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QUANTITATIVE CHANGES IN THE GOBLET CELLS OF THE RAT SMALL INTESTINE AFTER IRRADIATION

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Abstract

In order to evaluate the process of cell differentiation in the crypt of the rat small intestine the goblet cells were quantitatively studied in controls and after irradiation of the abdomen. The effect of a single dose, administered at 4 different times of the day, and multiple daily fractionations (MDF) of 6 and 12 Gy with different doses per fraction and different time intervals, were compared. Both regimens caused an initial increase of the goblet cells (both in absolute and relative terms), followed by a decrease and finally return to nearly control levels. After MDF the increase was more marked and the return to a normal level occurred earlier than after the single dose.

The effect of ionizing irradiation on goblet cells is not well known because attention has been concentrated above all on the digestive-absorption function of the small intestine and thus on the columnar cells. Instead, the origin of the goblet cells has been given much attention (12, 22) and according to some authors appears to be sufficiently clear (13, 15, 17).

According to the unitary theory of epithelial cell origin in the small intestine (19) only a single type of totipotent stem cell exists in the first four or five crypt positions. Intercalated among these are various types of daughter cells. The stem cells give rise to 'committed' cells which undergo mitosis a varying number of times according to the line of differentiated cells present in the intestinal epithelium (16).

In the lower crypt there are oligomucous cells with the ability to synthesize DNA and to divide, the only type of mucous cell known to do so (21). These give rise to the mature goblet cells present in the upper crypt. However, it cannot be excluded that the mucous cells may also derive from proliferating crypt-base columnar cells (14). The decision to study goblet cell behaviour was also made in order to examine the cellular differentiation process in the small intestine after irradiation. In the same animals the uptake of 3H-thymidine was also used to study the distribution and the frequency of proliferative cells along the crypt after various types of irradiation (2–4, 9).

Material and Methods

Female Wistar rats 10 to 12 weeks old and weighing 180 to 200 g were used. They were kept under rigidly controlled conditions in an L/D cycle (6.30 a.m. to 6.30 p.m.) and given food and water ad libitum.

A 5 cm×5 cm field covering the entire small intestine and abdominal cavity organs was irradiated by a telecobalt unit (dose rate 0.8–1 Gy/min). In different experiments some regimens were used: 1) 8 Gy in a single dose at 4 times of the day (0.00, 6.00 a.m., noon and 6.00 p.m.). Five animals were killed at 6, 12, 20, 36, 48, 72, 96, 120, 126, 132, 138, 144 and 150 h, and at 11, 20 and 29 days. 2) 6 Gy in multiple daily fractionations (MDF) with either 3 Gy at 12 h intervals beginning in the early afternoon (MDF A) or 2 Gy at 4 h intervals beginning in the late afternoon

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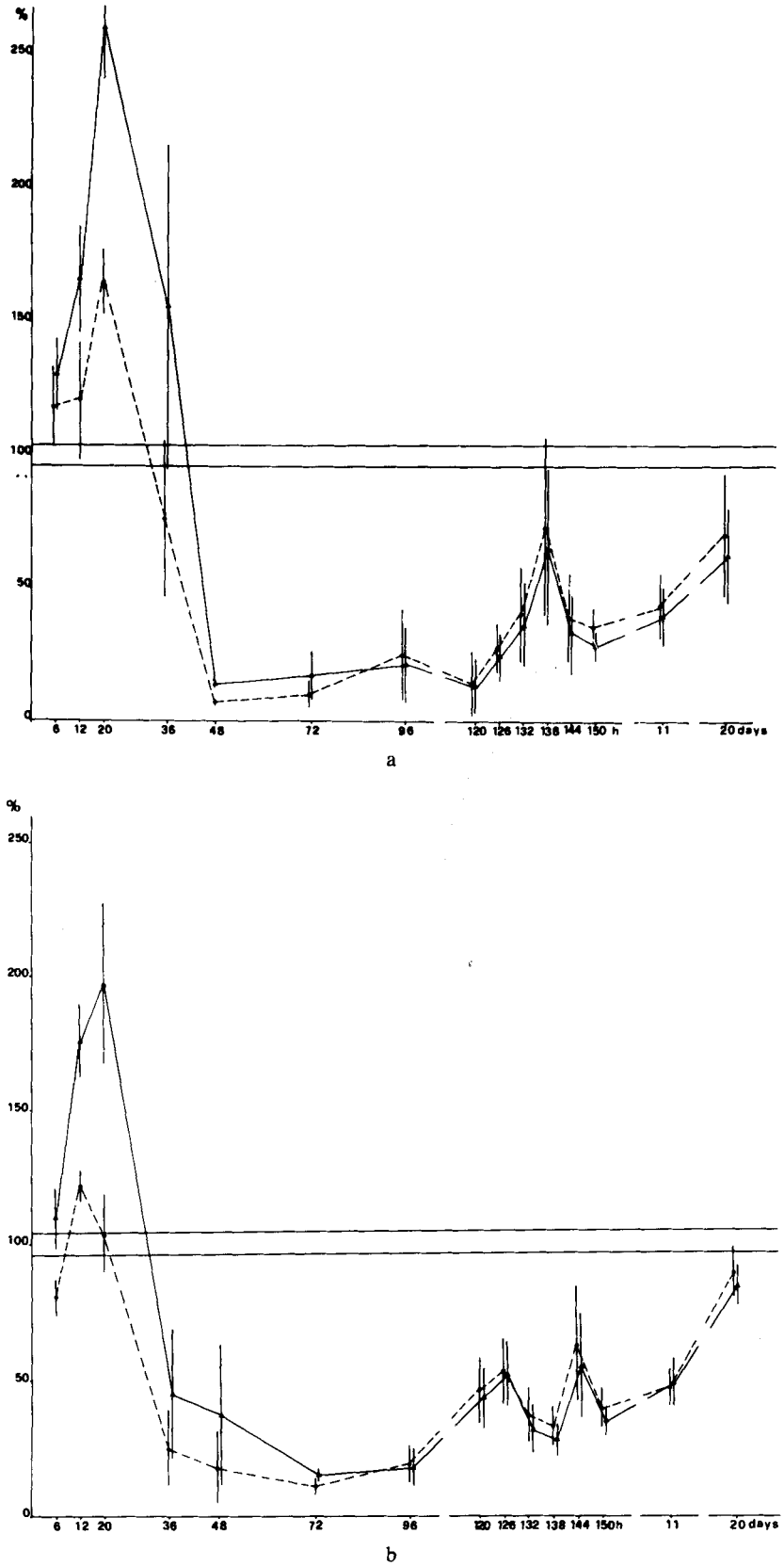
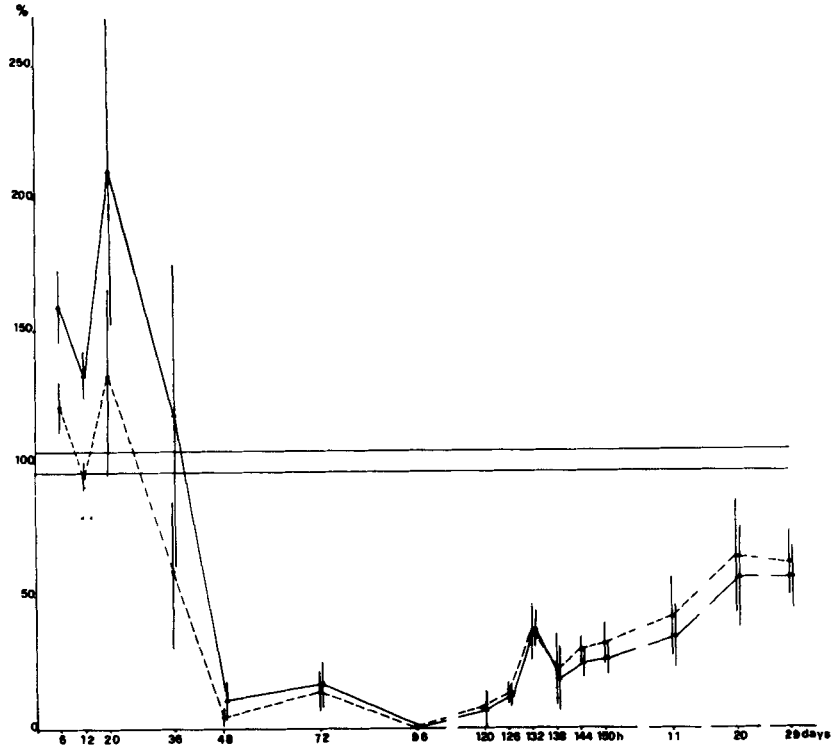


Fig. 1. Goblet cell number (---) and index (——) in the groups irradiated with 8 Gy single dose at a) midnight and b) 6.00 a.m. and at c) noon and d) 6.00 p.m.



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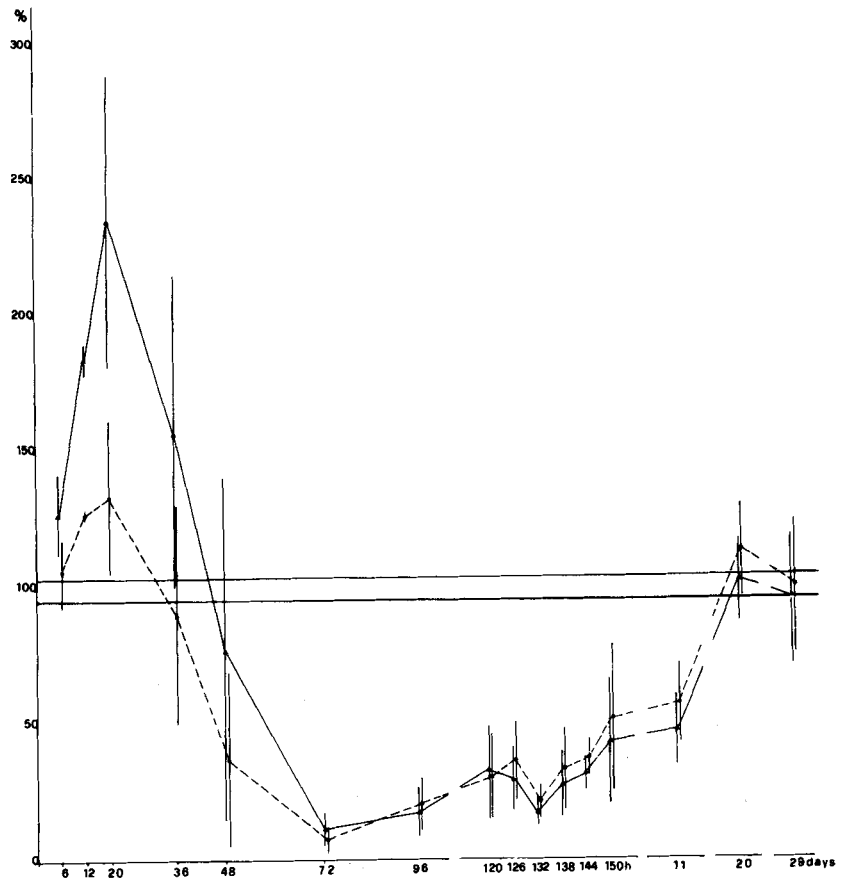

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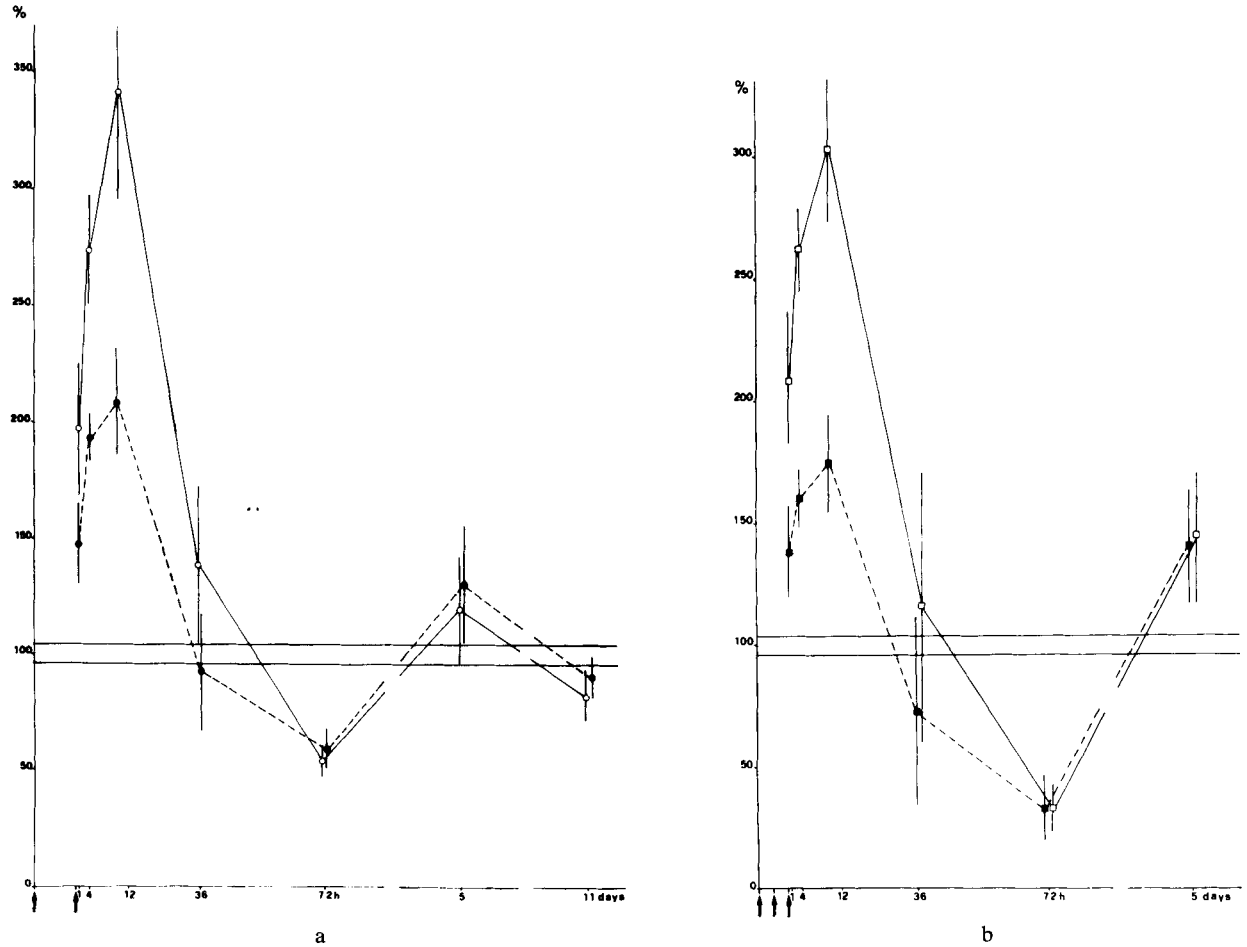

Fig. 2. Goblet cell number (– – –) and index (——) in the groups irradiated with a) 3 Gy×2 (MDF A) and with b) 2 Gy×3 (MDF B).

(MDF B). The animals were killed at 1, 4, 12, 36 and 72 h, and at 5 and 11 days after the last fraction. 3) 12 Gy in MDF with either 3 Gy at 12 h intervals (MDF C), 2 Gy at 4 h intervals allowing a pause of 16 h between the first and the last three fractions (MDF D), or 2 Gy at 8 h intervals beginning at midday (MDF E). The animals were killed at 1, 4, 24, 36 and 72 h, and at 5, 11 and 19 days after the last fraction. In MDF C and D, the animals were also killed after 7 days, and at 29 days in MDF D and E.

Groups of 8 to 10 control animals were killed at the same time as those irradiated with a single dose at midnight, 4.00 a.m., 8.00 a.m., noon, 4.00 p.m. and 8.00 p.m. and as those irradiated with MDF at 2.00 a.m., 6.00 a.m., 8.00 a.m., 2.00 p.m. and 4.00 p.m. In order to avoid the influence of circadian oscillations, irradiation and killing times were restricted to 50 min. Immediately after killing, the small intestine was removed and washed in cold 0.9% NaCl. A segment about 1 cm long was used for the histologic analysis and cut at a distance of 18 to 20 cm from the pylorus. The sections were stained with haematoxylin-eosin, PAS-haematoxylin and PAS-alcian blue-haematoxylin.

The following parameters were analyzed on 40 to 50 left sides of well-aligned crypt–villus formations from each animal: number of crypt epithelial cells along the column from the middle of the base to the crypt–villus junction, number of goblet cells, percentage of goblet cells among total crypt cells (goblet cell index), and localization of goblet cells along the column. As in previous investigations the quantitative morphology as well as the biochemical parameters were also studied (1–11).

The identification of crypts and villi is possible because crypt cells appear cuboid with a central nucleus, whereas the cells of the villus are cylindri-

cal with a basal nucleus. The presence of a knee is also usually visible at the mouth of the crypts. Only goblet cells occupying the entire thickness of the epithelium and having a flattened nucleus at the base were counted. Radiation injury was so severe at some intervals that the individuality of many crypt–villus formations was lost and sufficiently high counts could not be obtained. In such cases, particularly in the 48 to 72 h interval after a single dose and in the 24 to 36 h interval after MDF, only the formations with less damage or in which cell recovery was more efficient were counted.

The mean value ± SEM was obtained for each group of animals. Statistical differences among different groups were calculated with Student's t-test. Goblet cell distribution curves were elaborated using a Fortran language program (2).

Results

Morphologic changes. At the early intervals after a single dose of 8 Gy injury was evident at the base of the crypt while the goblet cells appeared to be normal. After 20 h a reduced number of cells with gross morphologic alterations occupied the base of the crypt and gradually migrated up along the villus. The goblet cells had increased in number but were normal in appearance. At 48 h the crypt–villus formations were blurred and disorganized, showing shortened conglutinated villi. There were fewer goblet cells which, like the other epithelial cells, had flattened out parallel to the villus axis to line a greater surface. The high proliferative activity evident at 72 h produced an epithelium morphologically similar to controls after 5 to 6 days.

In animals irradiated at different times of the day there were no marked differences at the corresponding killing intervals except in the group killed at the end of the dark period which showed greater recovery and faster return to the normal appearance (3, 4). After MDF the morphology changed with time in a similar way to the pattern observed after a single dose. At the end of irradiation, the area of the crypt–villus formation occupied by altered cells was proportional to the length of the treatment and to the dose. One h after a total dose of 6 Gy the alterations affected only the lower half of the crypt which contained abundant goblet cells so full of secretion that they compressed the neighbouring columnar cells. The alterations were generally less severe after 2 Gy fractions. Mitotic activity was partly inhibited. At 12 h swollen goblet cells reduced in number appeared in the villi, whereas at 36 h they were numerous in the whole formation. At the 72 h after 2 Gy per fraction crypts with normal cells appeared in the midst of damaged crypts. After 3 Gy per fraction the epithelium was similar to that of the controls and goblet cells were very rare. Later on the morphology of the intestinal epithelium returned to normal.

At the first intervals after 12 Gy MDF the alterations affected the crypt and most of the villus. The crypt–villus formations were notably shortened showing principal cells with globose thin nuclei and abundant swollen goblet cells clustered in the lower part of the crypt, particularly after MDF D.

After 24 to 36 h the entire formation was still injured and the goblet cells had practically disappeared. Proliferative activity started again and at 72 h the morphologic injury was partly restored, if not to the same degree in all animals of the same group or in all histologic sections of the same animal. The few goblet cells present were concentrated in the crypt–villus junction and in the villi. After longer intervals following all MDF regimens the epithelium was morphologically similar to that of the controls.

Quantitative changes. The number of goblet cells in the crypt of different groups of control animals killed at various times of the day did not show a trend which could be due to a circadian dependence. These values ranged between 1.00 ± 0.09 and 1.17 ± 0.07; in the same control groups the goblet cell index ranged between 2.6 ± 0.23 and 3.09 ± 0.18 so the values have been lumped together and reported as a band in the figures. Comparison among the different groups was facilitated by expressing the values as percentages of the respective controls.

Single dose. An initial increase in the number of goblet cells regardless of the hour of irradiation was followed by a decrease down to near zero (Fig. 1). The group irradiated at midnight showed the greatest increase ($p<0.001$ at 20 h) but also the other groups showed a statistically significant increase within 20 h. At 20 and 36 h the findings varied considerably. The group irradiated at the end of the dark period showed less evident increase, more abrupt reduction in the number of goblet cells, and less variability among the animals.

In all groups the lowest values were reached between 48 and 96 h. The group irradiated at the end of the dark period showed higher levels than the others 120 h after treatment; this group and the group

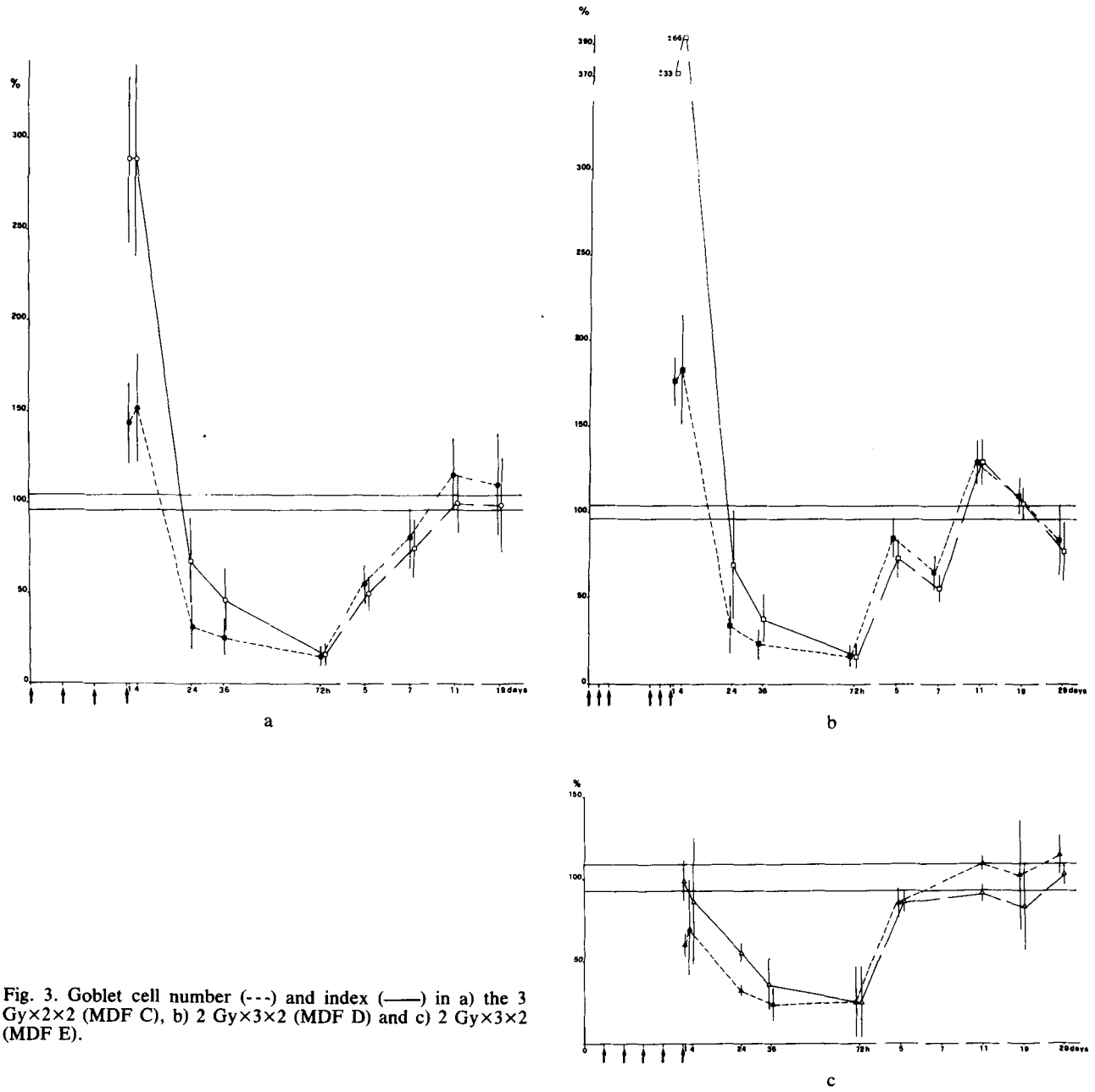

Fig. 3. Goblet cell number (---) and index (——) in a) the 3 Gy×2×2 (MDF C), b) 2 Gy×3×2 (MDF D) and c) 2 Gy×3×2 (MDF E).

irradiated at 6.00 p.m. reached normal levels already 20 days after treatment.

The goblet cell index and number followed the same trend but the index showed a much higher relative increase up to 20 h. Later on the two parameters differed only in the slightly higher relative number of goblet cells after 96 h.

MDF 6 Gy. The effects of irradiation were much more marked after this regimen than after a single dose. After MDF A (3 Gy×2) a significant increase in the number of goblet cells was already evident at the first killing interval, reaching a peak of more than 200 per cent after 12 h and decreasing to slightly more than 50 per cent of the controls at 72 h (Fig. 2a). At the next intervals the values of the controls were reached. The difference between goblet cell index and number increased progressively, peaking 12 h after the end of treatment.

The changes observed after MDF B (2 Gy×3) differed from those after the other MDF regimens only by a slighter increase and a more marked reduction in the number of goblet cells, even if values significantly greater ($p<0.02$) than in the controls were reached at 120 h (Fig. 2b).

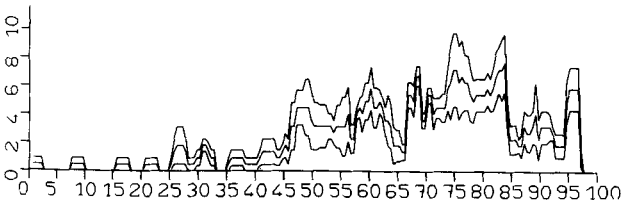

Fig. 4. Crypt goblet cell distribution in control animals killed at 2.00 p.m.

MDF 12 Gy. After MDF C (3 Gy×2×2) the number of goblet cells was significantly greater ($p<0.02$) than in the controls at the early intervals (Fig. 3a). At 24 h the number of goblet cells had decreased to about 30 per cent of the controls and continued to decrease up to 72 h. Later on it gradually increased, reaching control levels at 7 days. The goblet cell index followed the same trend as the number, showing notably higher levels up to 72 h when the two values coincided, and lower values during the recovery phase.

After MDF D (2 Gy×3×2) the initial increase in goblet cell number was much greater and reached values close to 180 per cent (Fig. 3b). A marked reduction at 24 h and a minimum at 72 h were observed. Then the number of goblet cells began to increase but without reaching control levels until 11 days, when it increased significantly ($p<0.02$). Later on the values were similar to those of the controls. Immediately after irradiation the goblet cell index increased to about 400 per cent of the control values. Later on the two curves were practically identical.

After MDF E (2 Gy×3×2 in 40 h) the number of goblet cells at all the first intervals was always less than the control values (Fig. 3c). A minimum was reached at 72 h and the values returned to normal at 5 days. The goblet cell index and number followed a similar trend, with only slightly higher levels for the index at the initial intervals. Index and number coincided at 72 h, after which the index remained lower than the number.

Distribution of goblet cells in the crypt. In control animals goblet cells were practically absent in the lower 25 per cent of the crypt, and then gradually increased in frequency till reaching a maximum of 8 to 10 per cent in the upper half. A somewhat lower cell frequency was observed near the junction with the villus (Fig. 4). The distribution curves in various groups of control animals killed at different times of the day did not differ significantly, perhaps due to the relatively low frequency and fairly high variability. For this reason the investigation at present has been limited to only a few animals.

Discussion

The importance of goblet cells in the production of mucus and thus in the protection of the intestinal epithelium from bacteria and alimentary material is well known (20). These cells may also accumulate Fe as ferritin and secrete it according to the needs of the organism (23).

Few reports concern the changes in the goblet cells after irradiation (18, 24–26). The present investigation on their behaviour after different modalities of irradiation gave the following information: a) No significant differences existed in the number of crypt goblet cells in control animals killed at various times of the day, in agreement with what has previously been reported concerning the number of crypt epithelial cells (2). b) The crypt goblet cells were practically absent in the lower 25 per cent and gradually increased in frequency up to the base of the villus. The distribution was the opposite of that of the S phase cells (2). c) After irradiation with either a single dose at different times of the day or with MDF, the goblet cell number and index followed a trend composed of three phases similar to observations made on small intestine brush border enzymes (1, 5, 6, 10, 11). These phases, due in part to the contemporary condition of the epithelium, consisted of an initial increase, a secondary reduction, and then a return to the control levels.

In all irradiated groups, and most marked after MDF, the number of goblet cells initially increased significantly. The contemporary increase in the goblet cell index was even more marked. This parameter is conditioned by the number of epithelial cells and, as irradiation destroys the proliferative cells and blocks mitosis, there is thus a relative increase in the more radiation-resistant differentiated cells. Similar observations have been made in man (25, 26).

As there is a lower percentage of goblet cells in the villus than in the crypt, the increase in absolute number may be due to early differentiation by which 'non-committed' or partially differentiated cells (which under normal conditions can still undergo mitosis) are transformed into differentiated cells. This explanation is also supported by the distribution of crypt cells labelled with 3H-thymidine (3, 9) and by the contemporary increase in the activity of

brush border enzymes synthesized during the differentiation process (1, 5, 6, 11).

The reduction phase corresponded to the period in which the morphologic changes of the epithelium were at a maximum. The number of goblet cells decreased to about 20 per cent of controls but was slightly higher after MDF 6 Gy. These data agree with observations concerning the activity of brush border enzymes and the distribution of the S phase cells in the crypt (3, 4, 9). All these data fit in with the assumption that during the phase of maximum epithelial injury only a limited number of cells undergo differentiation. Though epithelial cell morphology returned to normal after 5 days after all the doses, the differentiation process was not normalized. There was an evident difference in the return to normal values of goblet cells after irradiation with a single dose and after MDF. Thus, control levels were reached 5 days after 6 Gy MDF, 7 to 11 days after 12 Gy MDF, and 20 days after a single dose in two of the four groups of animals irradiated at different times of the day, demonstrating the different damage caused by the regimens.

Also these data are in agreement with previous results concerning brush border enzymes (1, 10) and the distribution of S phase cells (3, 9).

During the recovery phase the goblet cell values in the group irradiated at the end of the dark period with a single dose of 8 Gy were closer to the control values than in other groups irradiated with the same dose. Similar observations were previously made concerning the distribution of labelled cells and brush border enzymes. An MDF (6 or 12 Gy) regimen is obviously more efficient than a single dose one in inducing an increase in goblet cells.

A comparison between different fractionations showed that the increase was evident already at the early intervals after a total dose of 6 Gy and at 1 and 4 h after 12 Gy. The lack of an increase after MDF E could be due both to the long interval between the beginning and the end of irradiation and to the lesser effect of irradiation. This type of MDF was also followed by a more rapid return to control values. During the phase of maximum damage the decrease of goblet cells probably caused a reduced protective action of the mucus against bacteria and microtraumas produced by the transit of alimentary material in the lumen. This condition is not a negligible factor in the acute intestinal syndrome. The function of the mucous cells seems to be re-established much earlier after MDF than after a single dose.

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