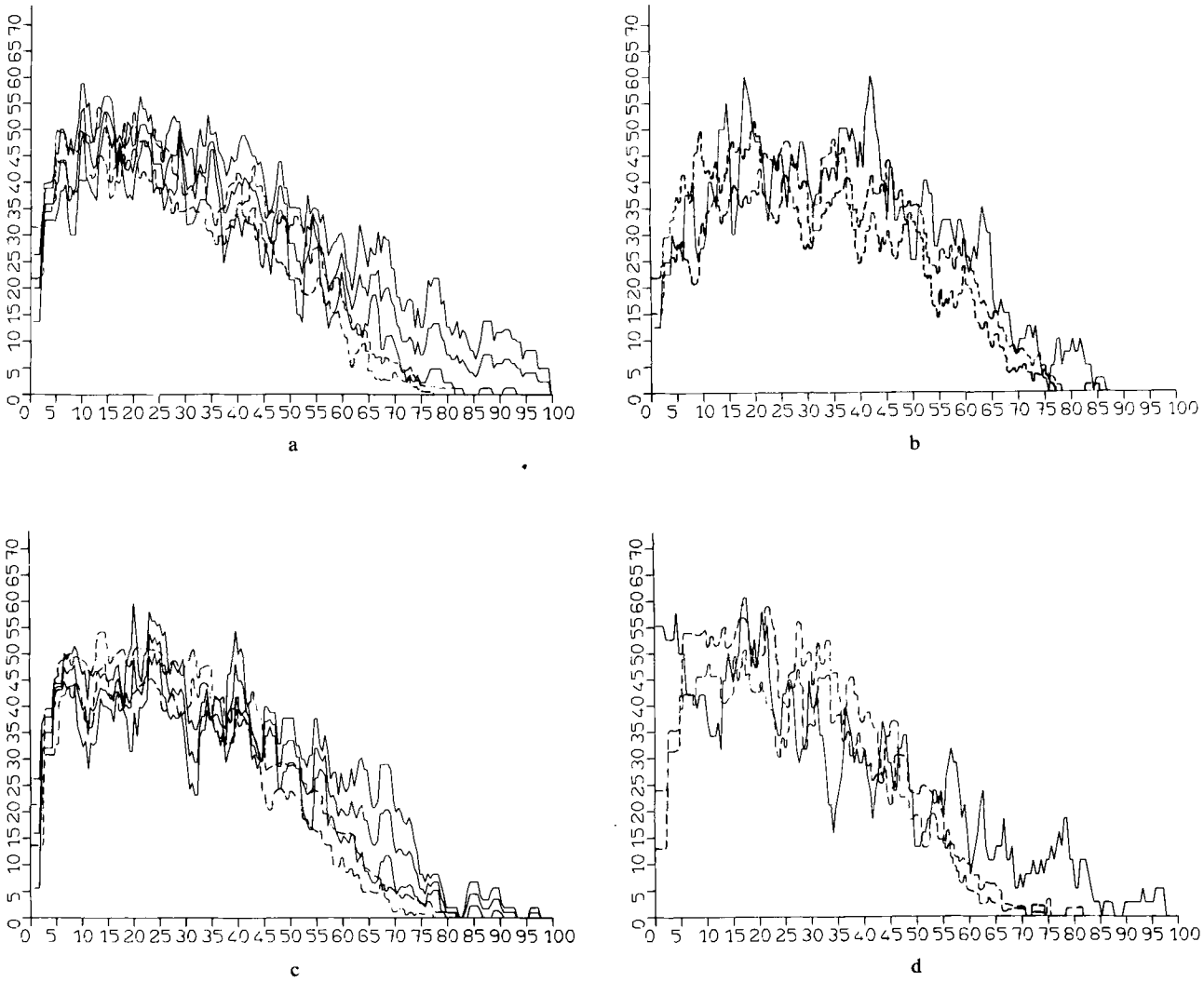

Fig. 3. The frequency, as per cent of labelled cells between the limits of SEM, in the animals killed at 132 h (cf. Fig. 1.). The mean number of epithelial cells in the left side of the crypt was 43, 44, 43 and 47, respectively.

height of the crypts, showing values significantly higher than in the controls, apart from group B where distribution was similar to the controls.

The frequency of S-phase cells in the upper half of the crypts decreased markedly in groups C and D at 132 h (Fig. 3). They were still numerous up to the top of the crypts in group A and in this case too their curve was similar to the controls in group B. At the next interval (Fig. 4), the labelled cell frequency in the upper positions had decreased still further.

This progressive reduction was not observed at 144 h (Fig. 5), when the distribution of labelled cells in the upper part of the crypts was significantly higher than in the controls in all groups except for group D and to some extent for group B.

An incomplete return to the S-phase cell distribution in the controls was also observed at 150 h in all groups except B.

In all animals killed 11 days after irradiation (Fig. 6) labelled cell frequency at the top of the crypts decreased substantially, although it still did not reach control values; this result was an indication of the persisting extension of the proliferative compartment. At this interval also, distribution in group B was similar to the controls.

At 20 days the curves appeared the same as in non-irradiated animals in all groups, and the differentiating cell compartment had a normal appearance.

Invertase activity. The activity of the brush bor-

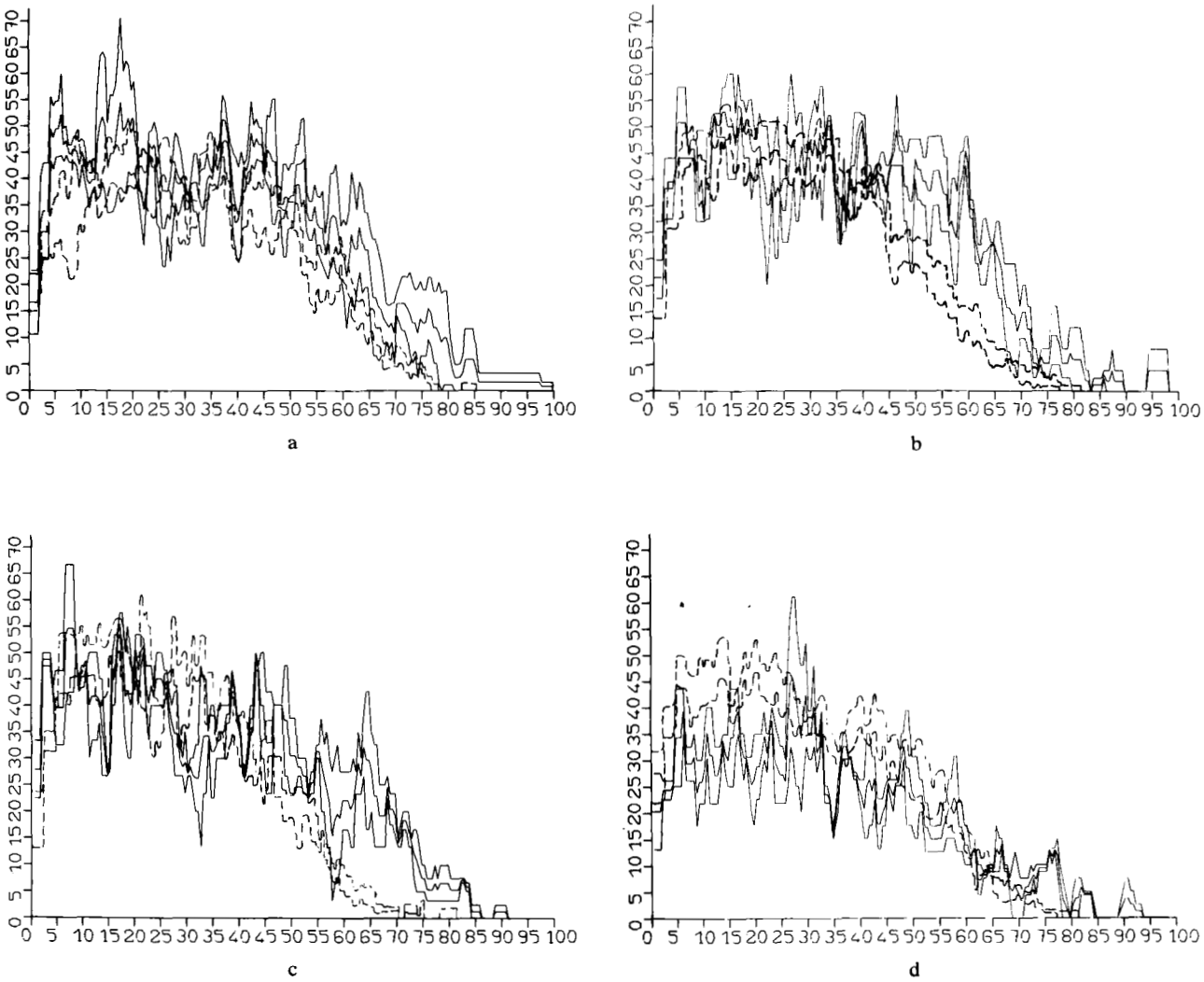

Fig. 4. The frequency, as per cent of labelled cells between the limits of SEM, in the animals killed at 138 h (cf. Fig. 1). The mean number of epithelial cells in the left side of the crypt was 44, 45, 44, and 46, respectively.

der enzyme in the second segment of the small intestine was assessed in order to investigate the behaviour of the enzymes responsible for membrane digestion as an index of the efficiency of the cell differentiation process.

During the acute radiation injury the activity appeared to be close to zero in all groups, and a partial increase was observed at 96 h (Fig. 7). Also at 120 h, in grous A, C and D, the enzyme activity appeared very low whereas in group B the values were similar to those of the controls.

During the 126 h to 150 h period, only modest oscillations of invertase activity appeared in groups A and D: the values remained at very low levels. Group B gave a curve with oscillations similar to the controls but still at statistically significant lower levels ($0.05<p<0.01$).

At 132 and 138 h the activity increased slightly in group C, but then decreased again.

Eleven days after exposure, group B reached control values whereas, among the other groups, group A only approached these values and group D reached them at longer intervals. It is worth noting that in group C activity persisted at a level half as high as those in non-irradiated animals as late as 29 days after exposure.

Discussion

Previously, investigations on the effect of ionizing radiation on the small intestine demonstrated that

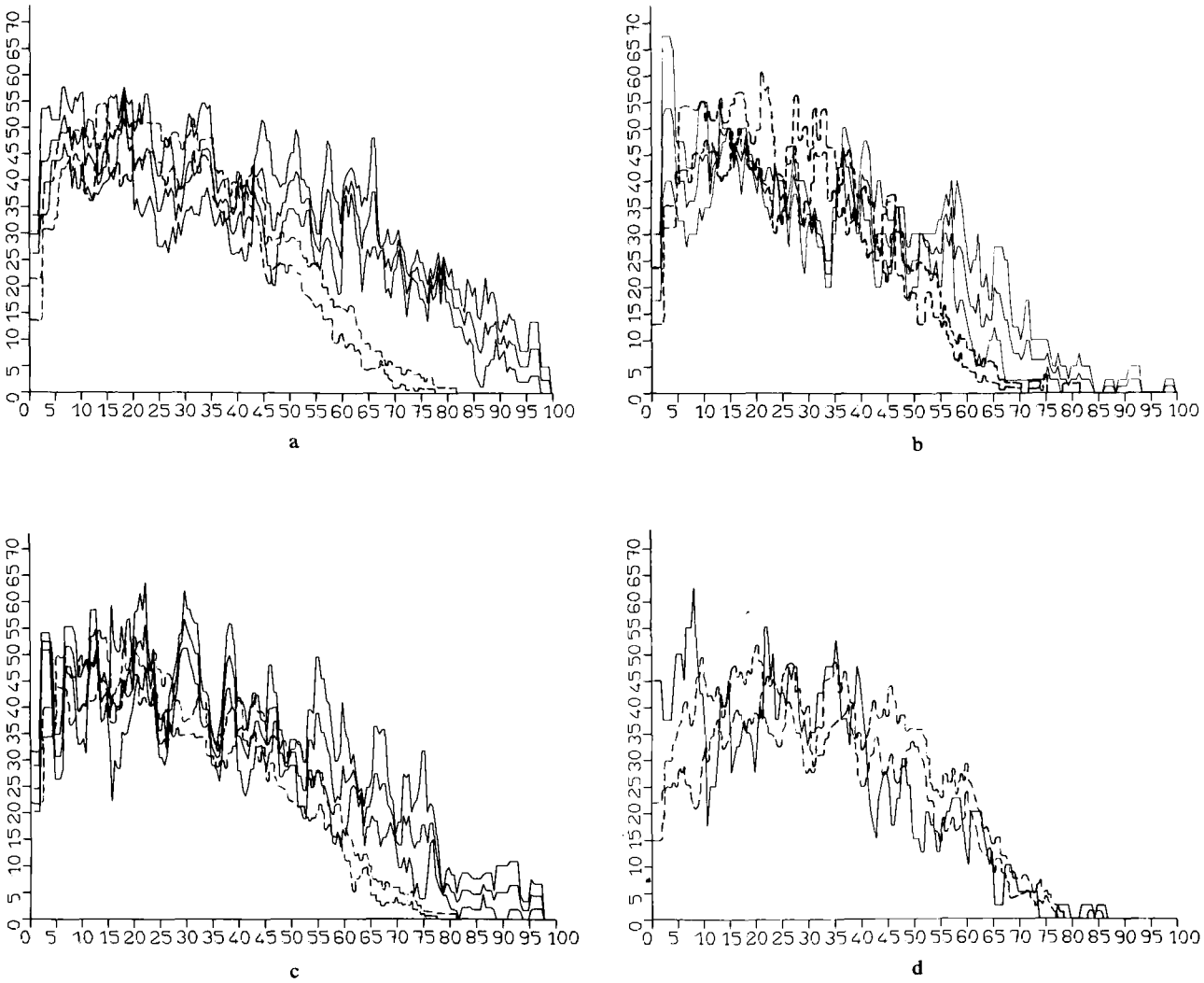

Fig. 5. The frequency, as per cent of labelled cells between the limits of SEM, in the animals killed at 144 h (cf. Fig. 1). The mean number of epithelial cells in the left side of the crypt was 46, 44, 45, and 46, respectively.

about 5 days after exposure complete recovery from morphologic injury had occurred, and epithelial cells had regained a morphology within normal limits (BECCIOLINI et coll, 1972, 1973, 1974, 1976).

Distribution of cells incorporating 3H Thymidine was analyzed in order to assess recovery. As variations in the distribution of S-phase cells over a 24 h period were observed depending on the time of the day when the animals were killed, data were compared at different intervals starting from the 120 h interval and continuing over the following 30 h.

In order to obtain biochemical information on the functional capacity of the epithelial cells an analysis was carried out on the brush border enzyme activity. These enzymes such as disaccharidase and dipeptidase are responsible for a membrane digestion process through which dimers and oligomers present in the intestinal lumen are hydrolyzed into monosaccarides and amino acids to be absorbed by specific transport mechanism (UGOLEV 1968, UGOLEV & DE LAEY1973). These enzymes, synthetized by the columnar cells during cell differentiation which occurs in the upper third of the crypts, are characteristic of the villus cells.

At the 120 to 150 h intervals a definite tendency to return to normal morphologic and functional conditions was observed, but the return was not complete. In fact, even if the crypts appeared to be formed by a significantly larger number of cells than in the controls, labelled cells were found also at

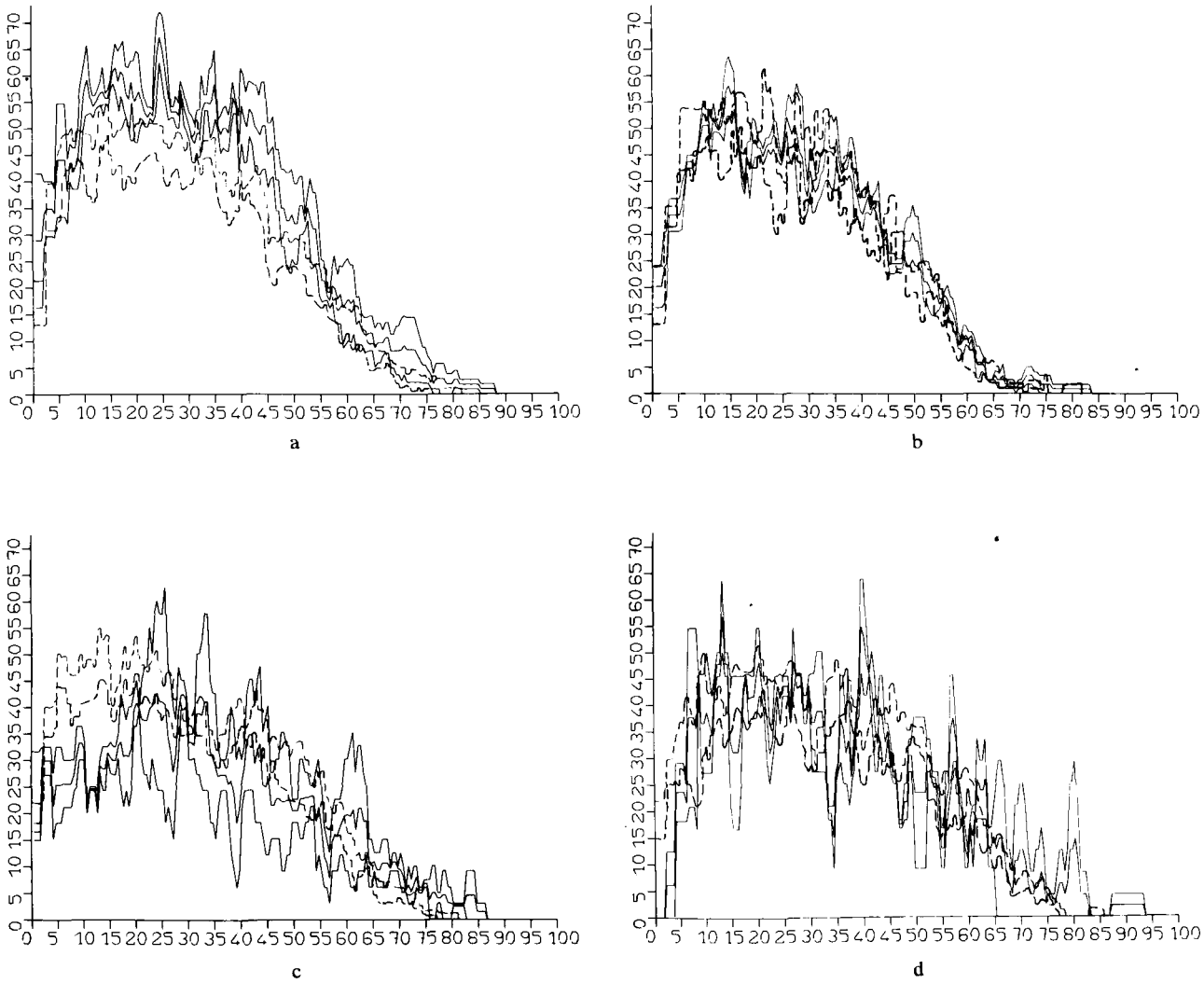

Fig. 6. The frequency, as per cent of labelled cells between the limits of SEM, in the animals killed at 11 days (cf. Fig. 1). The mean number of epithelial cells in the left side of the crypt was 43, 38, 46, and 46, respectively.

villus junctions. The analysis of labelled cell distribution, when compared with that of controls killed at the same times of the day, revealed an extension of the proliferative compartment and therefore a limitation in the size of the differentiating area.

Further confirmation was obtained from the brush border enzyme activity which, at these intervals, persisted at a significantly lower level than in the controls although the number of villus cells was similar (BECCIOLINI et coll. 1982a). The different relation between proliferating cells and differentiating as well as differentiated ones seemed to explain the reduced enzyme activity observed at these intervals.

Determination of brush border enzyme activity seems to be a valuable test in the analysis of cell differentiation after irradiation.

The animals irradiated at the end of the dark period recovered more effectively than the other animals. In fact, in group B, cell distribution along the crypt was similar to that in the corresponding controls as early as 120 h after exposure, and followed the same curve, although with some differences, till the end of the experiment. Variations related to the killing time, which might have been due to a renewal of circadian dependence, appeared again.

Invertase activity confirmed this result inasmuch as the data from group B were closer to the controls than any other data, although they did not quite

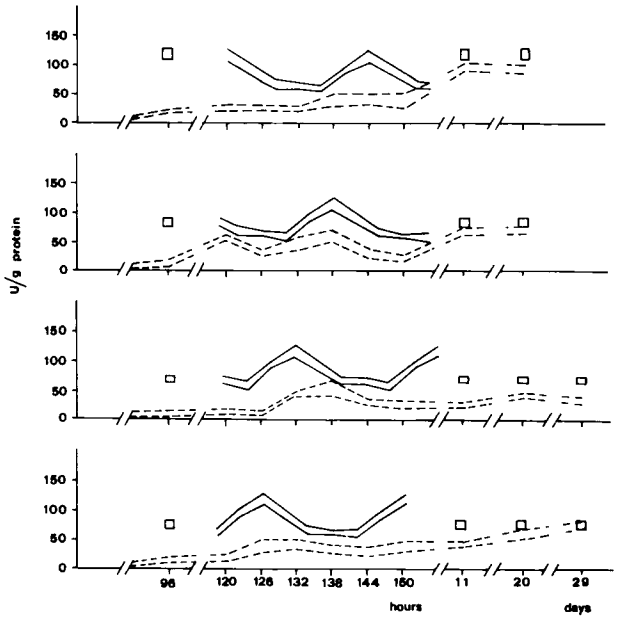

Fig. 7. Invertase specific activity in the second segment of the small intestine in the 4 groups irradiated at different times of the day (A, B, C, D, from the top) and killed starting from 96 h after irradiation. The continuous lines represent mean value ± SE of controls and the broken lines show values from irradiated animals.

reach the same values, and also the activity curve seemed to parallel oscillations at different times of the day.

This return to conditions more similar to the controls, in the group irradiated at the end of the dark period, seemed to indicate a better tolerance by the small intestine when irradiation was carried out at the end of the active period.

SUMMARY

Modifications occurring during recovery in the small intestine of animals exposed to the same radiation dose given at different times of the day were evaluated. S-phase cell distribution along the crypts and invertase activity were evaluated to ascertain the functional capacity of epithelial cells. In animals killed between 5 and 6 days after exposure, S-phase cell distribution and functional conditions tended towards normality although recovery was not complete. Labelled cells occurred also at villus junctions, demonstrating limitation in size of the differentiating compartment. This was confirmed by reduced activity of the brush border enzymes. Animals irradiated at the end of the dark period recovered more quickly and efficiently. In this group, labelled cell distribution was almost the same as in the controls starting from 120 h, and invertase activity was also closer to the controls than in any other group.

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