Organization
LUND UNIVERSITY
Department of Oncology
University Hospital
S-221 85 Lund
Sweden

Document name
DOCTORAL DISSERTATION

Date of issue
16 Nov, 1984

CODEN:
LUMEDW /(MEOK-1005)/1-48/(1984)

Sponsoring organization

Author(s)
Bo Baldetorp

Title and subtitle
THE RESPONSE OF THE CILIATED EPITHELIUM DURING AND AFTER EXPOSURE TO IONIZING RADIATION. A Physiological and Morphological Study.

Abstract
Irradiation of the ciliated tissues of the body gives undesirable sideeffects. In vitro irradiation (10 Gy) of the rabbit's trachea shows that 1.5 Gy of indirectly ionizing radiation (50 kV and 6 MV X-ray, ^{60}Co-gamma 1.25 MeV) causes a 20 per cent increase of the ciliary beat frequency lasting 5-10 seconds, followed by a decline to normal ciliary activity during the ensuing course of irradiation. Electron radiation (4 MeV) proved to be three times more effective than photon radiation in regard to the physiological response of the cilia to ionizing radiation. This finding led to introduction of the concept "Relative Physiological Efficiency" (RPE) in this study, complementing the Relative Biological Efficiency (RBE) concept. This momentary increase in frequency can be caused by a radiation-induced increased hydrolysis of the ATP available in the cilia. The ciliary activity was 20 per cent lower than normal at 45 min following irradiation (10 Gy, 50 kV X-ray), whereupon it increased to 12 per cent above normal activity at two hours after initial irradiation. At re-irradiation (10 Gy, 50 kV X-ray) administered two hours after initial irradiation, the cilia showed a constant rate of activity. In vivo irradiation (10 Gy, 160 kV X-ray) of the trachea of the rabbit caused a heightened activity (10%) during the first three days after irradiation, indicating a stimulation of the ATP-synthesis. During days 4 to 8 after irradiation, the ciliary epithelium's morphology was damaged resulting in reduced transport ability. Repair took place during days 9 and 10 after irradiation, i.e. the function of the ciliary epithelium appeared to be restored. The membrane potential of the ciliary cell, registered during irradiation (10 Gy, 50 kV X-ray) showed no changes, which supports the assumption that the increased ciliary beat frequency recorded during irradiation can be due to rapid radiation-induced biochemical changes that are connected to the motility of the cilia.

Key words
1. Trachea; 2. Ciliated cells; 3. Ionizing radiation; 4. Physiology; 5. Morphology
6. Relative Biological Efficiency (RBE); 7. Relative Physiological Efficiency (RPE)

Classification system and/or index terms (if any)

Supplementary bibliographical information

Language

ISSN and key title

ISBN

Recipient's notes

Number of pages 48

Price

Security classification

Distribution by (name and address)
Bo Baldetorp, M. Sc., Department of Oncology, University Hospital, S-221 85 Lund

Signature Bo Baldetorp

Date 2 Oct. 1984

THE RESPONSE OF THE CILIATED EPITHELIUM DURING

AND AFTER EXPOSURE TO IONIZING RADIATION

A physiological and morphological study.

BO BALDETORP

Civilingenjör, Ssk

Akademisk avhandling som med vederbörligt tillstånd av Medicinska Fakulteten vid Universitetet i Lund för avläggande av doktorsexamen i medicinsk vetenskap kommer att offentligen försvaras i Onkologiska klinikens föreläsningssal, Lasarettet i Lund, fredagen den 16 november 1984 kl 09.15.

LUND 1984

SE8500056

THE RESPONSE OF THE CILIATED EPITHELIUM DURING AND AFTER EXPOSURE TO IONIZING RADIATION

A Physiological and Morphological Study

Bo Baldetorp

Department of Oncology, University Hospital, 221 85 Lund
Sweden

Lund 1984

To Irma
Louise

The present thesis is based on the following papers, which will be referred to in the text by their Roman numerals.

I. Influence of temperature on the activity of ciliated cells during exposure to ionizing radiation.
Baldetorp L., Håkansson C.H. and Baldetorp B.
Acta Radiol. Ther. Phys. Biol. 16: 17-26, 1977.

II. Intracellular recording of potential oscillations in ciliated cells exposed to ionizing radiation.
Baldetorp B., Baldetorp L. and Håkansson C.H.
Acta Radiol. Oncology 23: 49-53, 1984.

III. A comparison between the immediate effects of photon and electron radiation on the ciliary activity of the rabbit's trachea. An in vitro study.
Baldetorp B.
Accepted for publication in Acta Radiol. Oncology 1984.

IV. The effect of re-irradiation on the tracheal ciliary cell activity. A radiobiological study.
Baldetorp B. and Baldetorp L.
Submitted for publication.

V. The effects of 10 Gy single-dose irradiation on the ciliated epithelium measured during and one-to-ten days following irradiation. A comparative physiological and morphological study.
Albertsson M., Baldetorp B., Håkansson C.H. and v Mecklenburg C.
Scanning Electron Microscopy 2: 813-824, 1984.

CONTENTS

INTRODUCTION

The response of cilia to ionizing radiation is of great interest and importance in radiation therapy, as the distribution of the cilia is such that they must inevitably be irradiated during the treatment of tumours, whether they be situated in the brain, the respiratory system or in the genital tract. The side-effects of irradiation on the ciliated mucousal membranes may inhibit the patient's recovery - and it is therefore of utmost importance to clarify how radiation interferes with the normal activity of the cilia.

Experiments with cilia and irradiation were begun at an early date. The first to describe this interrelation was M. G. Bohn in Paris (1903). He had borrowed a small quantity of radium from Madame Curie and intended to carry out microscopic studies of the influence of radiation on the development of sea-urchin eggs. These eggs already have cilia in their blastula stage, and Bohn wrote: "Les movements ciliaires deviennent beaucoup plus intenses. . ." As a matter of fact, his finding was a stimulation of the ciliary activity that was presumably caused by the irradiation.

The stimulating effect of ionizing irradiation has since then been a matter of dispute. The acceleration of vital processes caused by irradiation is generally accepted and therefore "stimulation" would seem to be more a purely semantic question.

Radiobiologists have been searching for the true explanation of the effects after irradiation for decades, but experiments regarding the causes of events occuring in the cells during irradiation are conspicuously lacking in the research thus far conducted.

Baldetorp et coll. (1976) examined the beat frequency of the cilia from the rabbit's trachea during exposure to X-rays, and thus presented a new system of measuring the influence of the radiation on the physiology of cells. Not only was it now possible to detect early effects of irradiation, but even differences between various qualities of radiation could now be elucidated. This gave further information of value for common radiobiological concepts, e.g. that of the relative radiobiological effect (RBE), that is based on the effects after irradiation.

Baldetorp and Håkansson (1977) found that the ciliary beating was stimulated during exposure to radiation, with a tendency to follow a particular pattern that is rather uniform at different dose rates.

The course of events found was difficult to explain, although simultaneous morphological studies were done. The hypothesis was presented that they were due to membrane-related changes influencing the ATP-concentration. Further investigation was called upon to elucidate unclear aspects of these events in connection with the immediate biological effects of irradiation.

EXPERIMENTAL GOAL

The field of radiobiology is so extensive that it ranges from the radiolysis of water - the immediate interaction between radiation and matter, the course of which is under 10^{-12} seconds - to the interaction of radiation with living tissue that has been investigated during many years after administered irradiation. These latter investigation are limited solely to analysing the effects after the dose has been delivered.

It is first during recent years that the events during irradiation have been investigated using a system where the activity of ciliated cells is studied. This research can be described as radiophysiological; physiology and morphology are, however, intimately interwoven, so that the present investigation has found it desirable to co-evaluate observations from both disciplines using modern ultrastructural methods.

The present investigation is a continuation of previously started work on the effects of irradiation on tracheal ciliated cells, and can be outlined in the following points:

- Examination of the influence of temperature on the early effects of radiation (I).

- Recording of the intracellular activity during irradiation (II).

- Comparison between the immediate effects of photon and electron radiation (III).

- Investigation of the biological reactions to two consecutive irradiations (IV).

- Correlation between physiology and morphology during and after irradiation (V).

THE MORPHOLOGY AND PHYSIOLOGY OF THE CILIA

There are chiefly two types of motile systems in eucaryotic cells which are dependent on the hydrolysis of adenosine triphosphate (ATP). That system which is represented in the muscle cells, among others, is made up of the protein actin and its mechanochemical connection with the ATP-ase myosin. The other system is based on the protein tubulin and its ATP-ase dynein. Ciliated cells are typical examples of those based on the latter system.

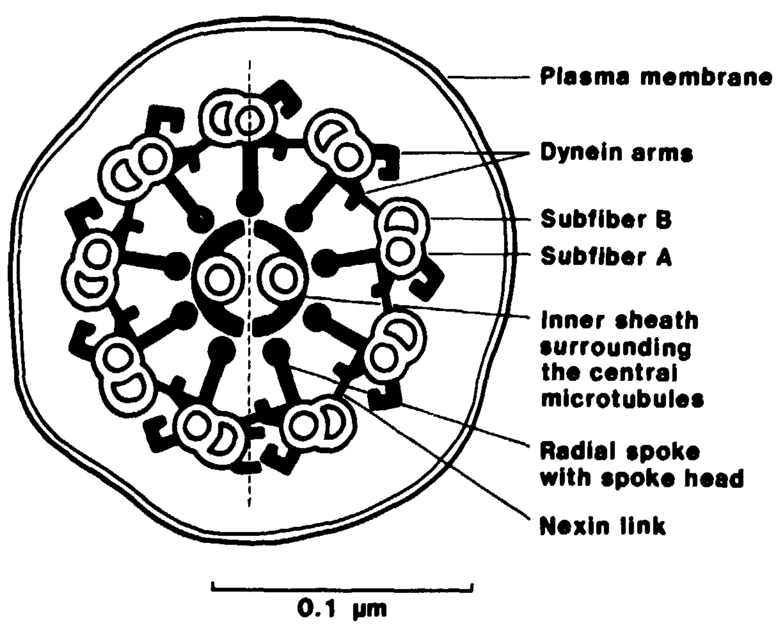

Figure 1. Cross-section of a cilium, viewed from the base to the tip. The dotted line defines the plane of the cilium's effective stroke. The outer doublets are designated clockwise with the numbers of 1 - 9 with respect to this line.

In order to demonstrate the structure and function of the cilia, a cross-section of a cilium as conceived today, seen from the above is shown in Figure 1. That part of the ciliary structure which lies within the ciliary membrane is called the axoneme and consists of a "9 + 2" pattern with nine outer doublets of microtubules and two single microtubules in the centre. Each outer doublet consists of a complete microtubule, subfiber A, and an imperfect subfiber B that is connected to it. Each subfiber A and the central pair of microtubules are made up of 13 protofilaments, while subfiber B consists of 10 - 11 protofilaments. The biochemistry and physiology of the microtubules have been described by Snyder and McIntosh (1976), among others.

The outer doublets are connected to each other by a protein called nexin (Stephens 1970, Linck 1973) . Two rows of arms (Afzelius 1959), dynein arms, extend from each subfiber A towards the subfiber B of the adjacent doublet. The outer doublets are numbered according to their position in relation to the central microtubules (Bradfield 1955, Afzelius 1959). Radial spokes extend from each subfiber A in towards the centre and have spoke heads on their ends that are associated with the central sheath, which in its turn surrounds the central pair of microtubules. The axoneme is surrounded by a double-structured plasma membrane which it has in common with the ciliary cell. The contents in a cilium is in direct contact with the cytoplasm of the cell via its basal body.

The cilia get energy for their movements from the hydrolysis of ATP (Gibbons 1965, Gibbons and Rowe 1965), which partly exists within the cilia themselves, and is partly synthesized from the abundantly occuring mitochondria in the apical part of the cell. It is believed that the ATP-ase activity is in the arms extending from subfiber A (Afzelius 1959, Gibbons 1963). The ATP-ase of the cilia is called dynein (Gibbons and Rowe 1965), and constitutes a part of these arms. Gibbons and Gibbons (1973) showed that dynein ATP-ase was involved in generating the energy for the strokes of the cilia, probably by some form of "cyclic cross-bridge mechanism" between the dynein arms and the microtubules. These arms interact with the doublet microtubules and generate ciliary beats by a sliding of adjacent doublets, substantiating the hypothesis of Afzelius (1959), which he based on experiments with sperm tails. This has later been verified by Satir (1965, 1968), Summers and Gibbons (1971) and Warner and Satir (1974).

The ultrastructural similarity between the cilia from the lower organisms and the cilia from the mammalian trachea, makes it quite likely that their motility is conducted in the same way. The sliding mechanism is now thought to be a cyclic course of detachment and attachment of the dynein arms to the microtubules that is governed by the hydrolysis of ATP (Summers and Gibbons 1971, 1973, Sale and Satir 1977, Warner and Mitchell 1978, Takahashi and Tonomura 1978, Zanetti et coll. 1979, Mitchell and Warner 1980, 1981 and Satir et coll. 1981).

The tracheal cilia are about 7 - 8 μm long and have the diameter of 0.2 - 0.3 μm. Each ciliary cell has about 250 - 300 cilia on its surface (Sanderson and Sleigh 1982). The ciliated epithelium consists of considerably more ciliated cells than the goblet cells. The cilia work in a layer of mucus that is a mixture of different kinds of secretions. Mucus is a viscoelastic, non-Newtonian liquid that consists of water, glucoproteins and other large protein complexes, to which oligo-sackarides are bound (Boat et coll. 1976). As early as 1934, Lucas and Douglas assumed that the mucus was divided in two layers, consisting of an upper mucousal layer covering a zone of mucus which is periciliary, serous and of low viscosity. This was later verified by Sadé et coll. (1970) and Sanderson and Sleigh (1981).

When the cilia perform active strokes, their apices reach up to the upper mucosal layer and move it while the recovery strokes takes place with the cilia bent down in the serous mucus. An effective transport of the mucus takes place, as the cilia work together in a coordinated metachronal course of action (Sanderson and Sleigh 1981). It is believed that the ciliary crown branching out from the ciliary apex, observed with transmission electron microscopy (TEM) by Dirksen and Satir (1972), and by Kuhn and Engleman (1978), can further assist in the transport of the mucus (Jeffrey and Reid 1977).

MATERIAL AND METHODS

This work is based on experiments performed on the trachea of the rabbit. A total of 194 healthy animals were used, weighing between 1.9 and 2.5 kg; they were thus full-grown and sexually mature. As no difference has ever been demonstrated between male and female animals as far as these experiments are concerned, the sexual distribution of the experimental material is not accounted for.

Tracheal specimens from 169 animals were irradiated with 10 Gy of photon energy and the tracheas from 10 other animals were irradiated with 5 Gy of electron energy, after which they were placed in an experimental chamber (I - IV). A further ten animals were irradiated (10 Gy) in vivo under anaesthesia (penthobarbital, 40 mg/kg) (V). In one of the investigations (II), five tracheas were used as controls.

When the trachea is removed from the animal's body, there is a sudden collapse of the preparation due to contraction of the connective tissue and the smooth muscles. The tracheal specimen must therefore be stretched to the length it had in the body of the animal prior to the excision. This manipulation causes physiological changes, so that an adaptation period in the experimental chamber was necessary. Conditions were stabilized within the 30 minutes chosen for this period, with quite good margins.

The studies consisted of recording the beat frequency of the cilia using a "light-beam" reflex method that has been common during the last twenty years. The ciliary surface of the trachea was illuminated with "cold light" and the ciliary movements were seen under the microscope as blinking reflexes. The microscope used for studying the preparations was equipped with a photodiode that transformed the light reflexes to electrical signals which were registered on the mingograph. This light beam reflex method enables registrations of the beat frequency while the tracheal preparation is in a horizontal position in the experimental chamber and is being irradiated with a vertical beam. The electrophysiological measurements performed with a glass capillary electrode mounted on a micromanipulator in accordance with accepted methods also permitted registration during the course of irradiation.

The ciliary beat frequency was measured each second in all of the preparations. The frequency was stable after the adaption process, and the average value of the measurements done 30 seconds immediately prior to irradiation in each animal was taken as a reference value. Each rabbit was thus its own control.

The results of these second-by-second measurements during irradiation were used for plotting the figures in all of the reports. The Department of Statistics at the University of Lund performed the statistical analysis of the curves.

Specimens of both irradiated and non-irradiated trachea were taken for both scanning electron microscopic (SEM) and transmission electron microscopic (TEM) studies. These specimens were taken immediately prior to and following dose administration. The procedure used in preparation of the tracheal samples was in accordance with accepted ultrastructural methods and is described in paper V. The studies were performed on modern instruments (Zeiss EM 10 and Zeiss Nanolab EM, and a Cambridge Stereoscan Mark II).

The animals irradiated in vivo were examined as follows: ten animals were irradiated under anaesthesia. On the following day, one of the animals was sacrificed and its trachea was placed in the experimental chamber, where the ciliary beats were registered and the morphological specimens were taken. This procedure was repeated during ten consecutive days.

Irradiation in vitro was performed using a Philips contact therapy unit in part of the material (I, II, IV and part of V) and in the other part linear accelerators (Siemens Mevatron 6 and Philips M.E.L. SL 75) for high-energy photon and electron radiation (III) were used. For in vivo irradiation, a conventional roentgen therapy unit with 160 kV acceleration was used (Siemens X-ray unit). As irradiation at different energies was used, it was not possible to avoid variations in the dose rate. The irradiative technique is described in detail in the respective reports.

Radiation Geometry: This has not been uniform in all of the experiments due to the radiation energy. The "build-up" effect of the radiations must be considered. For 50 kV roentgen radiation an absorption in the ciliary epithelium is calculated to 100 per cent (the mucus layer = 150 μm), when the beam is directly aimed at the lumen of the trachea. In order to get the available build-up when ^{60}Co and 6 MV photons were used, irradiation was performed beneath the bottom of the chamber. The irradiation thus struck the outside of the trachea before it struck its inner surface (the ciliary cells were irradiated from below). The irradiation had in these cases passed the lower part of the experimental chamber, which consisted of a perspex vessel containing water maintained at desired temperature, and the upper part of cotton wad soaked in Ringer's solution, before it reached the trachea. Thus, there was no extra build-up phenomenon in the pathway of the beam. The path of the irradiative beam within the chamber was 88 mm.

In vivo irradiation with 160 kV radiation was performed as described in paper V. In this case, the irradiation had to pass through the fur of the animal, its skin and connective tissue before reaching the trachea.

Irradiation with 4 MeV electrons was carried out directly towards the lumen of the trachea.

Dosimetry: In order to control the different radiation conditions, dosimetry was performed with thermoluminescent dosimeters (TLD) for the different qualities of irradiation. At in vitro irradiation, the dosimeters were placed in corresponding positions at the sides of and under the trachea, as well as in its lumen. The dosimetry of the in vivo irradiations was performed with ionizing chambers (V). The percentage of error in the dosimetric measurements including those in the dosimeters placed in the radiation source was approximated to be about 3.5 per cent.

RESULTS

Influence of temperature on the activity of ciliated cells during exposure to ionizing radiation (I).

Irradiation of the trachea at different temperatures (ranging from $20^{o}C$ to $40^{o}C$) showed a stimulation of the beat frequency at all levels. A dose-rate of 0.34 Gy/s was used, and in comparison with irradiations done afterwards with a lower dose-rate, the frequency pattern differs from that of these latter trials in that it follows a slowly heightened and successively reduced curve-like function not having any true "peak" value, as found in the experiments III, IV and V. The curves were the whole time above the reference level. It can be asserted that the integration of the surface covered by the function of this curves reflects the effective work of the cilia. It is therefore concluded that more work is done at $20^{o}C$ than at $40^{o}C$.

To interpret this finding, it must be assumed that the cilia need ATP in order to perform their work; more ATP is required at higher beat frequencies. The irradiation ought to have contributed the same amount of energy to the working system at all of the four temperatures used. Why, then, do the cilia beat faster at lower temperatures? It is only possible to present certain hypothetical explanations: 1. The mucus is more viscoelastic at the lower temperature, and as it is known that an increased burdening of the cilia gives - perhaps via signals from the ciliary crown (Dirksen and Satir 1972)- impulses to increase the work of the cilia, this together with stimulation from the irradiation may cause the increased beat frequency. 2. A higher availability of ATP due to the triggering of enzymes is another possibility (Ohyama et coll. 1974). 3. An increased hydrolysis of ATP in the pool already existant, by the radiation-induced enzyme release is a further possible explanation (Bacq and Alexander 1961). These experiments touch the field of radiobiochemistry, and therefore trials with ATP measurements during irradiation must be performed.

Intracellular recording of potential oscillations in ciliated cells exposed to ionizing radiation (II).

Ciliated cells have a membrane potential of 30 to 40 mV, inside negative. This potential fluctuates with an amplitude of about 1 mV and a frequency lying some few Hz above the ciliary beat frequency. Many studies consider this to be intemately connected to the mechanical activity of the cilia (Håkansson and Toremalm 1966, Yanaura et coll. 1979). In order to ascertain if it could be influenced by ionizing radiation, measurements of the intracellular potential oscillations were performed with glass capillary microelectrodes. The examinations were initiated prior to irradiation in order to get the reference value, and continued during the course of irradiation. No effect on the electrical activity could be demonstrated. The frequency was completely stable during the entire course of irradiation. The beat frequency, on the other hand, increased during this period in the usual manner.

The conclusion to be drawn from the results of this experiment must be that irradiation does not cause any effects via an eventual electrical initiation of the ciliary beats. It appears more likely that the irradiation directly affects the cilium itself. It must here be pointed out that the ciliary beat frequency has never during any of the experiments been higher than the frequency of the intracellular oscillations.

This is interpreted to imply that the cilia are caused to beat according to a constant biological impulse rate, they must work in surroundings that offer strong resistance. The cilia are thus possibly not able to respond to every impulse.

<u>A comparison between the immediate effects of photon and electron radiation on the ciliary activity of the rabbit's trachea (III)</u>.

A distinct pattern in the ciliary response could be observed when the trachea was irradiated with 6 MV photons and 4 MeV electrons. A stimulation effect was found, as at earlier radiation trials. The curves had in principle a similar course: first, a momentary increase to 5 - 10 per cent above the normal value, then a relative rapid increase up to about 20 per cent, followed by a decrease of the frequency increase.

There was, however, a difference between the curves: the maximal response to stimulation from electron radiation occured at an absorbed dose of 0.5 Gy, while it took place at 1.75 Gy during photon radiation.

There was also another difference between the curves: the declination phase during photon radiation was the whole time <u>above</u> the reference level, while at electron radiation it fell below the reference level already at an absorbed dose of 1.5 Gy.

The results of the trials described above show that electron radiation can be asserted to be three times as effective as photon radiation.

The effect of re-irradiation on the tracheal ciliary cell activity (IV).

As it was thought that two irradiations with a certain interval of time between them might give information on the consumption and eventual restitution of energy to the ciliary beats, two irradiations were performed on the same trachea with 10 Gy administered initially and after two hours had elapsed. Another trachea served as a control; it was stored two hours, after which it received initial irradiation. In each specimen where 10 Gy was given initially, the irradiation caused the same characteristic curve of stimulation (Figure in IV). None of the specimens given two irradiations showed the well-known "peak" response at the second irradiation.

As the beat frequency was continuously recorded in the two preparations, this lead to the discovery that when irradiation was stopped in those specimens given initial irradiation, there was a decrease of the ciliary beat frequency - greatest after 45 minutes following irradiation - by 20 per cent (Table in IV). The course of the curve then changed and there was an ensuing overshoot which reached 12 per cent immediately prior to the next irradiation. The pattern of the curve during the second irradiation indicated a reduction of the ciliary activity. At its lowest point this was 3.8 per cent above the normal level at an absorbed dose of 2.25 Gy, and towards the end of the irradiation period it slowly increased to 8.8 per cent, which was significantly above the reference level.

The effects of 10 Gy single-dose irradiation on the ciliated epithelium measured during and one-to-ten days following irradiation (V).

This paper accounts for a comparative physiological and morphological study. The ciliary beat frequency was measured during roentgen radiation (50 kV) and ^{60}Co radiation, and showed a rapid increase up to 22 - 25 per cent above the reference value after an absorbed dose of 1.3 - 1.5 Gy, followed by a gradual decrease during the remaining parts of these exposures (Figures 1 and 2, V).

The ciliary beat frequency of the area of the trachea irradiated in vivo was measured during ten consecutive days after the exposure and was related to the activity of a non-irradiated part of the same trachea. The beat frequency quotient was shown to be higher than 1.0 during at least three days after the irradiation. This was called the "stimulation" phase. During the following days, however, only parts of the mucous membrane exhibited ciliary motion, and the quotient decreased in the active areas to about 1.0 during days 4 - 8 (Figure 3, V). India ink applied to the mucous surface indicated a non-effective transport function during these days. This was regarded as a phase of radiation-induced "damage". During days 9 and 10 following irradiation, the mucociliary activity increased again, however, and the beat frequency in the irradiated part of the trachea showed the same values as those of the non-irradiated parts. The conclusion must be drawn that there was a "repair"-mechanism present.

Already 24 hours after irradiation, abnormal structural changes were observed in the form of blebs on the cilia, which could be seen both in the SEM- and the TEM-micrographs. These changes became even more apparent during the following days, and SEM studies showed a clear tendency toward progressive damage that was most pronounced during days 4 - 8 after irradiation. The specimens showed less and deleterious effects during the ensuing days, however, and on day 10 after exposure the tracheal cilia appeared to be completely restored.

In order to get a more certain estimate of the extent of this damage in relation to time, a scoring of the SEM-micrographs was done as to the deviations from normal appearance found on them. This scoring was done by four persons quite independently of one another. The average value of the scores given by these four persons was then plotted (Figure 9, V), the plotted graph showed a pattern that indicated a heavy process of damage around days 4 and 5 after irradiation. The concepts of "stimulation", "damage" and "repair" in their physiological implications were then included in Figure 9 (V), and their agreement was found to be mainly acceptable.

The diameter of the cilia was measured both on the TEM- and the SEM-micrographs. The value measured on the TEM-micrographs of a normal trachea was about 0.23 µm, which is the correct value, and it increased to 0.25 µm on day 1 after irradiation. The diameter was 0.19 µm on day 4, and on day 10 the cilia again showed their normal diameter of 0.23 µm. Statistical analysis showed that the differences were significant ($p < 0.002$).

The results found confirmed the description of the morphological process as it was described above.

DISCUSSION

All radiobiological effects on living objects are primarily to be found in the effect radiation has when it is absorbed in a cell or in a cellular system. It is, however, not certain that an immediate effect can be clearly measured. The heart does not change its frequency when the chest is submitted to radiation treatment; the EEG shows no change in the frequency pattern when the brain is irradiated. Radiation-induced changes can be detected first after a certain lapse of time (Garcia et coll. 1963, Garwicz et coll. 1975, Håkansson et coll. 1969). As the clinically interesting effects are naturally those to be observed after irradiation, there have only been a few reports published dealing with the effects on cellular systems during radiation treatment.

The ciliary cells are, from a radiobiological standpoint, a unique testing system, permitting studies with physiological methodics (i.e. the beat frequency), with electrophysiological methodics (intracellular potential measurements) and with biochemical methodics (ATP-measurements) at the same time as the ciliary epithelium is being irradiated. Ciliary cells are found in many parts of the body, and basic radiobiological research in this field therefore has clinical relevance, as it is nearly impossible to avoid the irradiation of cilia during the course of a radiation therapy.

Figure 2 illustrates how the physiological activity of the cilia (in the rabbit's trachea) in respect to different parameters of radiation physics has thus far been charted. Electron-microscopic studies have also been conducted in all of the trials in order to permit the detection of eventual changes occurring parallel to the physiological response.

It was found in all of the experiments that ionizing radiation stimulates the ciliary beat frequency, though not at re-irradiation. This phenomenon is no new discovery, having been described as early as 1903 by M. G. Bohn. It is, however, the charting of the morphology of the cilia (Afzelius 1959, 1961 a, b, Bradfield 1955, Dirksen and Satir 1972, Fawcett and Porter 1954, Gibbons 1960, 1961 a, b, Gibbons and Grimstone 1960, Kuhn and Engleman 1978, Manton 1952, Satir 1963, Watson and Hopkins 1962) and of their physiology (Dalhamn 1955, Frenckner and Richtner 1939, Goldhager and Back

1941, Håkansson and Toremalm 1965, 1966 a, b, 1967, 1968, 1971, Krueger and Smith 1957, Lucas and Douglas 1934, Mercke 1975, Proetz 1932, Reimer and Toremalm 1978) done during recent years that has enabled meaningful studies of the early radiobiological effects on the cellular level to be conducted.

The interpretation of the results is based on the assumption that the energy available to the cilia comes from adenosine triphosphate (ATP) (Gibbons 1965, Gibbons and Rowe 1965), and that the supply of ATP governs the ciliary beat frequency (Hoffman-Berling 1955, Brokaw 1961, 1975, Satir 1974).

It is of great interest for the entire field of radiation therapy to have knowledge of the effects of temperature, especially since hyperthermia has been introduced in the treatment of tumours. The investigations reported are in nearly all cases based on measurements at different temperatures after the administration of radiation. The results of these trials show that radiation lesions are greater when higher temperatures are applied on a tissue (Barth and Wachsmann 1948, Belli and Bonte 1963, Lindholm et coll. 1982, Martin and Caldwell 1922, Overgaard and Overgaard 1974). Hypothermic trials have mainly been conducted on cellular systems (Simpson 1916, Myers et coll. 1962, Myers and Sutherland 1962, Ohyama et coll. 1974). Early investigators have mainly found that low temperatures tend to promote cellular survival. It should be pointed out that the results treated here are from studies of the course also of events after irradiation.

Ohyama et coll. (1974) presented the hypothesis that the initial effect at 37^{o} C stimulated phosphofructokinase, resulting in an increased degradation of ATP, but the reaction of phosphofructokinase was considerably less at 25^{o} C, so that the cells had a normal supply of ATP and maintained their viability longer. "The initial lesion" discussed in their report is difficult to define, and this complicates applying their results to the present series of trials in order to see if an increased supply of ATP can occur at the lower temperature. An increased amount of ATP is possible at the lower temperature. Another question is whether or not ATP is equally susceptible to hydrolysis as it is at 37^{o} C. The surface that can be calculated from the curves using Simpson's formula (Figure 1, I) shows beyond doubt that the cilia perform more work at the lower temperature.

Other factors must also be considered. It is well-known that the ciliary beat frequency increases when the ciliary cells are subjected to increased mechanical loading. At lower temperatures (20^{0} C.) without irradiation there is a reduced ciliary beat frequency (Mercke et coll. 1974) This is not thought to be solely due to the usual reduction of physiological activity when temperature is reduced, but is also due to the fact that the cilia are beating in a viscoelastic medium, which further slows down the beat frequency at low temperature. This condition <u>during</u> irradiation is more difficult to comprehend. It must here be assumed that stimulation occurs. It can possibly reduce the mucous tenacity, the viscoelasticity of which is difficult to measure, as the layer of mucus covering the cilia is not a Newtonian liquid; the cilia can beat faster, but the effect can also be a direct effect on "sliding mechanism". Another possible reason can be the membrane effect on the mitochondria where the ATP-synthesis occurs (Baldetorp et coll. 1976). Further research is called for to shed light on this complex mechanism.

In an attempt to elucidate whether or not ionizing radiation causes changes in the electrical conditions of the ciliated cells, which eventually could contribute to an explanation of the increased beat frequency seen <u>during</u> irradiation, the membrane potential of the ciliated cells was examined simultaneously with the irradiation treatment (II).

The membrane potential of the rabbit's tracheal ciliated cell has previously been shown to be approximately -40 mV, and with a periodically fluctuating potential of 1 mV (Håkansson and Toremalm 1966 b). These earlier reports proposed an inter-connection between the oscillating signal, i.e. the so-called "pace maker" signal, and the ciliary beating, which has also been concluded by Yanura et coll. (1979).

The frequency of the potential oscillation was shown to be unaffected during the whole exposure of 10 Gy (Figure 2, II). The same pattern of the frequency change of the voltage oscillations was noted as in the untreated controls. This finding is not in agreement with the change in the beat frequency measured under the same conditions. The result presented may indicate that the ionizing irradiation interacts with other mechanisms involved in the ciliary motility machinery e.g. production of ATP in the mitochondria and diffusion of ATP from the cell up to the axoneme of the cilia.

These two mechanisms together with the biochemical activity involved in the ciliary motility determine the frequency of the ciliary beating (Brokaw 1966, Hoffman-Berling 1955). If the ionizing radiation thus stimulates one or all of these mechanisms, the beat frequency may increase.

The electrical conditions of the ciliated cells during irradiation were unchanged as far as the membrane potential was concerned. This is consistent with the findings of Bergeder (1958 a, b) and of Nachmannsohn (1957). They showed that high doses of ionizing radiation had to be administered to muscle and nerve cells before changes in their electrophysiology were detectable.

Previous investigations by Baldetorp and Håkansson (1977) showed that the beat frequency increase of the ciliated cells occurred nearly independently of the accumulated dose rates of photon irradiation 50 kV, 0.15 Gy/s and 0.05 Gy/s. In all of these experiments an absorbed dose of about 1.5 Gy was required before the maximal beat frequency appeared. To see if this biologic effect was influenced by the radiation energy, ciliated cells were irradiated with 6 MV photons (III). The dose rate was 0.07 Gy/s. This irradiation caused the same pattern of the ciliary reaction <u>during</u> the exposure as seen at low-energy irradiation.

From a physical point of view, the interaction between the radiation and the materia is not the same if 50 kV is compared to 6 MV. Photons below 20 keV interact mainly through photoabsorption, while high-energy photons interact via Compton effect and the pair-production process. The difference in energy did not see to be of any importance for the ciliary reactivity.

When the effects of 6 MV photons and 4 MeV electrons were compared (III), the physiological response of electrons was found to be greater than that of photons. 4 MeV electrons caused a maximal increase at 0.5 Gy, as compared to 1.5 - 1.75 Gy for photons. The decline of the electrons was also more rapid. This is not consistent with the common RBE-value. Therefore a new concept was introduced, the RPE (=relative physiological effeciency), and during the actual experimental conditions the value was stated for electrons/photons=3/1.

Cells exposed to 10 Gy photon irradiation showed weakened ciliary activity first after the treatment (IV). Such decline has also been observed by Fujiwara et coll. (1972), and was thought to be caused by disturbances of the ATP-production. Reduced ATP-production following irradiation has also been reported by Skog et coll. (1983) after the irradiation of Erlich ascites cells. The frequency variations after irradiation in relation to the reference level are also shown in the Figure (IV). It also appeared desirable to test a second irradiation in both the decline and the overshooting phases. The last period was first re-irradiated. No initial frequency increase was seen during this exposure, which was made two hours following initial irradiation. Tracheas kept as controls two hours showed the usual pattern of a 20 per cent frequency increase at initial irradiation.

The overshooting that occurs later on has not, as far as is known, been earlier described for photon radiation. Ogi (1959), however, described a similar phenomenon for electron radiation. This can be understood as an initiation of a resynthesis of ATP, but not to such a degree that a "peak increase" occurs at re-irradiation. These studies ought to be complemented in future with re-irradiation at the lowest period of the declination phase.

Electron microscopical studies during these periods of 10 Gy irradiation did not show any certain evidence of either stimulation or damage. Such evidence was, however, obtained in the trials where the rabbits were irradiated in vivo and examined 1 - 10 days after treatment (V). It was there possible to follow stimulatory effects in the form of multilobulated nuclei, visible during the first days following irradiation in the TEM-micrographs, and also to study signs of damage during the following days, which were most clearly seen in the SEM-micrographs, where degradation was greatest on days 4 - 8 after irradiation. Signs of repair were also noted during days 9 to 10. Four persons evaluated the micrographs, independently of one another, using a scoring system suitable for graphic presentation (Figure 9, V). The morphology was then compared with the results obtained from the measurements of the beat frequency. In this connection it is also seen that the changes in the ciliary beat frequency that occurred during the first two hours following irradiation (IV), where it was reduced by 20 per cent, were later amended by a rise to about 12 per cent above the reference level. It is possible that this rise reflects a resynthesis of the ATP having long duration, as a beat frequency of about 10 per cent above the reference level is recorded during the first three days following in vivo irradiation. Surfaces here and there were completely inactivated during the damage phase. A few other areas were intact but had reduced their beating frequency.

Radiophysical aspects

One of the most important concepts in the field of ionizing radiation is that of linear-energy-transfer (LET) (Zirkle 1954), which signifies the amount of energy per unit of length that the radiation beam deposits along its path. For electron radiation, which is directly ionizing, LET implies the amount of energy per unit of length deposited by the individual electrons, while for photon radiation LET indicates the value of the energy depositions made by the electrons that have been excited by the entering photons. LET is determined by the energy and the charge of the particles. The greater the charge and the lower the energy, the higher will LET be.

As the energy of roentgen radiation constitutes a spectrum, LET can vary over a large area, which implies that even a LET-concept using median values can be difficult to apply for comparing different irradiations with each other. The present investigation has used 50 kV and 160 kV roentgen radiation, ^{60}Co-gamma radiation (1.25 MeV), 6 MV photon radiation and 4 MeV electrons. The LET-values for the indirectly ionizing forms of radiation, as calculated on the basis of their track-average and absorbed dose-average, show much variance (ICRU Report No. 16, 1970). Despite this circumstance, the ciliary cells have an identical pattern of reaction during irradiation with the different qualities of indirectly ionizing radiation.

The 4 MeV electron radiation, which has the same LET-values as 6 MV photons, proved to be three times more effective for delivering the absorbed dose necessary to produce the maximal increase in the ciliary beat frequency. In radiation with higher LET, e.g. low-energy roentgen radiation, the ionizions take place very near each other. This causes a very high concentration of the free radicals $H^{\bullet}$ and $OH^{\bullet}$, but it is nonetheless not possible to get a higher degree of physiological effect using the higher LET at 50 kV roentgen radiation than is presented in the figure (V). A lower LET, e.g. 6 MV photon radiation, causes exactly the same ciliary response. According to HALL (1978), different radiations with the same LET can have different biological effects, so that electron radiation must have a special effect on the physiological activity of the ciliary cells, a fact that was seen in project (III) of this investigation.

The radiation from roentgen tubes and radionuclides is continuous as regards time, which is not always the case with high-energy electromagnetic and particle radiation generated in accelerators. By use of advanced electronic control-systems, the radiation is directed out in the form of "radiation packages" having an extremely high dose-rate and a certain durability. The desired rate of administered dose-per-second is decisive for how many "radiation packages" are to be gated per second.

The present study (III) has used 6 MV photon radiation and 4 MeV electron radiation generated in linear accelerators which release the radiation in a pulse-wise manner. The length of this pulse for the radiation qualities used in project (III) was 5 microseconds for both of the accelerators. The pulse frequency is 150 Hz. This gives an average dose rate of 0.07 and 0.05 Gy/s in trachea, respectively. The fact that photon radiation is distributed periodically does not change the ciliary response as compared to the response seen during continuous radiations. The pattern of reaction shown by ciliary cells is namely nearly identical for e.g. ^{60}Co and for 50 kV roentgen radiation (Figure 1 and 2, V). In these two latter cases, it is the absorbed dose of 1.5 Gy that stimulates the cilia to beat faster, despite the fact that the radiation qualities have different energies. The clear difference in the reaction of the cilia seen at electron radiation can thus not be explained by the radiation being distributed in pulsed form.

The difference can be due to the differing microdosimetric attributes of the types of radiation used, and possibly be due to release of the electricity carried by electron radiation. In this latter case, there may be some comparison with the response of excitable cells to electric current. It can possibly excite the ciliary membrane with increased potential fluctuation as a result, and thereby increase the beat frequency. It therefore appears necessary to carry out electrophysiological studies at electron radiation.

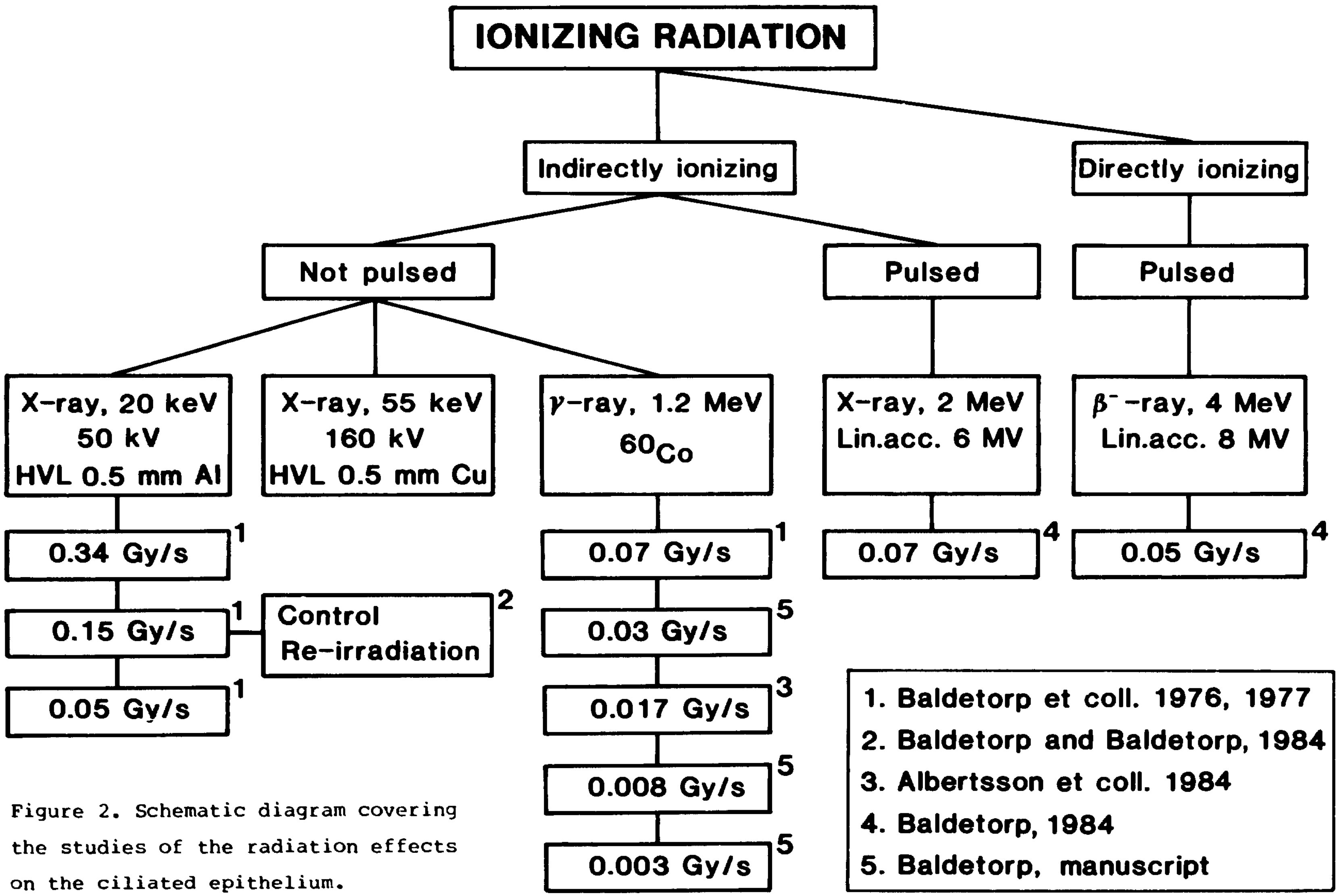

Figure 2. Schematic diagram covering the studies of the radiation effects on the ciliated epithelium.

SUMMARY

These studies on the effects of ionizing radiation on the ciliated epithelium of the rabbit's trachea have led to the following results:

The effect of temperature on cilia exposed to 50 kV roentgen radiation:

A decided stimulation of the beat frequency was seen during the entire course of irradiation at the temperatures investigated, i.e. at 20° C, 30° C, 37° C and 40° C. This effect was found to be greatest at 20° C. This finding raises the question of an eventual inactivation of phosphofructokinase at lower temperatures, or possibly of effects from other enzymes.

Intracellular potential activity compared with the beat frequency of the ciliary cells during irradiation:

Electrophysiological measurements of the fluctuations of the membrane potential were made during the irradiation of the ciliary cells. No significant changes in the electrical activity were observed. As the ciliary beat frequency was nonetheless stimulated at the dose-rate administered (0.15 Gy/s with 50 kV roentgen), it is concluded that a radiation dose of 10 Gy does not affect an eventual relation of potential fluctuation to the physiological effect.

Comparison of 4 MeV electrons with 6 MV photons during administration of radiation:

Both qualities of radiation (10 Gy) stimulate the ciliary beat frequency, but the effectivity of electron radiation was calculated as being three times greater than that of photon radiation. This led to the introduction of the concept "Relative Physiological Efficiency" (RPE), scored as 3 in this experiment.

Effect of re-irradiation on the ciliary beat frequency:

An initial irradiation (10 Gy) gave the usual stimulation pattern of a 20 per cent increase in the beat frequency. This had, however, been reduced to 20 per cent under the normal value at 45 minutes following irradiation, but then rose to about 12 per cent above the normal level at two hours after completion of irradiation. Re-irradiation with 10 Gy at that stage did not stimulate the beat frequency. Another tracheal sample that had been stored

in the experimental chamber two hours was given an initial irradiation (10 Gy) with the usual increase in the beat frequency as a result. If the stimulation during initial irradiation can be considered as an increased utilization of ATP, then the following decrease in the beat frequency must be attributed to a reduced supply of ATP, the later restitution of which causes the frequency to again rise above the normal level. The finding that re-irradiation has no stimulatory effect is interpreted as meaning that the cilia are already working at their maximal capacity after having received the additional new production of ATP.

In vivo irradiation and subsequent in vitro studies of the beat frequency and ultrastructure:

The upper part of the rabbit's trachea was irradiated with 160 kV roentgen (10 Gy) after the animal had been anaesthetized. The lower part of the trachea served as a control. Ten rabbits were irradiated, and on each of the following ten days the trachea of one animal was investigated. The irradiated part of the specimen showed an increased beat frequency during the first three days following irradiation, in comparison with the non-irradiated part. During days 4 - 8 there was reduced mucociliary activity that agreed well with the ultrastructural signs of damage observed. The tracheal mucus was restored to its normal state days 9 - 10. There were three distinct physiological and morphological phases reflecting the course of events occurring in the beat frequency and the ultrastructure: a stimulation phase, a damage phase and a repair phase, all taking place during the ten days ensuing after an irradiation with a single dose of 10 Gy.

ACKNOWLEDGEMENTS

I wish to express my sincere gratitude to

Dick Killander, head of the Department of Oncology, for a great deal good advices and constructive criticism, for his interest and never ending support during this study.

Carl-Håkan Håkansson, my teacher, who introduced me into the field of radiobiological science, for invaluable scientific training and generous support, for stimulating discussions throughout the study and for his warm friendship.

Lars Baldetorp, my brother, for valuable criticism and support, for interesting discussions and an excellent team-work.

Claes von Mecklenburg, who gave valuable contribution to the interpretation of the ultrastructural pictures and for interesting discussions.

Maria Albertsson, for ambitious performance and collaboration in one of the papers.

Marianne Palmegren, for very skilful and excellent technical assistance.

Ulla-Britta Nordberg, Inger-Lena Lamm and Per Nilsson,for help with radiophysical details.

Börje Blad, for his support in driving the linear accelerators.

Mårten Fernö and Åke Borg, for their interest and support during this time.

Gertrud Andersson, Eva Henriksson and Mona Martinsson, for excellent secreterial and technical assistance.

Kenneth Muir, for the linguistic revision.

This work was supported by grants from Konung Gustaf V:s Jubileumsfond,

B. Kamprad's Foundation, John and Augusta Persson's Foundation for Medical Scientific Research and from the Medical Faculty, University of Lund.

REFERENCES

AFZELIUS B. A.: Electron microscopy of the sperm tail: Results obtained with a new fixative. J. Biophys. Biochem. Cytol. 5 (1959), 269.

AFZELIUS B. A.: The fine structure of cilia from ctenophore swimming-plates. J. Biophys. Biochem. Cytology 9 (1961 a), 383.

AFZELIUS B. A.: Some problems of ciliary structure and function. In: Biological Structure and Function (T. W. Goodwin and O. Lindberg eds.). Acad. Press, N.Y., Vol. II (1961 b), 5 7.

BACQ Z. M. and ALEXANDER P.: Fundamentals of Radiobiology. Pergamon Press, N.Y. (1961), 263.

BALDETORP L., HUBERMAN D., HÅKANSSON C. H. and TOREMALM N. G.: Effects of ionizing radiation on the activity of the ciliated epithelium of the trachea. Acta radiol. Ther. Phys. Biol. 15 (1976), 225.

BALDETORP L. and HÅKANSSON C. H.: Response of ciliated cells during irradiation at different dose rates. Acta radiol. Ther. Phys. Biol. 16 (1977), 378.

BARTH G. and WACHSMANN F.: Über den Einfluss der Temperatur auf die Hautreaktion bei Röntgenbestrahlungen. Strahlentherapie 77 (1948), 87.

BERGEDER H. D.: Potentialmessungen am röntgenbestrahlten Muskel. Naturwissenschaften 45 (1958 a), 43.

BERGEDER H. D.: Kaliumverluste von Kaltblutermuskeln nach Röntgenbestrahlung. Naturwissenschaften 45 (1958 b), 61.

BELLI J. A. and BONTE F. J.: Influence of temperature on the radiation response of mammalian cells in tissue culture. Radiat. Res. 18 (1963), 272.

BOAT T. F., CHENG P. W., IVER R. N., CARLSON D. M. and POLONY I.: Human respiratory tract secretions. Arch. Biochem. Biophys. 177 (1976), 95.

BOHN M. G.: Influence des rayons du radium sur les oeufs vierges et fécondés, et sur les premiers stades du développement. C. R. des S. de L'ACAD. T. CXXXVI (1903), 1085.

BRADFIELD J. R. G.: Fibre patterns in animal flagella and cilia. Symp. Soc. Exp. Biol. 9 (1955), 306.

BROKAW C. J.: Movement and nucleoside polyphosphatase activity of isolated flagella from Polytoma uvella. Exp. Cell Res. 22 (1961), 151.

BROKAW C. J.: Mechanics and energetics of cilia. The Am. Rev. of Resp. Dis. 93 (1966), 32.

BROKAW C. J.: Effects of viscosity and ATP concentration on the movement of reactivated sea-urchin sperm flagella. J. Exp. Biol. 62 (1975), 701.

DALHAMN T.: A method for determination in vivo of the rate of ciliary beat and mucous flow in the trachea. Acta Physiol. Scand. 33 (1955), 1.

DIRKSEN E. R. and SATIR P.: Ciliary activity in the mouse oviduct as studied by transmission and scanning electron microscopy. Tissue and Cell 4 (1972), 389.

FAWCETT D. W. and PORTER K. R.: A study of the fine structure of the ciliated epithelia. J. Morphol. 94 (1954), 221.

FRENCKNER P. and RICHTNER N. G.: A method for study and filming of ciliary activity among animals and human beings. Acta Oto-laryng. 27 (1939), 668.

FUJIWARA K., HÅKANSSON C. H. and TOREMALM N. G.: Influence of ionizing radiation on the ciliary cell activity in the respiratory tract. Acta radiol. Ther. Phys. Biol. 11 (1972), 513.

GARCIA J., BUCHWALD N. A., BACH-Y-RITA G., FEDER B. H. and KOELLING R. A.: Electroencephalographic responses to ionizing radiation. Science (Wash.) 140 (1963), 289.

GARWICZ S., ARONSON A. S., ELMQVIST D. and LANDBERG T.: Postirradiation syndrom and EEG findings in children with acute lymphoblastic leukaemia. Acta Paediatr. Scand. 64 (1975), 399.

GIBBONS I. R. and GRIMSTONE A. V.: On flagellar structure in certain flagellates. J. Biophys. Biochem. Cytol. 7 (1960), 697.

GIBBONS I. R.: Observations on the structure of cilia and flagella. Proc. Eur. Reg. Conf. on Electron Microscopy, Delft 2 (1960), 929.

GIBBONS I. R.: The relationship between fine structure and beat in the gill cilia of a lamellibranch mollusc. J. Biophys. Biochem. Cytology 11 (1961 a), 179.

GIBBONS I. R.: Structural assymmetry in cilia and flagella. Nature 190 (1961 b), 1128.

GIBBONS I . R.: Studies on the protein components of cilia from Tetrahymena pyriformis. Proc. Natl. Acad. Sci. USA. 50 (1963), 1002.

GIBBONS I. R.: Chemical dissection of cilia. Arch. Biol. (Liège) 76 (1965), 317.

GIBBONS I. R. and ROWE A. J.: Dynein: A protein with adenosine triphosphatase activity from cilia. Science (Wash.) 149 (1965), 424.

GIBBONS B. H. and GIBBONS I. R.: The effect of partial extraction of dynein arms on the movement of reactivated sea-urchin sperm. J. Cell. Sci. 13 (1973), 337.

GOLDHAGER G. and BACK A.: Studies on radiosensitivity of animal cell in vitro. I. Radiosensitivity of muscular and ciliary movement. Proc. Soc. Exp. Biol. Med. 48 (1941), 150.

HALL E. J.: Radiobiology for the Radiologist. Second edition, Harper and Row, London 1978.

HOFFMAN-BERLING H.: Geisselmodelle und Adenosintriphosphate (ATP). Biochem. Biophys. Acta 16 (1955), 146.

HÅKANSSON C. H. and TOREMALM N. G.: Studies on the physiology of the trachea. I. Ciliary activity indirectly recorded by a new "light beam reflex" method. Ann. Otol. 74 (1965), 954.

HÅKANSSON C. H. and TOREMALM N. G.: Studies on the physiology of the trachea. II. Electrical potential gradients within the tracheal wall. Ann. Otol. 75 (1966 a), 33.

HÅKANSSON C. H. and TOREMALM N. G.: Studies on the physiology of the trachea. III. Electrical activity of the ciliary cell layer. Ann. Otol. 75 (1966 b), 1007.

HÅKANSSON C. H. and TOREMALM N. G.: Studies on the physiology of the trachea. IV. Electrical and mechanical activity of the smooth muscles. Ann. Otol. 76 (1967), 873.

HÅKANSSON C. H. and TOREMALM N. G.: Studies on the physiology of the trachea. V. Histology and mechanical activity of the smooth muscles. Ann. Otol. 77 (1968), 255.

HÅKANSSON C. H., LINDGREN M. and SULG I. A.: EEG effects of postoperative irradiation treatment of brain tumours. Acta radiol. Ther. Phys. Biol. 8 (1969), 301.

HÅKANSSON C. H. and TOREMALM N. G.: Ciliary activity recorded by TV- monitor and phototube. Acta Oto-laryng. 71 (1971), 508.

ICRU Report 16: Linear Energy Transfer. ICRU Washington, D. C. (1970).

JEFFREY P. K. and REID L. M.: The respiratory mucous membrane. In Braine, J. D., Proctor, D. F. and Reid L. M. (eds): "Respiratory Defense Mechanisms". N. Y.: Marcel Dekker, Pt. 1 (1977), 181.

KRUEGER A. P. and SMITH R. F.: Effects of air ions on isolated rabbit trachea. Proc. Soc. Exp. Biol. Med. 96 (1957), 807.

KUHN C. and ENGLEMAN W.: The structure of the tips of mammalian respiratory cilia. Cell. Tiss. Res. 186 (1978), 491.

LINCK R. W.: Chemical and structural differences between cilia and flagella from the lamellibranch mollusc, Aequipecten Irradians. J. Cell Sci. 12 (1973), 951.

LINDHOLM C.-E., KJELLEN E., LANDBERG T., NILSSON P. and PERSSON B.: Microwave-induced hyperthermia and ionizing radiation. Preliminary clinical results. Acta Radiol. Oncol. 21 (1982), 241.

LUCAS A. M. and DOUGLAS L. C.: Principles underlying ciliary activity in the respiratory tract. II. A comparison of nasal clearance in man, monkey and other animals. Arch. Otolaryng. 20 (1934), 518.

MANTON I.: The fine structure of plant cilia. Symp. Soc. Exp. Biol. 6 (1952), 306.

MARTIN C. L. and CALDWELL G. T.: The relation of temperature changes to roentgen-ray skin reactions. Amer. J. Roentgenol. 9 (1922), 152.

MERCKE U., HÅKANSSON C. H. and TOREMALM N. G.: The influence of temperature on mucociliary activity. Temperature range 20 ^{o}C - 40 ^{o}C. Acta Otolaryng. 78 (1974), 444.

MERCKE U.: The influence of varying air humidity on mucociliary activity. Acta Otolaryng. 79 (1975), 133.

MITCHELL D. R. and WARNER F. D.: Interactions of dynein arms with B subfibers of Tetrahymena cilia: Quantitation of the effects of magnesium and adenosine triphosphate. J. Cell. Biol. 87 (1980), 84.

MITCHELL D. R. and WARNER F. D.: Binding of dynein 21S ATP-ase to microtubules. J. Biol. Chem. 256 (1981), 12535.

MYERS D. K. and SUTHERLAND R. M.: Effect of temperature on the radiosensitivity of rat thymocytes. Can. J. Biochem. Physiol. 40 (1962), 413.

MYERS D. K., DEWOLFE D. E. and ARAKI K.: Effects of X-radiation and of metabolic inhibitors on rat thymocytes in vitro. Can. J. Biochem. Physiol. 40 (1962), 1535.

NACHMANSOHN D.: Nerve function and irradiation effects. III[e] Congr. Intern. Neuropath. Bruxelles (1957), 195.

OGI D.: Studies on the cellular effects by radiations: I. Effects on the cilia movements of beta-ray irradiation of human and rabbit ciliar epithelial cells in vitro. Nippon Acta radiol. 19 (1959), 819.

OVERGAARD K. and OVERGAARD J.: Radiation sensitizing effect of heat. Acta radiol. Ther. Phys. Biol. 13 (1974), 501.

OHYAMA H., YAMADA T. and INOUE Y.: Effects of post-irradiation temperature on viability of rat thymocytes. Int. J. Radiat. Biol. 26 (1974), 535.

PROETZ A. W.: Motion picture demonstration of ciliary action and other factors of nasal physiology. Trans. Am. Laryng. Assoc. 54 (1932), 264.

REIMER Å. and TOREMALM N. G.: The mucociliary activity of the upper respiratory tract. II. A method for in vivo studies on maxillary sinus mucosa of animals and human beings. Acta Otolaryng. 86 (1978), 283.

SADE J., ELIEZER N., SILBERBERG A. and NEVO A. C.: The role of mucus in transport by cilia. Am. Rew. Resp. Dis. 102 (1970), 48.

SALE W. S. and SATIR P.: Direction of active sliding of microtubules in Tetrahymena cilia. Proc. Natl. Acad. Sci. USA. 74 (1977), 2045.

SANDERSON M. J. and SLEIGH M. A.: Ciliary activity of cultured rabbit tracheal epithelium: Beat pattern and metachrony. J. Cell. Sci. 41 (1981), 331.

SANDERSON M. J. and SLEIGH M. A.: The function of respiratory tract cilia. In: The Lung and Its Environment. Plenum Publishing Corporation N. Y., (1982).

SATIR P.: Studies on cilia. The fixation of the metachronal wave. J. Cell. Biol. 18 (1963), 345.

SATIR P.: Structure and function in cilia and flagella: Facts and problems. Protoplasmatologia III. E. (1965), 1.

SATIR P.: Studies on cilia. III. Further studies on the cilium tip and a "sliding filament" model of ciliary motility. J. Cell. Biol. 39 (1968), 77.

SATIR P.: How cilia move. Sci. Amer. 231 (1974), 45.

SATIR P., WAIS-STEIDER J., LEBDUSKA S., NASR A. and AVOLIO J.: The mechanochemical cycle of the dynein arm. Cell Motil. 1 (1981), 303.

SIMPSON C. A.: The sensitizing effect of cold on roentgenized surfaces. Amer. J. Roentgenol. 3 (1916), 485.

SKOG S., TRIBUKAIT B. and SUNDIUS G.: Energy metabolism and ATP turnover time during the cell cycle in roentgen irradiated Ehrlich ascites tumour cells. Acta Radiol. Oncol. 22 (1983), 369.

SNYDER J. A. and McINTOSH J. R.: Biochemistry and physiology of microtubules. Ann. Rew. Biochem. 45 (1976), 699.

STEPHENS R. E.: Isolation of nexin - the linkage protein responsible for maintenance of the nine-fold configuration of flagellar axonemes. Biol. Bull. 139 (1970), 438.

SUMMERS K. E. and GIBBONS I. R.: Adenosine triphosphate-induced sliding of tubules in trypsintreated flagella of sea-urchin sperm. Proc. Natl. Acad. Sci. USA. 68 (1971), 3092.

SUMMERS K. E. and GIBBONS I. R.: Effects of trypsin digestion on flagellar structures and their relationship to motility. J. Cell. Biol. 58 (1973), 618.

TAKAHASHI M. and TONOMURA Y.: Binding of 30S dynein with the B-tubule of the outer doublet of axonemes from Tetrahymena pyriformis and adenosine triphosphate-induced dissociation of the complex. J. Biochem. (Tokyo) 84 (1978), 1339.

WARNER F. D. and SATIR P.: The structural basis of ciliary bend formation. Radial spoke positional changes accompanying microtubule sliding. J. Cell. Biol. 63 (1974), 35.

WARNER F. D. and MITCHELL D. R.: Structural conformation of ciliary dynein arms and the generation of sliding forces in Tetrahymena cilia. J. Cell. Biol. 76 (1978), 261.

WATSON M. R. and HOPKINS J. M.: Isolated cilia from Tetrahymena pyriformis. Exp. Cell Res. 28 (1962), 280.

YANAURA S., IMAMURA N., HOSOKAWA T., TAKEDA H. and MISAWA M.: Drug effects on electrical activities of the canine tracheal ciliated cells. J. Pharm. Dyn. 2 (1979), 266.

ZANETTI N. C., WARNER F. D. and MITCHELL D. R.: Effects of divalent cations on dynein crossbridging and ciliary microtubule sliding. J. Cell. Biol. 80 (1979), 573.

ZIRKLE R. E.: The radiobiological importance of linear energy transfer. In Hollaender A (edt): Radiation Biology Vol. 1. N. Y., McGraw-Hill. (1954), 315.