Effects of Fractionated Irradiation on the Esophageal Mucosa: A Scanning and Transmission Electron Microscopic Study

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EFFECTS OF FRACTIONATED IRRADIATION ON THE ESOPHAGEAL MUCOSA: A SCANNING AND TRANSMISSION ELECTRON MICROSCOPIC STUDY

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Abstract

The mucosa of rabbit esophagus was irradiated with daily fractions of 2 Gy to an accumulated dose of 20 Gy. Specimens were taken for scanning electron microscopy, transmission electron microscopy and light microscopy investigations. Examination was made 1-10 days after each fractionation schedule. Light microscopy showed dose-dependent edema of the irradiated mucosa which also could be seen and scored from SEM pictures. SEM investigations showed that this was accompanied by loosening of microridges and a slightly increased cell loss. By SEM, a varying amount of bacteria could be seen which did not make intimate contact with the surface cells.

During the first five days there was a steady decrease of the number of bacteria in relation to the absorbed dose. In the later period of examination, the amount of bacteria increased up to a given dose of 10 Gy. Thereafter, the number faded off to about zero when 20 Gy had been administered.

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Introduction

Treatment of malignancies within the thoracic cavity in most cases includes the esophagus in the irradiation field. One of the most troublesome side-effects in these cases is esophagitis (Earlam and Cunha-Melo 1980).

According to clinical reports, esophagitis is observed during fractionated treatment. Esophagitis is defined as pain, swallowing difficulties and weight loss and is believed to run parallel to an acute inflammatory reaction in the esophageal mucosa (Michalowski et al. 1983). Late effects, after treatment of esophageal tumors, include stricture and fibrosis (Michalowski and Hornsey 1986). In animal experiments, after thoracic irradiation with high single doses, death due to esophagitis is observed within 30 days. Microscopically, denudation of the esophageal epithelium within this dose range has been shown (Hornsey and Field 1979), as have perforation and mediastinitis (Phillips and Ross 1974). These serious radiation effects are not seen after fractionated doses of 20 Gy and below. Very little is known either about the early effects of fractionated irradiation course on the esophageal mucosa or about the mechanisms of repair and repopulation. Therefore, the esophageal mucosa of the rabbit has been investigated after a fractionated irradiation course (2 - 20 Gy).

Scanning electron microscopy (SEM), transmission electron microscopy (TEM), and light microscopy (LM) were used in this investigation.

Materials and Methods

Sixty full-grown rabbits weighing 1.8 - 2.3 kg were selected for this study. Ten animals acted as controls; fifty rabbits received fractionated irradiation according to schedule (Fig. 1).

Irradiation

Each rabbit was anaesthetized for about 15 minutes during the administration of irradiation by intraperitoneal injection of pentobarbital (40 mg per kg body weight).

Irradiation was delivered by a Siemens X-ray machine operating at 160 kV X-ray, filtered by 4 mm Al, at a focus-skin distance of 50 cm, giving an absorbed dose of 2 Gy to 2 cm of the esophagus just beneath the larynx. The absorbed dose in the esophagus was controlled by thermoluminescent dosimeters. From four repeated measures in four rabbits the following results were found. Absorbed dose in

Gy: Within the irradiation field 1.98 ± 0.12, 4 cm beyond the caudal part of the irradiated area 0.01 ± 0.004, 5 cm beyond the caudal part of the irradiated area 0.009 ± 0.0004. The distance between irradiated area and control area was 40 mm.

Experiments

The rabbits were treated with fractionated irradiation (2 Gy/F), with a total dose ranging from 2-20 Gy. The rabbits were laid on their backs and the upper part of the esophagus was irradiated. The animals were treated in groups of ten. After completion of irradiation, one animal was removed from the group on ten consecutive days. The animals were sacrificed by a blow on the skull in order to avoid pharmacological side-effects. The esophagus was dissected out on its entire length (9 cm). Samples for SEM, TEM and LM were taken from the upper part of the esophagus, (irradiated area: E_x) and the lower part of the esophagus, (control area E_o). Control investigations were also performed in the same way on untreated animals.

Preparation for SEM

The segments for SEM examination were not rinsed, but were placed directly in 2.5% glutaraldehyde (in 0.15 M cacodylate buffer) for fixation for 12 hours. The pH of the solution was 7.3. They were then transferred into the same buffer, and were later osmium-fixed in 1% osmium tetroxide in 0.15 M cacodylate buffer for 2 hours. After dehydration with graded series of ethanol, the preparations were transferred to Freon TF 618.

The specimens were later critical point dried in Balzer - 000 critical point dryer. They were sputter-coated with gold and palladium in Polaron coating unit E 5000; and examined in a Cambridge Stereoscan Mark II A or a Zeiss Nanolab Electron Microscope. The microscopes were operated at 20 kV.

Preparation for TEM

The samples were fixed as for the SEM preparations and also in 1% osmium tetroxide in 0.15 M cacodylate buffer (pH = 7.3) for 2 hours, rinsed in 0.15 M cacodylate buffer, dehydrated in ethanol and embedded in Vestopal W or Epon. Sections of 1 um thickness were cut on an LKB-ultrotome, stained with toluidine blue and examined in a light microscope. Ultrathin sections were cut and contrasted with lead citrate and uranyl acetate or en bloc with 0.5% uranyl acetate. A Zeiss EM 10 electron microscope was used to examine the sections.

Bacterial control

Cultivation of bacteria from the upper and lower end of the esophagus was performed in ten normal untreated animals.

pH-measurements:

These were made from the upper and lower end of the esophagus with a PHM 62 Standard pH Meter.

Statistical analysis:

Statistical analysis was performed with a three-way analysis of variance with repeated measurements on one factor.

Scoring system

The score for loosened microridges was based on the number as calculated from SEM pictures (5000x) at an area of 17 x 11 cm; score 0 = 0-50, score 1 ∿ 100, score 2 ∿ 200, score 3 > 250.

The score for cell loss was based on the number of loosening cell flakes at an area (17 x 11 cm) on a SEM picture (100x); score 0 < 50, score 1 = 51-100, score 2 = 101-150, score 3 > 150. The

number of bacteria was calculated from SEM pictures (1000x) at an area (17 x 11 cm); score 0 = 0-25, score 1 ∿ 200, score 2 ∿ 400, score 3 ∿ 600.

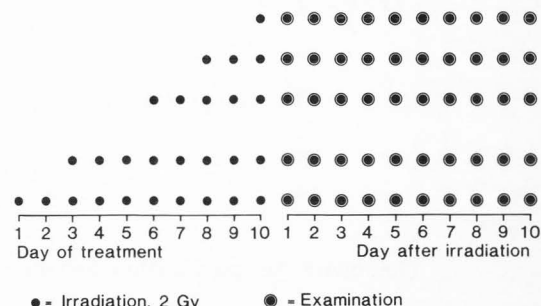● = Irradiation, 2 Gy   ◉ = Examination

Fig.1. Fractionated irradiation schedule: 2 Gy/F was given daily, with the total dose ranging from 2 to 20 Gy. Examinations were made every day after the completion of irradiation from day 1 to day 10.

Results

Untreated control animals

SEM. The esophageal mucosa consisted of flat epithelial cells of polygonal shape connected to each other in irregular flakes. (A in Fig. 2). The cells were joined by discrete cell lines (B in Fig. 2) and each flake had an area of 50-70 µm, composed of many epithelial cells. The unity of these flakes seemed to form an essential part of the normal desquamation process which maintains a steady state in growth and cell-loss. The epithelial cells had numerous microridges on the surface with a width of 0.2-0.3 µm. The microridges were generally arranged in parallel rows of varying length (A in Fig. 3) but in some areas they curled or showed circular formations (A_2 in Fig. 3). The whorled pattern of microridges differed from one cell to another and even within the same epithelial cell surface.

As has been shown earlier (Robinson et al. 1981) these microridges are outfoldings of the cell membrane on both sides of the epithelial cells possessing a large amount of microdots which make physical contact with other points of a ridge belonging to an adjacent underlying cell (desmosomes). Occasionally (small) knobs on the microridges could be seen (arrow). Sometimes the microridges were branched and connected with small interdigitations. Bulges like those described by Robinson et al. (1981) could not be detected in rabbit esophagus. Openings varying in appearance with a width of 3-5 µm were seen on the surface especially where 3-5 cells converged and made a corner with an elevated edge (Fig. 4). These holes were gland openings in the esophageal wall (Bloom and Fawcett 1975).

TEM. The epithelial mucosa was about 20-25 cells thick. The basal cells were columnar and rested on a thin basement membrane. Their nuclei occupied the major part of the cells. The nucleoli were dense, and the cytoplasm showed a multitude of ribosomes and tonofilaments. A large number of mitochondria was observed. The cells were attached to each other by desmosomes, which connected the microridges from one cell to another. The desmosomes were situated on several sites of the convex surface of the microridges (Fig. 5a). Hemidesmosomes connected the basal cells to the basal lamina.
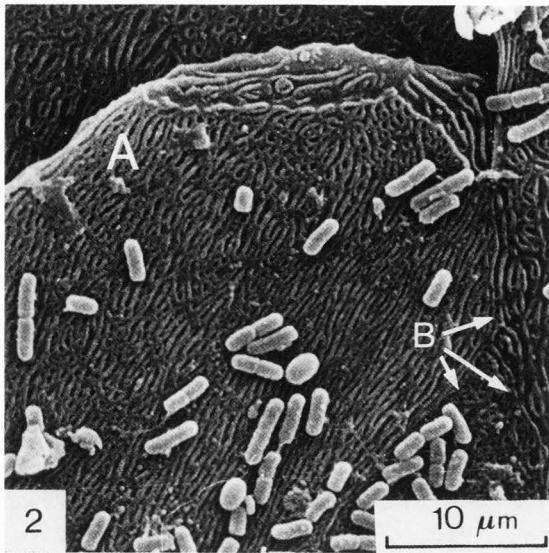
Fig.2. SEM-micrograph of normal esophageal mucosa. A = Loosening cell flake. B = Borderlines between epithelial cells.
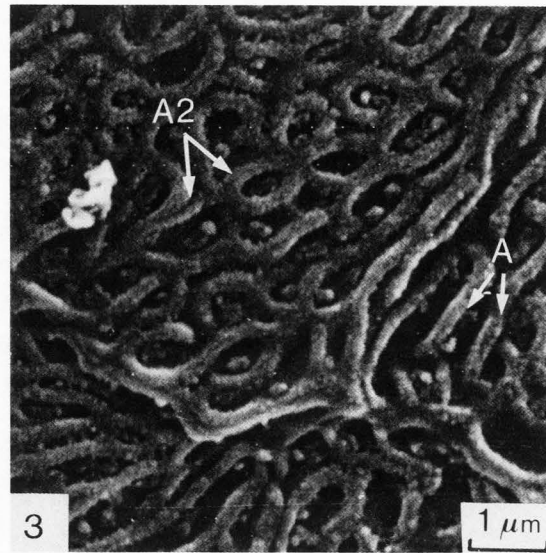
Fig.3. SEM-micrograph of normal esophageal mucosa. Regularly arranged microridges are seen, a small amount of bacteria and occasionally small knoblike structures on the microridges (arrow). Loosening microridges score = 0. A = Microridges in parallel rows. A_2 = Microridges in circular formations.
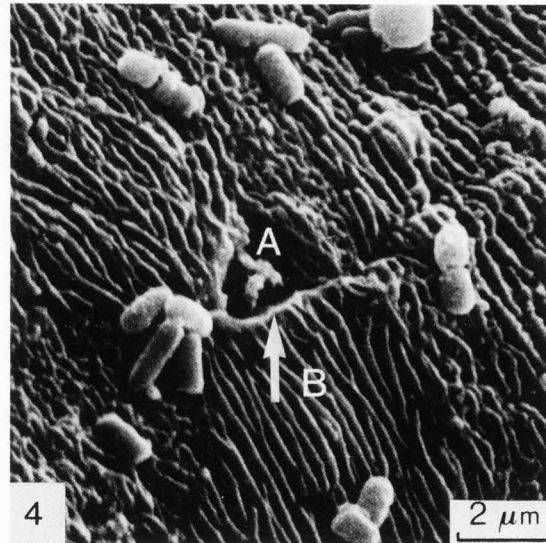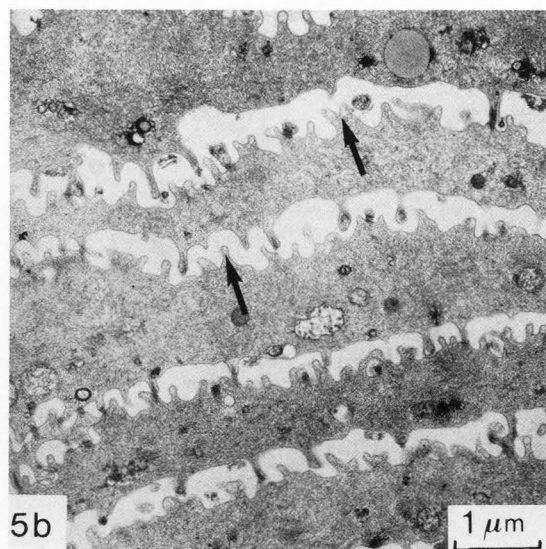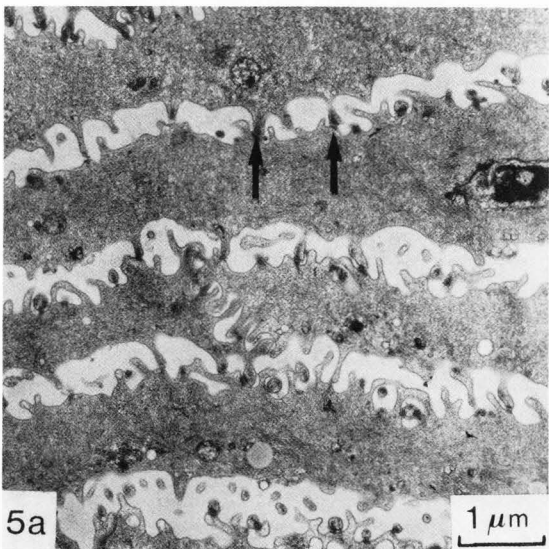
Fig.4. SEM-micrograph illustrating gland opening in the esophageal wall (arrow A). Arrow B illustrates the elevated edge.

Fig.5. TEM-micrographs illustrating **(a)** attachment of the microridges to each other by desmosomes (arrows), and **(b)** microridges, which seem to be vacuous (arrows).

Apically, some microridges had an empty space (Fig. 5b).

In close association with the desmosomes a large number of tonofilaments was observed in the cytoplasm. The desmosomes gradually decreased in number and size as the surface of the epithelium was approached until they finally disappeared.

As the cells migrate towards the surface they undergo a transformation to a more flattened appearance. The number of nuclei appeared to be reduced and the intercellular spaces gradually closed up. The cell organelles in the upper layers were very few or totally absent. The findings reflected the physiology of the mucosa where the ultimate process is the desquamation of cells.

LM, SEM, and TEM investigations were performed on ten control animals. There was no difference in the ultrastructural pattern in the upper or lower part of the esophagus. The bacterial content was the same, and pH measured about the same value in the upper and lower part. The ratio $E_X/E_O = 1$.

Fractionated irradiation

Edema. Ultrathin sections from the upper irradiated part of the esophagus (E_X) and the control area in the same animal (E_O) were routinely examined. The thickness of the epithelium within the irradiated area was measured and the epithelium was found to be swollen in comparison with the epithelium in the control area. The values from all ten days of observation (Fig. 1) were collected to form a mean value since no significant time effect was seen in each of the dose group (Fig. 6).
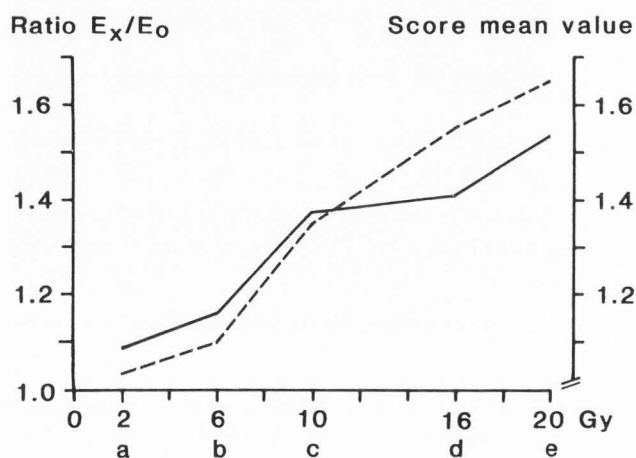

Fig.6. The relationship between the height of the esophageal mucosa within irradiated area compared to the control area for each dose group a-e 1-10 days after irradiation. - - - - edema as measured from LM-pictures. _____ score mean value.

The edema of the irradiated epithelium was noticed already after 2 Gy, with a ratio, E_X/E_O of 1.09 for the whole group. The edema was positively dose-dependent, and after 20 Gy the ratio E_X/E_O was 1.54. The edema was also observable in SEM where it could be scored from SEM pictures and graded on a scale from 0 - 3 (Figs. 7 and 8). For comparison the result of the scoring is also plotted in Fig. 6, and the result of scoring and actual values, as calculated from LM pictures, are parallel.

Figs.7-8. SEM-micrographs illustrating the edema of the esophageal mucosa 16 Gy the first day **(Fig.7)**, and the second day **(Fig.8)**, after irradiation.

Fig.9. TEM-micrograph illustrating microridges loosened from the surface.

Figs.10-12. SEM-micrographs illustrating loosening microridges: score = 1 **(Fig.10)**, score = 2 **(Fig.11)**, and score = 3 **(Fig.12)**.

Table 1. Estimations of the number of loosened microridges at different doses on the surface of the irradiated area (E_X) and non-irradiated area (E_O).

Total dose	Area	Day of examination 1-5 Smv	6-10 Smv
2 Gy	E_X	1.0	0.8
	E_O	0.3	0.15
6 Gy	E_X	1.5	1.0
	E_O	0.05	0.05
10 Gy	E_X	1.1	0.5
	E_O	0.15	0.1
16 Gy	E_X	2.2	0.5
	E_O	0.15	0.15
20 Gy	E_X	2.4	0.9
	E_O	0.2	0.1
Controls	E_X	0.1	0.1
	E_O	0.1	0.1

Smv = Score mean value.
0 = 0-50   2 ∿ 200
1 ∿ 100   3 > 250

Process of loosened microridges

SEM of the normal esophageal surface showed microridges which had loosened and been raised from the underlying structure. The raised end was formed like a small knob or curled up with a snake-like appearance having a length of 0.5 - 3 μm. As a common denomination, the term S.A.K.s (snakes and knobs) was used for lack of a better expression. The appearance could also be deduced from the TEM-micrographs (Fig. 9). The phenomenon was most pronounced on the loosening epithelial flakes and not to the same extent on the underlying cell surface. These may represent a course of events preceding the subsequent desquamation indicating that the microridges are a sensitive indicator of the general condition of the epithelial cells. S.A.K.s were found in the normal untreated esophageal mucosa although to a much lesser extent than in those animals treated with fractionated irradiation. A scoring system considering dose and time, based on the number and size of the S.A.K.s as calculated from the SEM pictures (5000x) was made up and is presented in Fig. 3 and Figs. 10-12. The result from the irradiation is shown in Table 1.
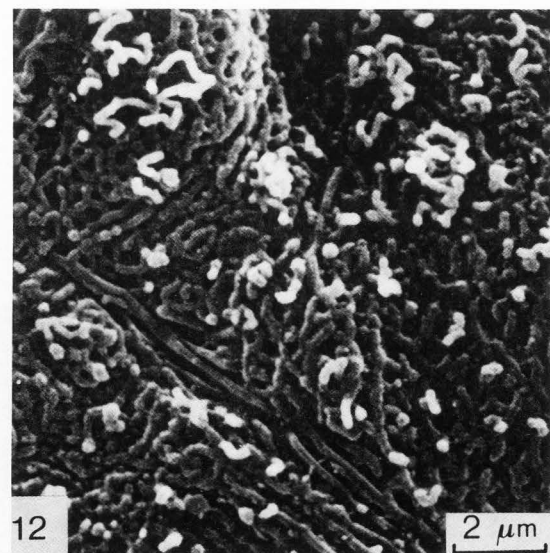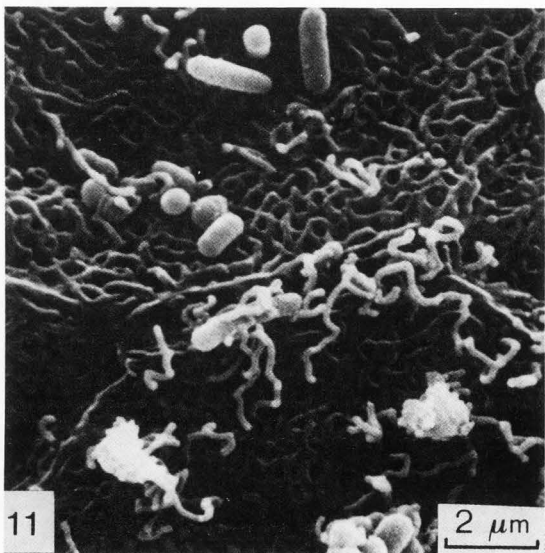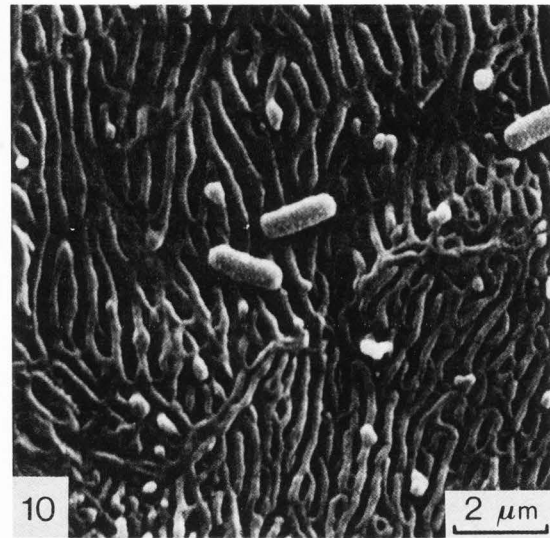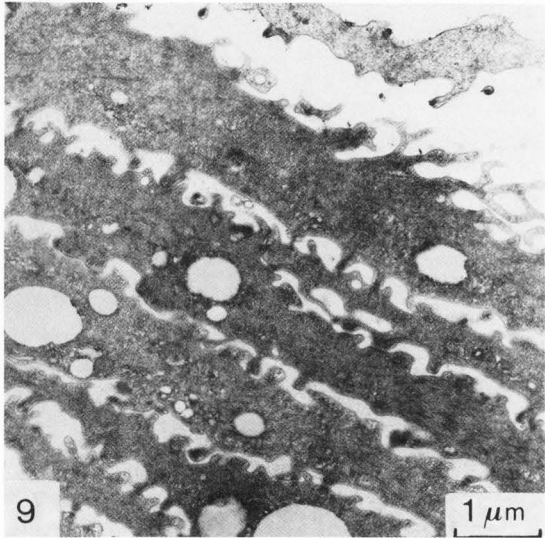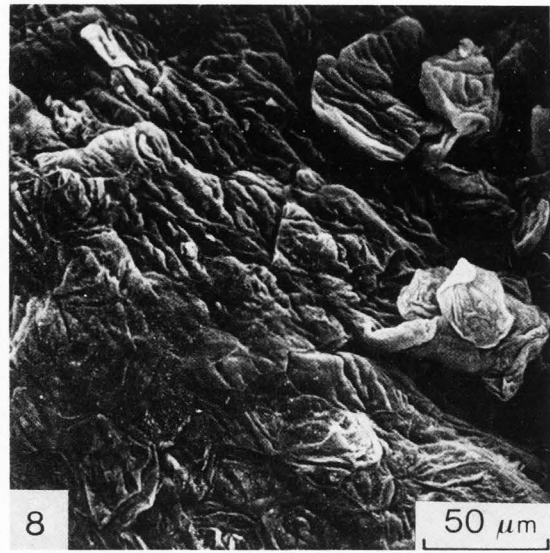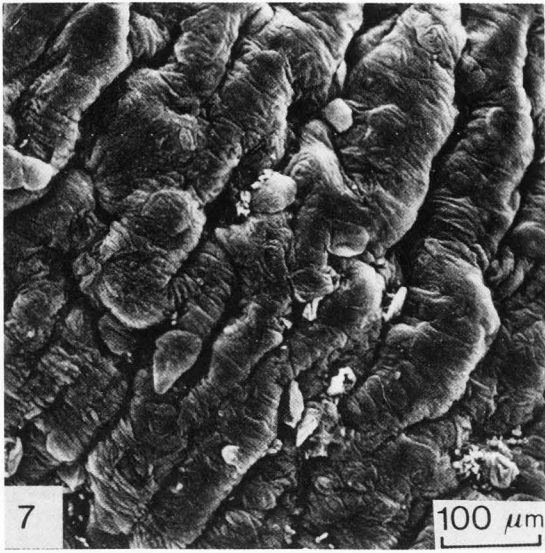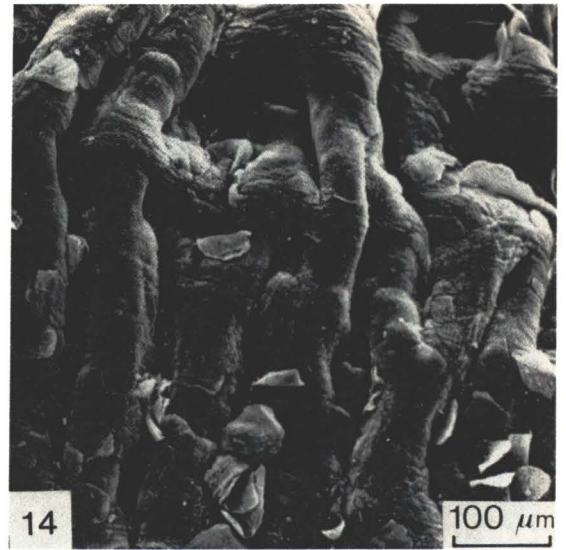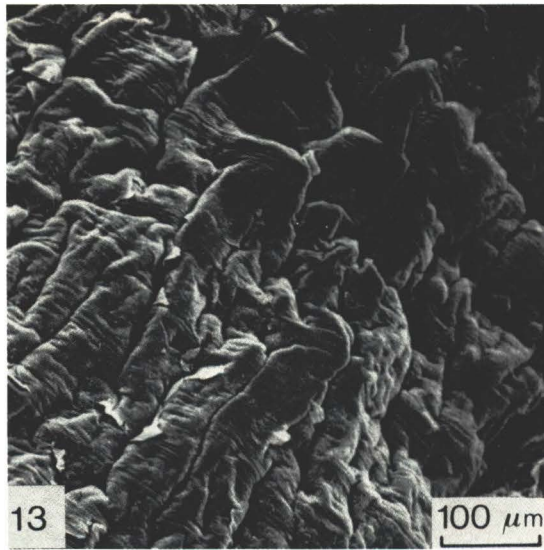
Figs.13-16. SEM-micrographs illustrating cell loss: score = 0 **(Fig.13)**; score = 1 **(Fig.14)**; score = 2 **(Fig.15)**, and score = 3 **(Fig.16)**.

Table 2. Estimation of the number of cell loss at different doses on the surface of the irradiated area (E_x) and non-irradiated area (E_o).

Total dose	Area	Day 1-10 Smv
2 Gy	E_x	0.8
	E_o	0.6
6 Gy	E_x	1.1
	E_o	0.55
10 Gy	E_x	1.45
	E_o	0.65
16 Gy	E_x	1.55
	E_o	0.85
20 Gy	E_x	1.6
	E_o	0.7
Controls	E_x	0.65
	E_o	0.70

Smv = Score mean value.
0 < 50   2 = 101-150
1 = 51-100   3 > 150
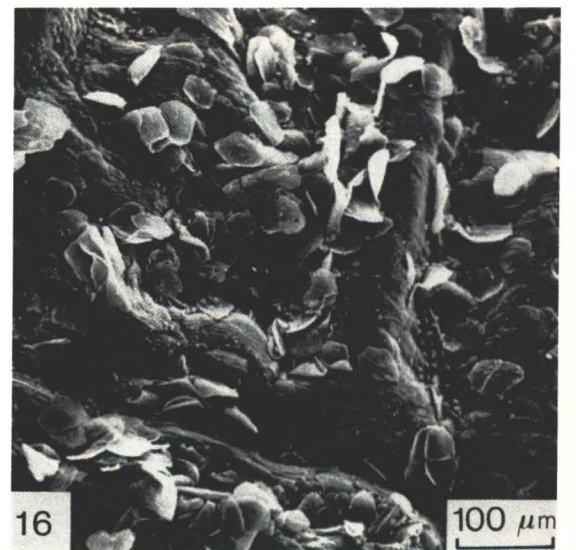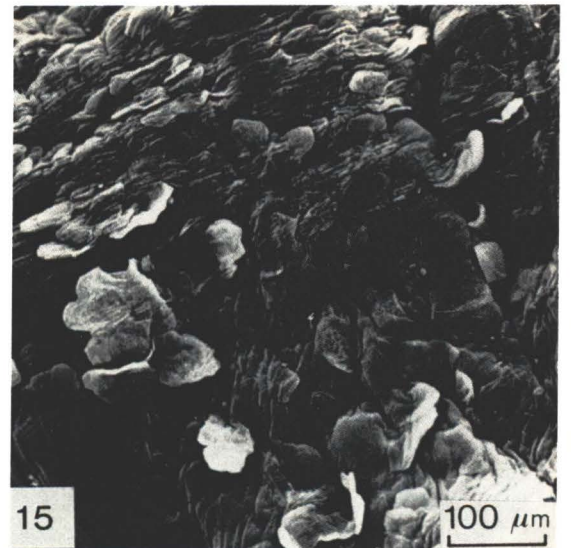
After 2 Gy the number of S.A.K.s was slightly increased. The score mean value was judged to be 1.0. Increasing the total dose was followed by an increase in the number of S.A.K.s. During the first five days of examination these were more pronounced than during the last five days. Therefore, the table is divided into two parameters: The events during day 1-5 and during day 6-10 (Table 1).

During the last days of observation in the higher dose range, (16 and 20 Gy) i.e., about two weeks after start of the fractionated irradiation, the number of S.A.K.s decreased to less than 100 in a defined area. This probably reflects the turnover rate of the cells in this tissue with an exfoliation of the upper layers of the epithelium.

Cell loss

The normal physiological activity of the esophagus includes a certain amount of cell loss (Fig. 13).
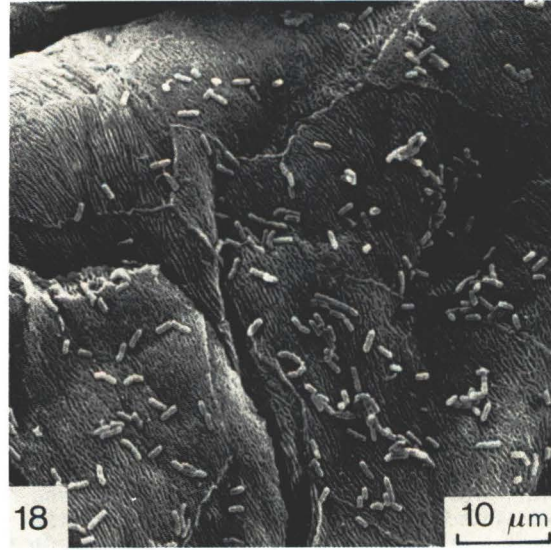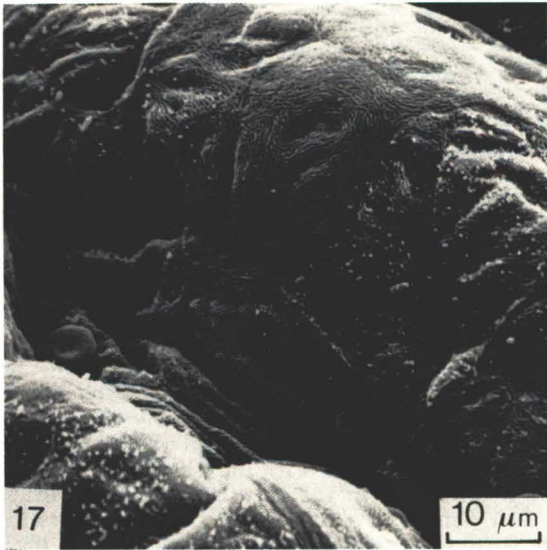
Figs.17-20. SEM-micrographs illustrating bacteria: score 0 **(Fig.17)**; score 1 **(Fig.18)**; score 2 **(Fig.19)**; and score 3 **(Fig.20)**.

Table 3. Estimations of the number of bacteria at different doses on the surface of the irradiated area (E_x) and non-irradiated area (E_o).

| Total dose	Area	Day 1-5 Smv	Day 6-10 Smv
2 Gy	E_x	0.9	1.3
	E_o	1.0	1.0
6 Gy	E_x	0.7	1.1
	E_o	1.1	1.5
10 Gy	E_x	0.6	2.1
	E_o	0.7	1.4
16 Gy	E_x	0.5	0.6
	E_o	0.5	0.6
20 Gy	E_x	0.7	0.1
	E_o	0.6	0.1
Controls	E_x	1.0	1.0
	E_o	1.0	1.0

Smv = Score mean value.
0 = 0-25   2 ~ 400
1 ~ 200   3 ~ 600

In the untreated control animals less than 100 loosening cell flakes could be detected on a defined area on a SEM-picture (100x). Figs. 14-16 illustrate a score of 0-3. In the untreated control part of the esophagus the desquamation was normal (score = 0). After treatment with fractionated irradiation the cell loss increased within the treated area. No significant variation was found during the ten days of examination and therefore all values for each dose group were collected and are presented in Table 2. Within the irradiated part of the esophagus the cell loss in the dose group 2 Gy was scored to 0.8. Thereafter the cell loss increased with the radiation dose and reached a maximum after 20 Gy with a score mean value of 1.6.

Bacteria

The surface of the esophageal lining was normally covered with a varying amount of bacteria
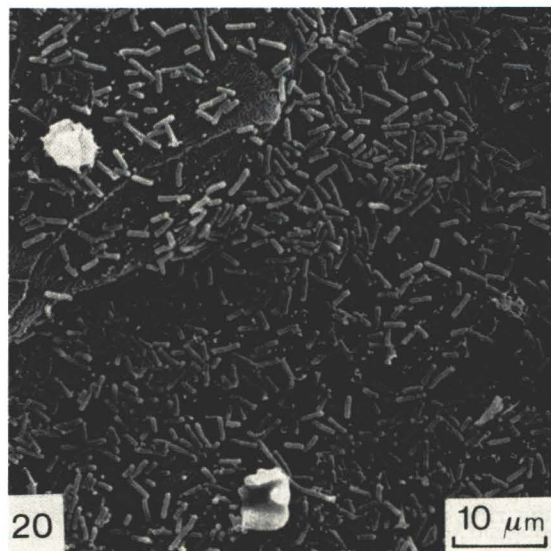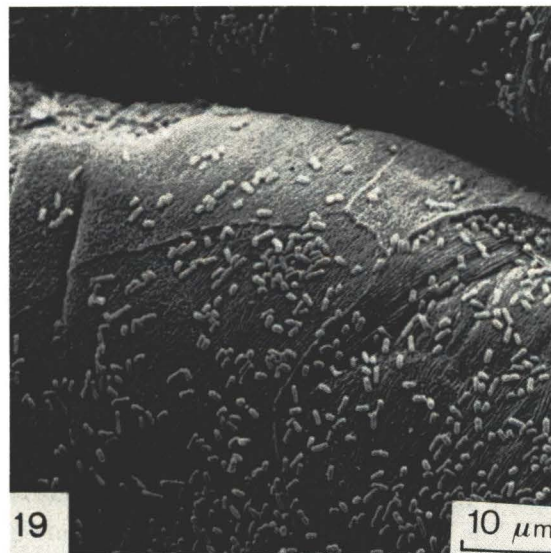
belonging to the cocci or coliform microorganisms (Fig. 17). Cultivation from ten normal and untreated rabbits gave the following results: E. coli, B. catarrhalis, Haemophilus sp, and Acinetobacter sp. Samples were taken both from the upper end of the esophagus, E_x, (pH = 7.4), and from an area 4-5 cm lower, E_0, (pH = 7.3). No predominance for one species of the microbes was found on the surface of either of the two areas examined.

The adherence to the epithelial cells seemed to be rather superficial and a penetration of the microbes into a cell was never seen. On TEM pictures, the bacteria could be seen attached to the fuzzy coat on the cell surface like the fusiform bacteria of the intestine (Nelson and Mata 1970). In any case, the bacteria appeared as individuals in symbiosis with the epithelial cells. Mucopolysaccharides in the fuzzy coat may play a role in the mutual action between cells and bacteria (Savage et al. 1967). However, whether such saccharides existed was not investigated. The effect of ionizing radiation on the amount of bacteria was investigated on both the irradiated area (E_x) and on the control area (E_0), 4-5 cm lower. The amount was scored from SEM micrographs and the results during the 10 days after irradiation were collected in a mean value, for day 1-5, and for day 6-10, respectively (Table 3). Figs. 17-20 illustrate bacteria scored 0-3. A relationship between the upper part (E_x) and the lower part (E_0) seemed to exist for both days 1-5 and 6-10. This may indicate an action of radiation outside the irradiated area (reflexion action?, "diffusion flare"). The main result from Table 3 is an accumulation of bacteria 6-10 days after a fractionated dose of totally 10 Gy. At higher doses, the amount dropped and after 20 Gy, less than 200 per unit area could be detected. The increase in amount of bacteria the second week after 10 Gy was not accompanied by an invasion of the epithelial cells which could be verified by TEM.

Discussion

Rabbit esophagus

The reaction to ionizing radiation applied to the esophagus can, for several reasons, best be compared with a reaction in the skin. However, useful comparisons can also be made with other parts of the digestive tract, e.g., the buccal mucosa and the intestinal epithelium. From a phylogenetic and embryologic point of view, the alimentary canal consists of a tube developed from the very beginning as a fold during the early gastrula stage with cells provided with cilia. Later on, squamous cells appear and the cilia show a complete regression and an esophageal mucosa is created which, like the skin, consists of a basal layer of germinative cells covered by a stratified squamous epithelium.

However, there is an important difference between the skin and esophagus of the rabbit. The esophageal lining is not keratinised although this feature can be seen in ruminants (Desmet and Tytgat 1974). In rats, keratohyaline granules can be seen in the surface layer whereas in rabbits the superficial cells contain folded filaments which react to keratin-markers (unpublished results).

The rabbit esophagus begins at the caudal cartilage ring and passes through the mediastinum 6-7 cm to the diaphragm and then another 3 cm to the cardia of the stomach.

In the current experiments, the sample used for control (E_0) was a safe distance from the acid part of the esophagus. The pH-measurements further confirmed this fact. E_x and E_0 had the same appearance as was verified both by SEM and TEM.

Irradiation effects

One of the first reactions in tissues exposed to ionizing radiation is generally an erythema and an edema. As far as rabbit skin is concerned, Rigdon and Curl (1943) were able to show that these phenomena were based on a radiation effect on the permeability of the vascular endothelial cells. The esophageal mucosa in the current experiments produced a similar reaction, with an edema which directly could be measured from the epithelial layers as examined by LM and from the scoring of the SEM micrographs. The edema was shown to be dose-dependent, becoming larger with increasing dose. This has also been shown in the skin by Mount and Bruce (1964) and points to a damage of the squamous epithelium with an accumulation of fluid based on a change of the osmosis and a secondary diffusion. The same changes were found in the irradiated trachea (Albertsson et al., 1983).

Membrane damage may also be responsible for the phenomenon of loosening microridges and may be explained in the following way: The microridges on the surface layer lose desmosomal contact with the underlying cells. On the TEM pictures the tonofilaments seem to have contracted leaving the damaged microridges apically with an empty space (Fig. 5b). The damaged microridges have a very variable appearance from small knob-like structures to long snake-like formation. The number of these structures as calculated from SEM micrographs is clearly increased with increasing dose, most marked during the first observation days. In the higher dose range (16, 20 Gy), when about two weeks have passed since the start of the fractionated irradiation treatment, a repopulation from the basal cell layer may have occurred since the damaged microridges are most clearly seen on the cells that are about to loosen. This probably explains why the number of S.A.K.s is reduced during the last five days of observation. From other reports concerning thoracic irradiation of mice with high single doses (20 Gy) Philips and Ross (1974) could show with LM investigation of the esophagus, that one to two weeks after irradiation, there was "a mixed pattern with foci of proliferating basal cells and regenerating epithelium mixed with complete esophageal denudation with absence of any cellular layer". Michalowski and Hornsey (1986) also found after thoracic irradiation of mice with high single doses (27 Gy), ulcerative esophagitis "which raised from nil to 100% during the 7th and 8th days after irradiation, remained at this level for two days and subsequently decreased to 10% by day 14."

These investigations illustrate that the esophageal epithelium is a fairly rapidly proliferating and renewing tissue (1-2 weeks). The damage effects observed in this study with edema and loosening microridges are moderate effects. With increasing time, probably the tissue normalizes completely with fractionated irradiation in this dose range. The cell loss was most pronounced after 20 Gy, positively correlated to the dose. Cell loss and desquamation of the surface epithelium is a normal process in proliferating tissues like skin, intestine, trachea and

esophagus. Since the number of cell layers calculated from TEM pictures did not seem to decrease within the whole dose range, the squamous epithelium is apt to respond to the irradiation effects with an increase in its proliferative activity. Such a compensatory proliferation mechanism is known to start within two days in the intestine (Withers and Elkind 1969, Withers 1971), and in the skin within 1-2 weeks (Denekamp et al. 1969, Denekamp 1973, Denekamp et al. 1976).

Bacteria

The presence of microorganisms in the esophageal lumen is by no means unique. On the contrary, the esophagus shares this state of affairs with most of the alimentary canal where bacteria normally live in a balanced ecological system regulated, however, within certain limitations that cannot be superseded. The physiological activity of the luminal cells influences the occurrence of bacteria. Fasting and non-fasting animals have different amounts of microbes, i.e., fewer in the fasting animal (Friberg 1980). Access to mucopolysaccharides has been shown to be a factor of importance (Savage et al. 1967; Takeuchi and Zeller 1972) as a medium for life of the bacteria in the lower ileum, but as far as the esophagus is concerned, data on the attachment of the bacteria and membrane physiology are lacking.

Drastic changes in the environment, e.g., after treatment by ionizing radiation have a pronounced effect on the bacteria. Thus, Friberg (1980) found that - after a single dose of 25 Gy to the small intestine of the rat - a swarm of bacteria came already five minutes after the irradiation, especially in the extrusion zone of the villi. Maybe this shows a preference for cells in the destruction phase or membrane damage. However, 30 minutes after the irradiation the bacteria had disappeared completely. The interpretation of the phenomenon is difficult. Perhaps the bacteria followed the process of desquamation. Possibly the nutritional basis had changed.

Fractionated irradiation in the current experiments showed an increase in the amount of bacteria 6-10 days after a total dose of 10 Gy (2 Gy x 5). In these cases, it is impossible to use the word attack, since neither penetration nor close connection between bacteria and epithelial cells existed. With continued fractionation and a higher total dose the amount of bacteria decreased to virtually nil. During the first week after all fractionations there was a continuous decrease, and after 20 Gy (2 Gy x 10) very few bacteria could be found during all 10 days after irradiation. In the non-irradiated this may indicate a secondary effect (reflex action from the irradiated area?). The bacteria possibly follow the epithelial cells in their desquamation process.

Friberg (1980) showed that in the ileum of the non-fasting rat 2 Gy x 5 produced a great attack of bacteria on the top of the villi and of the same magnitude as after 2 Gy x 10. One explanation may be that the cells in the crypts of the Liberkuehn are capable of maintaining a steady state of new cells migrating to the top of a villus where the bacterial flora is unchanged.

As the epithelial cells of the esophagus were heading for extinction, the bacteria on the surface seemed to suffer at the same time. This effect of increasing dose was easily shown by TEM, where the bacteria seemed to be exposed to a process of deterioration showing a shaggy surface and a non-homo-geneous cytoplasm. Using greater magnification in the SEM (25,000 x) the rugged surface could be verified. The reason for this phenomenon may be that the content of disaccharides in the fuzzy coat had fallen below the subsistence level of the bacteria.

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Discussion with Reviewers

K.E. Carr: How does the treated control material compare with that from the untreated animals?
Authors: For comparison, scoring of the number of cell loss, the number of loosened microridges and the number of bacteria are presented in Tables 1-3. No significant differences are observed between untreated animals and treated control material.

K.E. Carr: Why did you assess edema using SEM?
Authors: This was done in an attempt to compare an objective method (measurements of the height of the mucosal epithelium from LM-pictures) to a subjective score of the edema from SEM-pictures.

J. Reitan: The anaesthetic pentobarbital is a membrane active drug. The combined effects of membrane active drugs and radiation have been investigated in various experimental systems. Barbiturate anesthesia has pronounced effects with hypothermia, hypotension and hypoxia. No sham irradiated animals are mentioned in your paper. Do you think that barbiturate anesthesia may have influenced your results?
Authors: A possible effect of the anesthesia to be considered in the interpretation of the result described here, cannot be excluded. Therefore, separate analyses have been performed of the ultrastructure on 10 animals that had received the anesthetic but had not been irradiated. However, these investigations showed normal ultrastructure.

J. Reitan: In clinical practice, the radiation doses applied are generally higher than those used in these experiments. Doses in the range 40-60 Gy with daily fractions of 2 Gy are commonly applied for target volumes encompassing parts of esophagus. As the clinical relevance of the experiments seems to be of concern, why haven't you used higher doses?
Authors: In the clinic concerned with chemotherapy treatment, patients with squamous cell carcinoma of the esophagus are treated with cis-DDP and 5-fluorouracil concomitant with preoperative radiotherapy TD: 24 Gy (2 Gy/F). This experimental design is chosen from our clinical point of view.