Scanning Microscopy

Volume 3 | Number 4 Article 17

11-20-1989

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Scanning Microscopy, Vol. 3, No. 4, 1989 (Pages 1169-1176) 0891-7035/89$3.00+.00
Scanning Microscopy International, Chicago (AMF O'Hare), IL 60666 USA

THE EFFECT OF 10 AND 20 Gy SINGLE DOSE IRRADIATION ON THE ESOPHAGEAL MUCOSA OF THE RABBIT. AN ELECTRON MICROSCOPIC STUDY

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(Received for publication August 20, 1989, and in revised form November 20, 1989)

Abstract

The mucosa of rabbit esophagus was irradiated with single doses of 10 and 20 Gy respectively. Specimens were taken for scanning electron microscopy (SEM), and light microscopy investigations. Examinations were made 1-14 days after 10 Gy and 1-17 days after 20 Gy. Irradiation resulted in edema during the first days. The thickness of the epithelium was at its lowest value around day 5 after the administration of 10 Gy, and on days eight to ten after the administration of 20 Gy. Damage of the epithelium surface, as scored on SEM micrographs, was most pronounced on days eight to eleven after irradiation, showing a clear dose dependency. The second week after irradiation repair was seen concluding in an overshoot of the mucosal height. The amount of bacteria on the surface increased during the period of edema, and decreased when the damage was most pronounced.

Key Words: Esophagus, mucosal epithelium, single dose irradiation, light microscopy, scanning electron microscopy.

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Introduction

Esophagitis is one of the most irritating side effects observed after irradiation of malignancies within the thoracic cavity. In order to optimize radiotherapy treatment, different models have been tried based on various radiobiological principles, e.g., accelerated hyperfractionation (Herskovic et al., 1988), sensitization with chemotherapy (Wendt et al., 1987), or concomitant boost (Richmond et al., 1987). However, the different models have in common an aggravation of the reaction of the normal tissue included in the radiation field, which thus constitutes a limiting factor in the treatment.

Earlier work with fractionated irradiation on rabbit esophageal mucosa, with doses equivalent to those used clinically, has been published from this laboratory (Albertsson et al., 1987). Ultrastructural investigations by light microscopy (LM), scanning electron microscopy (SEM), and transmission electron microscopy (TEM) were made on the first ten days after completion of irradiation. Dose-dependent edema, loosening microridges, and a slight increase in cell loss were seen.

When investigating radiobiological phenomena, it is necessary to exceed clinical dose limits and use doses that are not directly applicable in the working-day reality. In animal experiments, sufficiently large single doses have been applied, so that the course of events within a tissue could be observed (Hornsey and Field, 1979). Michalowski and Hornsey (1986) examined the esophagus of rats and found esophagitis after thoracic irradiation using a single dose of 27 Gy. As an ineluctable complement to our earlier work, the results of 10 and 20 Gy single dose irradiation of the upper part of the esophagus are presented in this paper. The interest was focused on the acute damage effects. Furthermore, LM, SEM, and TEM examinations were performed for 14 and 17 consecutive days (for 10 and 20 Gy single doses, respectively) in order to study the pattern of desquamation and restitution.

Materials and Methods

Forty-five full grown rabbits were selected for this study. Fourteen animals served as controls. Fourteen rabbits received 10 Gy single dose irradiation, and seventeen received 20 Gy single dose irradiation to the esophagus, since it was assumed that the repair process would be delayed after 20 Gy doses.

Experiments

Each rabbit was anesthetized for about 45 minutes during the administration of irradiation by an intraperitoneal injection of pentobarbital (60 mg per kg body weight). The rabbits were laid on their backs and 2 cm of esophagus, just beneath the larynx, was irradiated. After completion of irradiation, one animal was removed from the group each day. The animals were sacrificed by a blow to the neck in order to avoid pharmacological side effects. The trachea and esophagus were dissected out en-bloc (8-9 cm). Sample for SEM and LM were taken from the upper part of the esophagus (irradiated area, A) and the lower part of the esophagus (control area, B), 4 cm from the lowest borderline of irradiation. Control investigations were also performed in the same way on untreated animals.

Irradiation

The groups of rabbits were treated with single dose irradiation of 10 and 20 Gy respectively. As mentioned above, the upper 2 cm of the esophagus was irradiated. The field of width was 3 cm. When the animals are laid on their back before irradiation with a dorsal flexion of the neck, the difficulty is to have the esophagus in a sagittal position just above the columna vertebrae. Excessive backward sloping of the head causes sliding of both the trachea and esophagus to one side. Therefore, the head had to be fixed in an ideal position with the esophagus in the middle. This was controlled by X-ray examination. Irradiation was delivered by Siemens X-ray machine operating at 160 kV and filtered by 4 mm Al, at a focus - skin distance of 50 cm. The dose rate was 0.013 Gy per second. The dose in the whole esophagus was controlled by thermoluminescent dosimeters (rods, 1 x 5 mm, in a plastic catheter with an outer diameter of 2 mm) (Albertsson et al., 1987).

Preparation for SEM

The specimens for SEM examination were not rinsed, but were placed directly in 2.5% glutaraldehyde (in 0.15 M cacodylate buffer, pH = 7.3) for fixation for 12 hours. They were then transferred into the same buffer and were later osmium-fixed in 1% osmium tetroxide in 0.15 M cacodylate buffer for two hours. After dehydration with graded series of ethanol, the preparations were transferred to Freon TF 618.

The specimens were later critical point dried in a Balzer-000 critical point drier. They were then sputter-coated with gold and palladium in a Polaron coating unit (E 5000) and examined in a Philips SEM 515 electron microscope operating at 20 kV.

Measurement of epithelial thickness

The thickness measurements were performed by a Leitz 12.5x measurement ocular (1 mm divided into 100 parts). From each animal 8-10 sections were investigated. Due to undulations of the epithelium 20 measurements at various sites from each animal were made.

Results

Morphology - epithelium thickness measurements

LM observations and measurements were made each day and the height of the esophageal mucosa was calculated both in the irradiated area and control area for each animal. In the normal untreated controls the height of the esophageal epithelium, as measured in 14 rabbits, was 126 ± 20 micrometers.

After 10 Gy single dose irradiation, the first day after the treatment, the height of the irradiated mucosa was practically the same as in the control animal. Measurements each day after irradiation showed, in their entirety, a tendency to increase (curve A, Fig. 1). Analyzed day by day, the curve showed an increase with a maximum on day 3 (155 micrometers) and a transient decrease to day 5 (116 micrometers). Thereafter, the height of the epithelium rose again and reached a maximum on day 12 (212 micrometers). The values during days 7-14 are higher than the control values but a tendency to normalization seemed to occur after day 12.

After 20 Gy irradiation, an initial increase in the mucosal height was seen (curve A, Fig. 2) which was sustained for the first seven days. A minor deviation was seen as a dip on day 6, but no importance was attached to this. However, a similar dip was observed on the 10 Gy curve. During days 8-10 the epithelium was so desquamated that no calculations could be made (Fig. 2). At the end of the observation period, days 12-17, the height of epithelium was steadily increasing above the normal level.

The height of the epithelium with the lower part of the esophagus (outside of the irradiation field) is presented in curves B of Figs. 1 and 2. A comparison of A versus B values for each day shows, a tendency, although not statistically significant, to co-variation (Jolles, 1950). The trend, however, is noticeable and perhaps implies a possible influence of the irradiation outside the irradiation field.

Morphology - scoring of epithelial surface from SEM

Scoring of the surfaces from SEM-microridges for evaluation of the irradiation effects was performed according to the criteria described below. The dynamic course of development of the post-irradiation (20 Gy) morphological changes could not be scored in the same way on the SEM micrographs when the time just after treatment was compared to the status 10-12 days later. Therefore, it seemed necessary to create two separate scoring criteria: one used for days 1-11 after irradiation, classified as the 'first phase' or 'damage phase', and one for the second phase during days 12-17, the 'repair phase'.

The first phase (day 1 to day 11):

Score 1 = normal SEM surface. A slight loss of cell flakes; a small number of loosened microridges and interdigitations were observed (Figs. 3a-c).

Score 2 = Cell loss increased, edema of microridges, and increased number of loosened microridges (Figs. 4a, b).

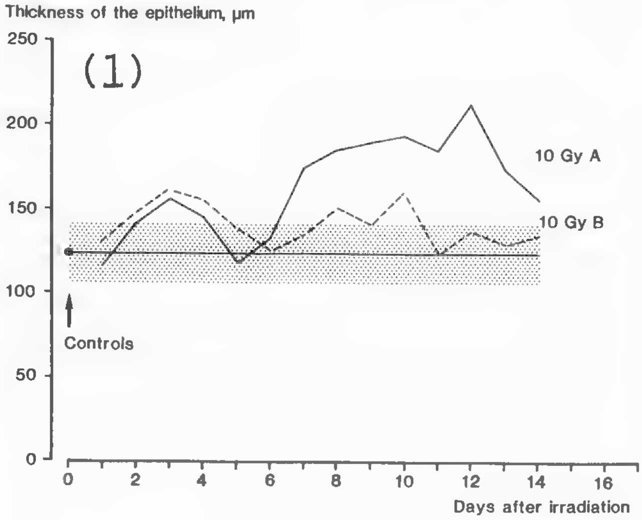
Score 3 = Fissures between cell groups. Cracked cell flakes with folded edges. Edema of the microridges. Damaged microridges even beneath the surface layer (Figs. 5a, b).

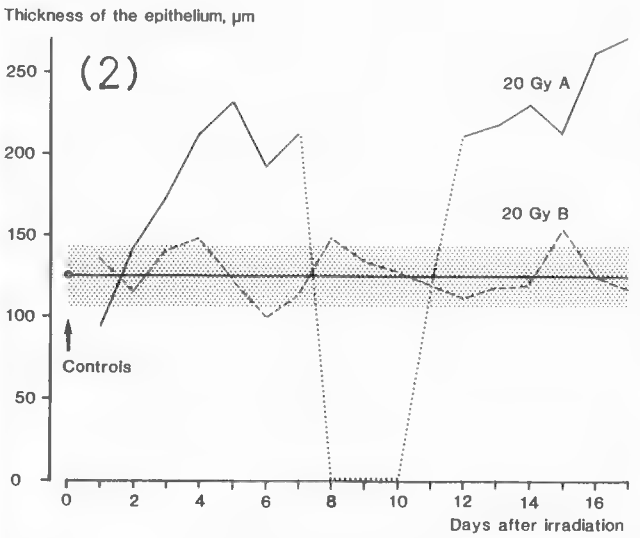
Score 4 = Desquamation, (Figs. 6a, b), but in some places a small number of severely damaged cells dispersed on the surface (Figs. 7a, b).

Second phase (days 12 to 17):

Score 3 = The surface is partially covered with newly formed epithelial cells. No clear edema. Some damaged cells are observed on the surface. Normal microridges are absent and no interdigitations are seen (Figs. 8a, b).

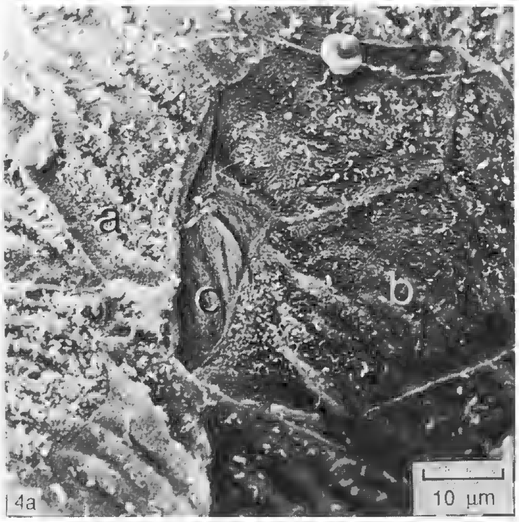
Score 2 = The surface is covered with cells. Remaining single damaged cells are seen on the surface. The borderline between cell flakes is clearly

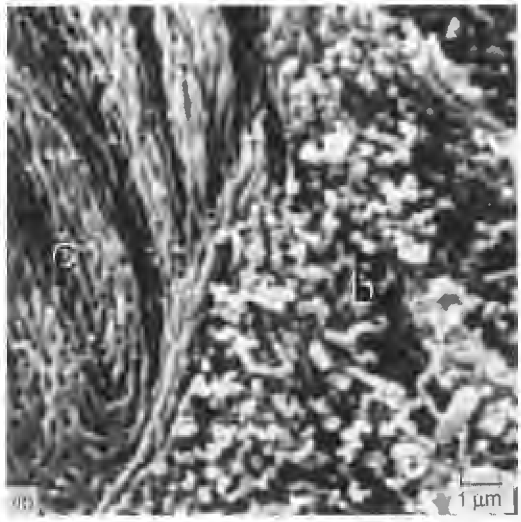




Figs. 1 and 2 (above). Height of the esophageal epithelium as calculated from LM micrographs after 10 Gy **(Fig. 1)** and 20 Gy **(Fig, 2)** single dose irradiation. Curves A and B are for the irradiated areas and for the control areas outside the irradiation field respectively. Line represents the mean value of untreated animals (both for areas A and B), and shadowed area represents the standard deviation.

Fig. 3 (at right). SEM micrographs of:
(a). Normal rabbit esophagus. Cell loss (arrows) with flakes. Score 1. A moderate amount of bacteria, mainly rods (E.coli) is seen.
(b) and (c). Normal mucosa.
(b). Bacteria. Esophageal gland opening (arrow). **(c)**. Microridges. Small knobs (arrows).


a
c
b
10 µm
4a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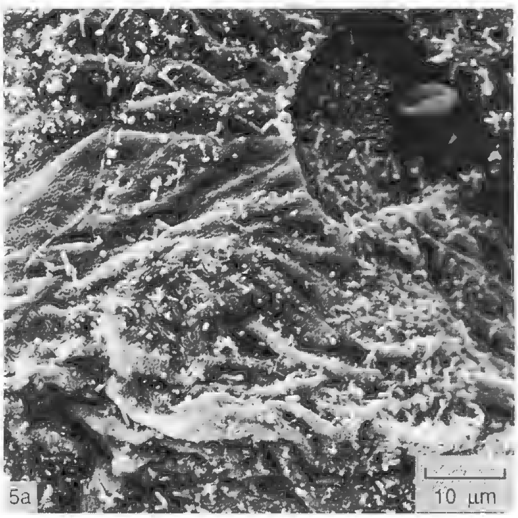
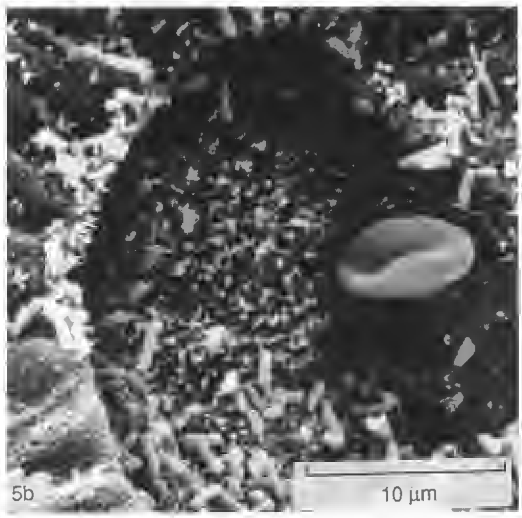
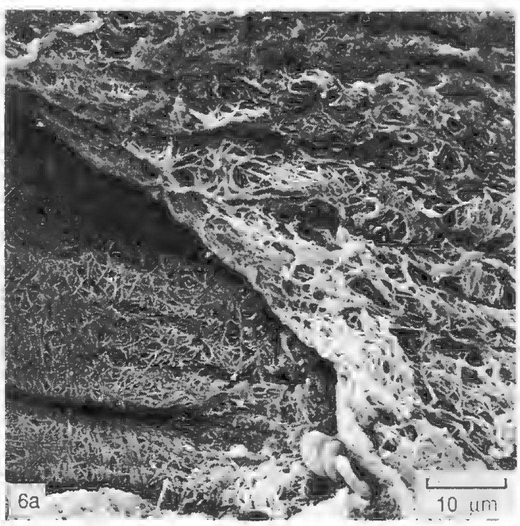

10 µm
5a


10 µm
5b


10 µm
6a


1 µm
6b

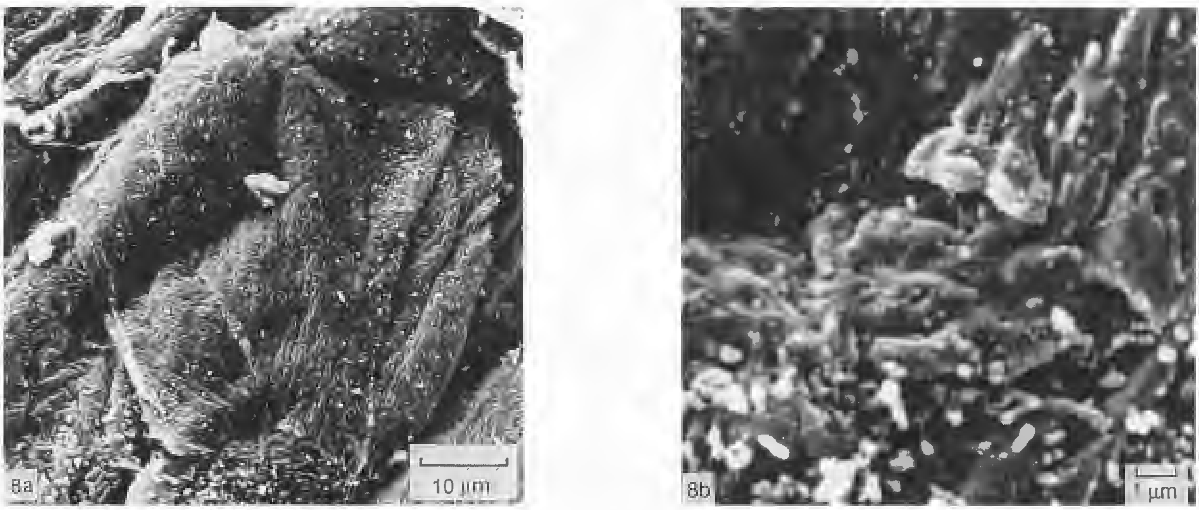


Fig. 4a. SEM micrograph illustrating a rich amount of loosened microridges on a surface cell (a). Underlying layers are less damaged (b and c). Score 2. Day 2 after irradiation. **Fig. 4b.** Enlargement of Fig. 4a.

Fig. 5a. SEM micrograph. Score 3. Day 6 after irradiation (see text). **Fig. 5b.** Enlargement of Fig. 5a.

Fig. 6a. SEM micrograph illustrating total desquamation. Subepithelial tissue. Score 4. Day 8 after irradiation. **Fig. 6b.** Enlargement of Fig. 6a. Fibrillar supporting network.

Fig. 7a. SEM micrograph. Score 4. Repair. Newly formed epithelial cells (a). Desquamating cells (b). **Fig. 7b.** Enlargement of Fig. 7a.

Fig. 8a. SEM micrograph. Score 3. Repair. Newly formed epithelial cells. **Fig. 8b.** Enlargement of Fig. 8a. Absence of microridges and interdigitations.

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marked. In some places there are fissures between cells (Figs. 9a-c).

Score 1 = Normal SEM surface (Figs. 3a-c).

Cell loss and loosening microridges are phenomena normally occurring in the esophageal epithelium, and seem to be associated with cell death and desquamation. To establish one scoring system for the whole period of time did not seem satisfactory. Instead, more attention was paid to the time dependency of cell loss, damaged microridges, edema and denuded cells.

A scoring was made each day after irradiation and the result is presented in Fig. 10. A damage effect could be observed already on day one after irradiation, with a score mean value of 1.3 for 10 Gy and 2.0 for 20 Gy. Thereafter, the score mean value seemed fairly constant during the first four days. However, the maximum epithelial damage occurred during days 6-11, with a mean score value of 2.2 day 8 after 10 Gy and 4.0 day 8 after 20 Gy. During the last days of observation a repair seemed to take place leading to normalization. Evidently, the two phases overlap. Nevertheless, the method of describing the changes used here appears adequate.

Bacteria

The esophagus in the non-fasting rabbit contains a certain quantity of bacteria consisting of Haemophilus, Actinobacter, and mostly E. coli. This has been confirmed by repeated cultures from the esophageal mucosa. By SEM the bacteria are clearly visible, and show no firm attachment to the mucosa. This can also be verified on TEM. So far, no penetration through the cell membrane has been observed by TEM. The attachment to the cell surface seems to indicate that the bacteria in the esophagus, in conformity with the conditions in the gastrointestinal tract, live in co-existence with the individual, and are typical of the normal physiological state of the surface cells. Scoring of the relative occurrence of bacteria from 0-3 (as earlier presented, Albertsson et al., 1987) was made and is presented in Fig. 11. This figure shows how the bacteria content, after a single dose of 20 Gy, reaches its highest level during days 2 and 6-7 after irradiation. On days 3-5 the number was reduced. On days 8-11, when the surface was desquamated, no bacteria was seen. After day 12 the amount of bacteria increased. Occasionally bacteria were clustered together in very remarkable formations (Fig. 12). There was a larger number of bacteria after 10 Gy irradiation than after 20 Gy.

Discussion

In these series with single dose irradiation of 10 and 20 Gy, damage to the esophageal mucosa was developed, which reached its maximum during days 8-11. After 20 Gy, the mucosa was denuded during this period. The surface damage was scored on SEM micrographs and was clearly dose dependent with a mean score value twice as high after 20 Gy than after 10 Gy. However, within both series the maximal injury was found on day 8. This time pattern fits in well with other radiobiological end points for the esophagus reported in the literature, e.g., Phillips and Ross (1974) during a study of early deaths after thoracic irradiation found a maximal occurrence between one to three weeks after irradiation. Also Michalowski and Hornsey (1986), after thoracic irradiation of mice with high single doses (27 Gy), found ulcerative esophagitis which rose from nil to 100% at days 7 and 8 after irradiation, remained at this level for two days, and subsequently decreased to 10% by day 14. The esophageal mucosa is a rapidly renewing system and the damage is mainly the result of effects on the normal proliferation pattern. However, even after a single dose as high as 20 Gy, the esophageal mucosa recovers.

Calculations on the height of the mucosal epithelium on LM showed a slight increase initially after irradiation, a dip in the middle of the time period, followed by an increased epithelial height seen again during the last observation days (Figs. 1, 2). The changes in the height of the mucosal epithelium are probably multifactorial. The initial increase in height may depend on an edema, not only intracellularly, but also interstitially. The reaction in the basal layer may play a role. Theoretically, a large number of these cells may be affected by the radiation which also can be seen on TEM when calculating the number of basal cells. Even if the surface looks totally denuded after 20 Gy, some cells must have remained and these begin to grow. An overshoot is seen in the second week and later. In the 10 Gy group the overshoot lasted until day 12. Thereafter a normalization seemed to occur. In the 20 Gy group there was an overshoot from day 12 to the end of the examination time (17 days).

A closer examination of variation in the epithelial height within the irradiated area, as compared with the unirradiated area, showed a tendency to co-variation reminiscent of the "old diffusion flare theory" (Jolles 1950, Ellinger 1951). If the co-variation exists (which has to be proven on a larger sample), the deduction of such results point rather towards a reflex-mechanism perhaps via neuropeptides. This will be examined in future studies.

Observations of the normal untreated esophageal mucosa show bacteria on the surface in a moderate amount. Ofek et al. (1977) showed that the mucosa was an excellent substrate for E.coli in the intestine and Friberg (1980) presented results which showed that the attachment of fusiform bacteria was, in a similar way, dependent on galactose, fructose, glucose and mannose. From histochemical studies of mucosubstances and lipids in the normal human esophageal epithelium (Hopewood et al., 1977), we know that a large amount of glycogen is present in the cytoplasm of the intermediate and superficial cell layer. The intracellular space of the most superficial cells contains neutral and sialic-rich acid mucopolysaccharides. In the small intestine an initial increase in the amount of bacteria with a heavy attack and penetration of the mucosal cells after a single dose of 25 Gy was described by Friberg (1980). The bacteria, however, disappeared after 30 minutes perhaps due to an exhaustion of the cells. The esophageal mucosa, however, has many epithelial cell layers and the explanation of the actual happening may be the following: the initial increase in bacteria observed during the first days may depend on a leakage of a substrate, which allows them to multiply. When the mucosal cells vanish, they may be accompanied by the bacteria and the number of bacteria observed on the surface is dramatically reduced. The dip in the amount of bacteria during days 3-5 of irradiation (Fig. 11) may be the result of a desquamation, due to

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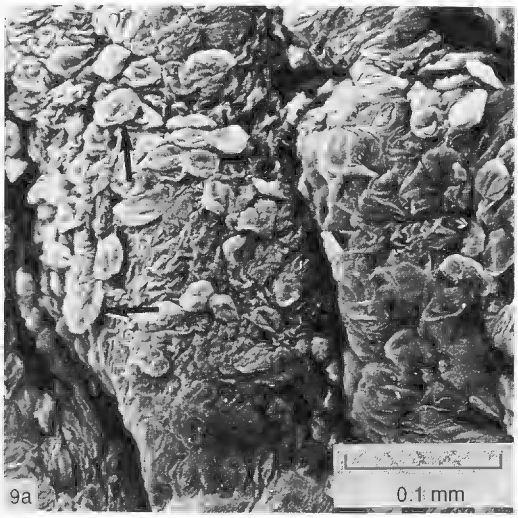
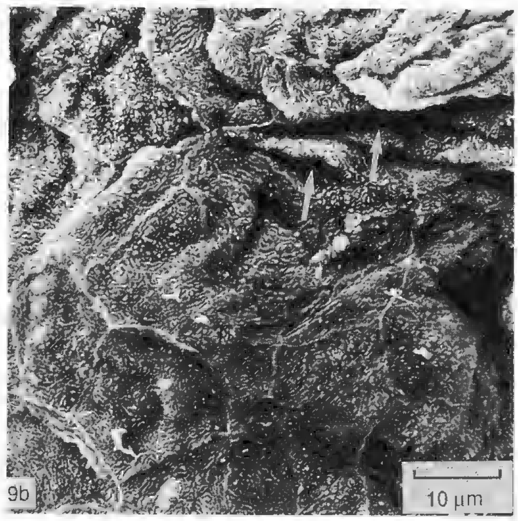
Fig. 9. SEM micrographs. Score 2. Repair. **Fig. 9a.** Flakes pushed up by the new epithelium (arrows). **Fig. 9b.** Enlargement of Fig. 9a. New epithelial cells with fissures. Borderlines clearly marked between cells (arrow). **Fig. 9c.** Enlargement of Fig. 9b.

Fig. 10. The average reaction of the esophageal epithelium each day after a single dose radiation. The smoothed curves were calculated by computer using the least square method. Solid circles represent 20 Gy single dose during the damaging process. Solid squares represent 20 Gy single dose during the process of repair. Solid triangles represent 10 Gy single dose.

Fig. 11. Scoring of the relative occurrence of bacteria after 20 Gy.

Fig. 12. SEM micrograph illustrating bacteria on the surface.


9a
0.1 mm


9b
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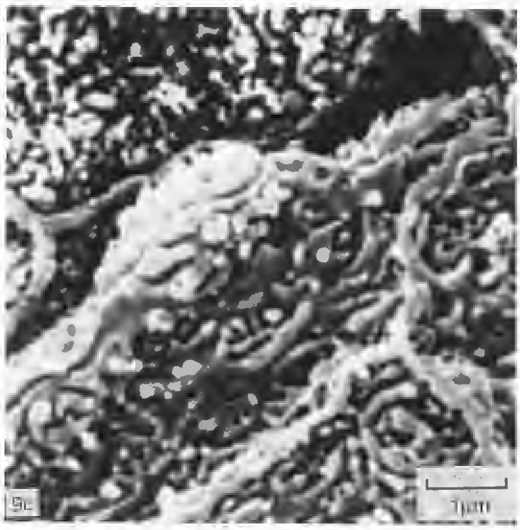
9c
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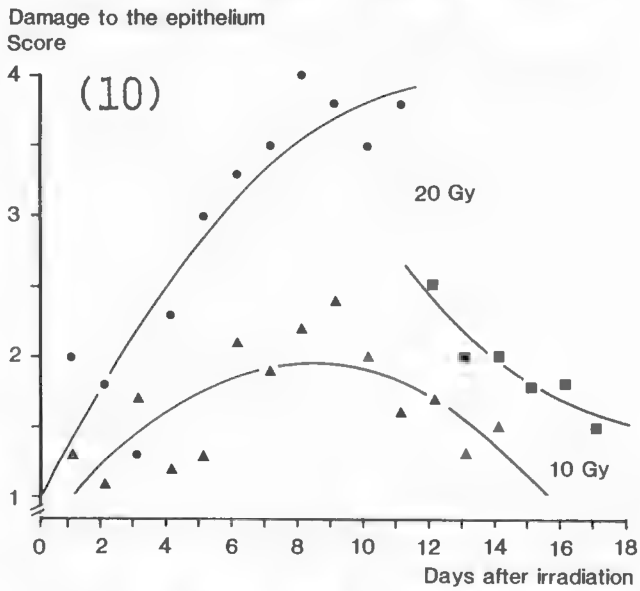
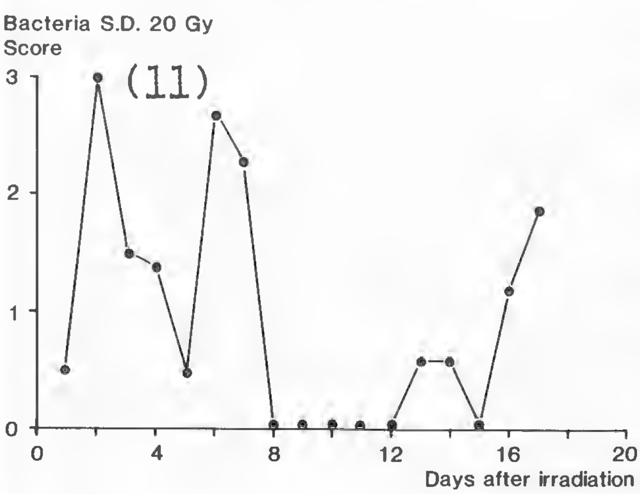

Damage to the epithelium
Score
(10)
20 Gy
10 Gy
Days after irradiation


Bacteria S.D. 20 Gy
Score
(11)
Days after irradiation


12
1 µm

edema, of the superficial epithelial layers which are swallowed, leaving the new surface as nourishment for bacteria until all epithelial cells have disappeared (Fig. 10). The new accumulation of microbes after day 12 after irradiation indicates fresh access to nutrition (from the newly formed epithelial cells, cf Fig. 9a-c).

Conclusion

Ten and twenty Gy single dose irradiation of the upper part of the rabbit esophageal mucosa resulted during the first days in an edema, and thereafter in damage, most pronounced day eight to eleven after irradiation as scored on SEM micrographs, showing a clear dose dependency. The second week after irradiation a repair was seen concluding in an overshoot of the mucosal height. The amount of bacteria on the surface increased during the period of edema, and reduced when the damage was most pronounced.

Acknowledgments

This work was supported by the grants from John and Augusta Persson's Foundation for Scientific Medical Research, from the Medical Faculty, Lund University, from Lund's Hospital Research Foundations and from B. Kamprad's Foundation.

This work has been performed at the Electron Microscopy Unit, Faculty of Medicine, Lund University.

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Editor's Note: All of the reviewer's concerns were appropriately addressed by text changes, hence there is no Discussion with Reviewers.