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EFFECTS OF IONIZING RADIATION ON THE ACTIVITY OF THE CILIATED EPITHELIUM OF THE TRACHEA

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The late effects of ionizing radiation on the mucociliary activity of the tracheal epithelium have been thoroughly investigated in patients who had been irradiated for bronchial carcinoma or Hodgkin's disease (RUCKES & HOLLSTEIN 1968, FERNHOLZ & MÜLLER 1969, BOUSHY et coll. 1970, LANDBERG et coll. 1972), and also in experiments on the rabbit (DANIELSSON et coll. 1971). Both the production of mucus and the ciliary activity were reduced by irradiation. The duration of the effects was related to the absorbed dose. The effects of irradiation on the movements of the cilia have been investigated previously using different methods (UMEDA 1927, HEINE 1936, FRENCKNER 1939, OGI 1959, FUJIWARA et coll. 1972) but the immediate effects are largely unknown and controversial.

Therefore, it was felt to be of interest to attempt to record the immediate physiologic effects of irradiation on the tracheal mucous membrane from rabbit in vitro using a light reflection method (MERCKE et coll. 1974), which registers the mucociliary activity. The physiology of the tracheal epithelium has been investigated previously by HÅKANSSON & TOREMALM (1965–1968); their results constitute the background for the evaluation of the effects of irradiation in the present report.

Methods and Materials

The trachea of the rabbit has been used in all experiments. A total of 20 animals were used, 5 for each dose level. The animals were killed by a blow on the skull. The

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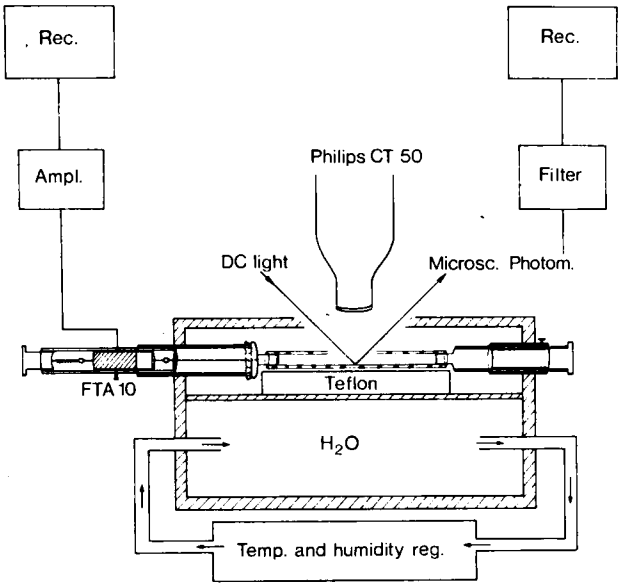

Fig. 1. Experimental chamber with the possibilities for simultaneous irradiation and recording.

trachea was then immediately dissected and placed on a layer of teflon in an experimental chamber where an even temperature and humidity could be maintained at the level desired (Fig. 1). The preparations, on an average 3.5 cm long, were placed in the chamber between two identical adjustable holders and stretched out to their calculated natural length. The trachea was then opened by a longitudinal incision in the membranous part, which was turned upwards in the chamber. Humidity was kept constant above 90 per cent to prevent drying out, and the temperature was maintained at 30°C. The mucociliary activity was registered with a 'light beam reflection method' (HÅKANSSON & TOREMALM 1965, MERCKE et coll.). Heat filtered DC light from a cold light source (Zeiss KL 150) illuminated the mucous membrane of the trachea via the incision and was reflected by the mucous ciliary border at the 'bottom' of the trachea. The blinking light reflection which arose thereby due to the mucociliary activity was observed through a laboratory microscope. A photomultiplier attached to the lens of the microscope was connected to an ink plotter (Mingograf 34, Siemens-Elema) through a frequency filter (Krohn–Hite 3550). This apparatus enabled registration and documentation of the intensity variations of the light reflexes. The contractions and relaxations in the preparation were registered continuously by aid of a transducer (Sanborn FTA 10) connected to a plotter (Servogor RE 511).

The preparation was irradiated by Philips' contact therapy apparatus at 50 kV, 2 mA, HVL 0.5 mm Al, focus-object distance 40 mm, dose rate 0,34 Gy/s (34 rad/s). The administered dose was measured in the chamber using thermoluminescent dosemeters (TLD) placed at the bottom of the trachea, on a teflon sheet under the prepa-

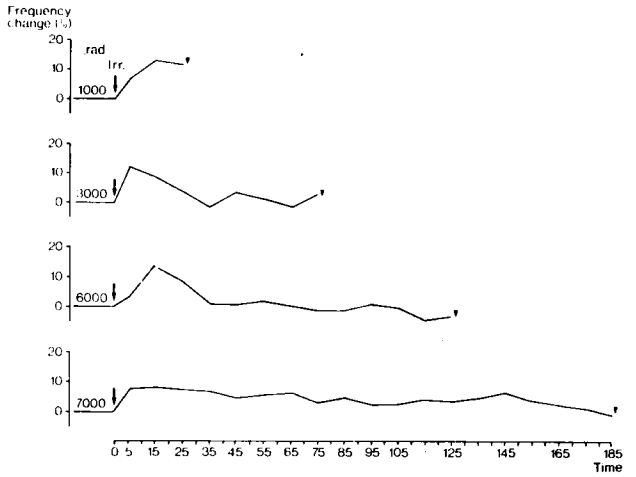

Fig. 2. Mucociliary wave frequency in the trachea after ionizing irradiation at different dose levels (1 Gy = 100 rad). Time in seconds.

ration and in lead shielded control preparations placed in the chamber on each irradiation. The following doses were given: 10, 30, 60 and 70 Gy (1 000, 3 000, 6 000 and 7 000 rad), respectively, with continuous registration of the mucociliary activity and the contractions and relaxations of the musculature. Sections from the irradiated epithelium and the control sample were taken for scanning electron microscopy (SEM) and transmission electron microscopy (TEM) immediately after the end of irradiation and fixed in glutaraldehyde.

Results

The mucociliary activity increased at all dose levels within 5 seconds after the start of irradiation (Fig. 2). A return to the level registered before irradiation (reference value) took place after 35 s for 30 Gy, after 65 s for 60 Gy, and after 185 s for 70 Gy. For 10 Gy the exposure was too short to permit adequate observation.

The mean value could be derived from the observations in the entire series of 20 animals during the first 15 s of the irradiations (Fig. 3), as the dose rate was the same at all dose levels and exposures. The average increase in frequency after 5 s was 8 ± 2 per cent. The maximum increase in frequency occurred after 10 s and was 11 ± 1 per cent; after 15 s the increase was 7 ± 2 per cent.

As it is important to establish how this frequency increase reflects the biologic processes causing the response to irradiation, the variations of the frequency increase have been analysed in detail. This has been done from the basis of the reference value before start of irradiation and by aid of a computer. The k-value has been calculated in five-second intervals for 0 to 5, 5 to 10 and 10 to 15 s after start of irradiation (Fig. 4). The regression line is built up of 6 dots in each interval. The k-value for the first 5 s, which is 0.82, is followed by a k-value of 1.40, and the third k-value (be-

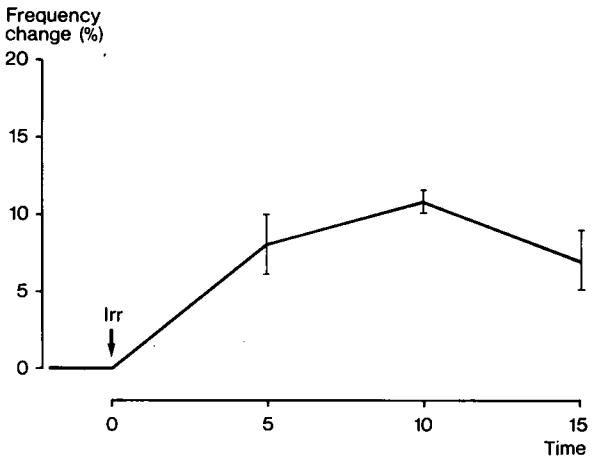

Fig. 3. Mucociliary wave frequency during the first 15 seconds of irradiation at a dose rate of 0.34 Gy/s (34 rad/s).

tween 10 and 15 s) was 0.26. The analysis indicated a higher influence, i.e. a higher response, in the cell during the second phase, that is after 5 to 10 s of irradiation.

Changes of contractions and relaxations of the tracheal musculature were registered in approximately 30 per cent of the experiments. These changes arose immediately following irradiation. (These results as well as the observations from SEM and TEM of the irradiated mucous membrane, will be described in future publications.)

Discussion

The ciliated epithelium of the trachea is an ideal object for analysing the effects of irradiation. The apical cilia of the epithelial cells maintain constant activity for at least 3 hours after the trachea has been extirpated and placed in an experimental chamber (FUJIWARA et coll.) and their activity can be registered (MERCKE et coll., TOREMALM et coll. 1974). Thus it is possible, by recording the changes in the mucociliary activity during that length of time, to analyse the effects of ionizing radiation on the vital activity of the living cells.

Previously it has been demonstrated that the effect on the movements of the cilia is related to the dose and that high doses are necessary to obtain complete inhibition of the movements (GOLDHABER & BACK 1941). FUJIWARA et coll. reported reduced mucociliary activity 15 min after irradiation. The activity normalized within 1.5 and 2.5 hours following doses of 5 and 30 Gy (500 and 3 000 rad), respectively. After a dose of 70 Gy (7 000 rad) no recovery of ciliary activity occurred during 3 h observation. Similar results have previously been published by OGI; no observations of the immediate effects were made. HEINE observed an increased beating frequency of the cilia shortly after exposure of the tracheal mucous membrane to radium but this could not be reproduced by exposure to roentgen rays.

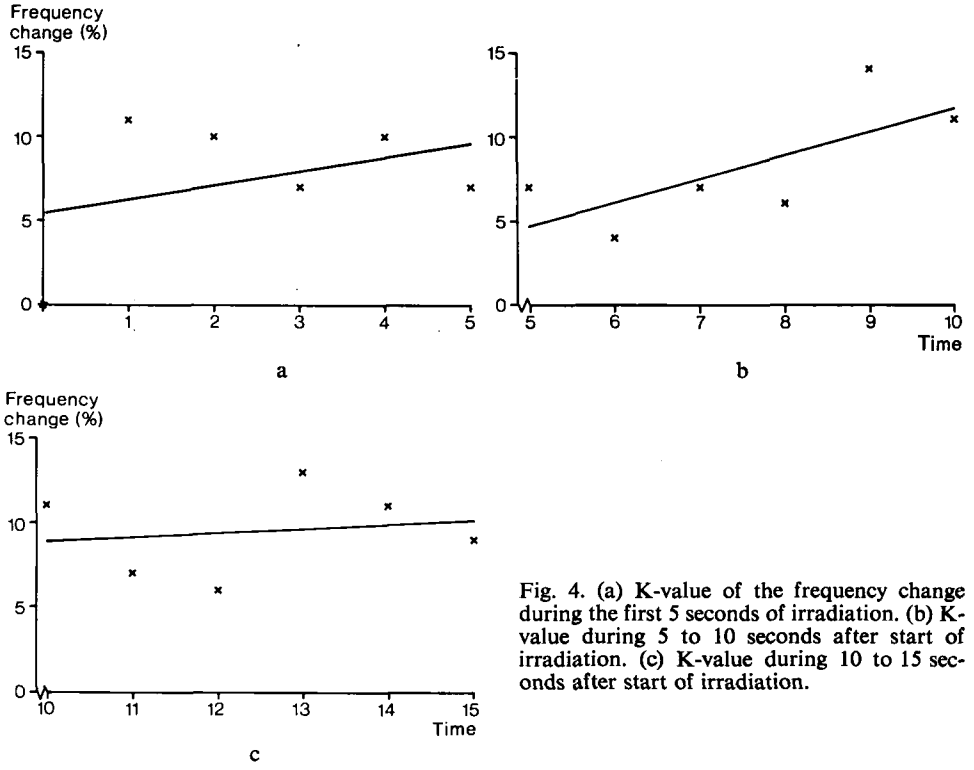

Fig. 4. (a) K-value of the frequency change during the first 5 seconds of irradiation. (b) K-value during 5 to 10 seconds after start of irradiation. (c) K-value during 10 to 15 seconds after start of irradiation.

The present method enables continuous registration of the epithelial mucociliary activity thus permitting an analysis of the influence of ionizing radiation on the functions of the epithelial cells second by second. Under stable conditions the mucociliary activity increased initially after the beginning of irradiation, discernible after 2 to 3 s, reaching its maximum after 10 s and then returning to the original activity. This occurred after a varying time, up to 185 s after beginning of irradiation.

The mechanism behind the initial effects of irradiation are not known but some theoretical factors may be considered. The cilia require adenosine triphosphate (ATP) as a source of energy for their activity. ATP is produced by the mitochondria, which usually lie apically in the epithelial cells. Diffusion of ATP is assumed to take place towards the rootlet of the axoneme. A suitable concentration of ATP and certain essential ions are maintained around the axoneme through the function of the ciliary membrane. The ATP concentration is believed to determine the beat frequency of the cilia (SATIR 1974). Ionizing radiation may cause injuries to the mitochondria resulting in an increased outflow of the ATP available to the cilia. It may influence the dyneinmolecules, which are attached to the axoneme and which are active in breaking down ATP into ADP (adenosine diphosphate) and phosphoric acid, whereby energy is released. HAMBERGER et coll. (1970) have observed an increase of succinoxidase activity in isolated nerve cells as soon as one hour after 30 Gy

(3 000 rad) and an increase of ^{3}H-leucine incorporation into proteins soon after the beginning of irradiation. It may be due to an increase in the enzyme content of the cells, or be caused by an activation of previously existing enzymes. However, it is also possible that ionizing radiation destroys the physiologic intracellular barriers and thereby influences the enzyme activities. It is possible that the observations are expressions of compensating intracellular metabolic processes, replacing the consumed energy in the epithelial cells during irradiation.

Microdosimetry has shown that the specific energy which certain particles deposit in some of the subcellular structures may have a range between almost zero to several hundred rad (LINDBORG & BENGTSSON 1973). Such a localized deposit of energy could possibly change the energy status of the cilia. However, in these experiments the energy thus brought to a cell is probably quite small.

ECKERT & MURAKAMI (1972) found that the beating frequency of the cilia was directly dependent on the concentration of energy available, probably ATP. Their finding was confirmed by SATIR. They also demonstrated that the beat frequency of the cilia was related to the concentration of intracellular calcium: when the calcium concentration increased, the beat frequency also rose. Calcium is assumed to influence one or several metabolic steps in the production of ATP by aiding in uncoupling oxidation and phosphorylation, whereby energy is released as heat. It may be that ionizing radiation affects the permeability of the cellular and mitochondrial membranes, resulting in an intracellular redisposition of calcium, which in turn affects the ciliary activity. On the other hand, increased extracellular calcium ions are known to decrease the velocity of the action potential and to make the membrane more resistant to stimulation (AXELSSON 1961). Electromagnetic radiation of a wavelength different to the roentgen radiation used in the present material is believed to affect the ciliary activity. SAIER & GIESE (1966) found increased beat frequency of the cilia of the *Protozoa paramecium* initially after irradiation with ultraviolet light. With the low energy spectrum used in the present experiments, excitation phenomenon cannot be excluded.

The time elapsed from the beginning of irradiation until the maximum effect is reached is of great interest. The maximum appears in the second period (Fig. 4 b). This problem covers many unknown parameters, such as different capacity, membrane sensitivity etc., and requires further investigation.

It seems to be worthwhile to apply the method presented to an analysis of the influence of the dose rate, different radiation energies and temperature on the effects of ionizing radiation on vital tissues. Such investigations may be of value for the clinical radiation therapy.

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SUMMARY

The immediate effect of ionizing radiation on the activity of the ciliated epithelium of the trachea has been investigated using a light reflection method. This method enables continuous registration of the mucociliary activity both during and after irradiation. A notable increase of the mucociliary activity occurred within 5 seconds after beginning of irradiation and this activity reached its maximum 10 seconds after initial exposure with a dose rate of 0.34 Gy/s (34 rad/s). The mechanism causing the phenomena observed is not clear but theoretically it might be due to ATP, the source of energy of the cilia being freed by the irradiation, possibly through disturbances of the permeability in the mitochondrion membranes.

ZUSAMMENFASSUNG

Der unmittelbare Effekt ionisierender Strahlen auf die Aktivität des Zilienepithels in der Trachea ist mit Hilfe einer Lichtreflexionsmethode studiert worden. Diese Methode ermöglicht eine kontinuierliche Registrierung der mucoziliaren Aktivität sowohl während als auch nach der Bestrahlung. Eine bemerkbare Zunahme der mucoziliaren Aktivität erfolgte innerhalb von 5 Sekunden nach Beginn der Bestrahlung. Diese Aktivität erreichte ihren Höchstwert nach einer Expositionszeit von 10 Sekunden mit einer Dosisrate von 0,34 Gy/s (34 rad/s). Eine ursächliche Erklärung für die beobachteten Erscheinungen steht noch aus. Eine theoretisch mögliche Energiequelle für die Zilienaktivität wäre ATP, das durch die Bestrahlung freigesetzt wird, möglicherweise durch Störungen in der Membranpermeabilität der Mitochondrien.

RÉSUMÉ

L'effet immédiat de radiations ionisantes sur l'activité de l'épithélium cilié de la trachée a été étudié en utilisant une méthode de réflexion de la lumière. Cette méthode permet un enregistrement continu de l'activité mucociliaire aussi bien pendant qu'après l'irradiation. L'activité mucociliaire augmente notablement dans les 5 secondes après le début de l'irradiation et elle atteint son maximum 10 secondes après l'exposition initiale avec un débit de dose de 0.34 Gy/s (34 rad/s). Le mécanisme qui cause les phénomènes observés n'est pas clair, mais il pourrait théoriquement être dû à l'ATP, la source de l'énergie des cils étant libérée par l'irradiation, peut-être par des perturbations de la perméabilité des membranes mitochondriales.

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