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EFFECTS OF THERAPEUTIC PROTON DOSES ON HEALTHY ORGANS IN THE NECK, CHEST AND UPPER ABDOMEN OF THE RABBIT

by

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Experimental and clinical investigations on the effect of therapeutic proton doses have been mainly concerned with malignant growth and healthy tissues in the pelvis minor (NAESLUND et coll. 1959, FALKMER et coll. 1962, FORS et coll. 1964, DANIELSSON et coll. 1968) and in the head (GRAFFMAN, HUGOSSON et coll. 1967, GRAFFMAN, JUNG et coll. 1967). The single dose used in the investigation now reported, 3 000 rad, or its fractionated equivalent, seems to represent an upper limit for doses applicable in tumour therapy. At this dose level, the proton radiation seems to affect the primary tumour and its metastases in a way similar to other sparsely ionizing radiation in therapeutic use. The situation in the healthy tissues is less clear in the above-mentioned reports but the protons seem generally to have caused little damage. This agrees with the results of early experiments on rabbit ears (NAESLUND et coll. 1958) and, in particular, recent investigations by STÉNSON (1969) who found that the ratio between roentgen and proton doses creating histologically equivalent damage in the rat rectum, was in the range 0.6—0.7.

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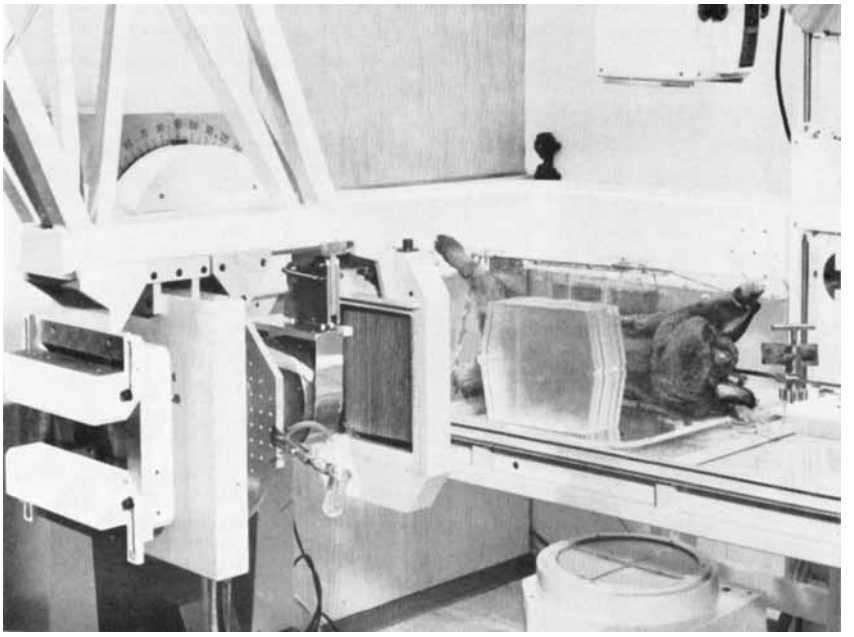


Fig. 1. Set-up for irradiation of the stomach. The collimated 185 MeV proton beam passes through a 3 cm × 4 cm aperture, a parallel-plate ionization chamber, a ridge filter and a Lucite absorber. The plateau of the depth-dose curve contained the thickness of the animal.

The present report concerns screening for possible untoward effects of proton radiation on healthy tissues in organs not previously investigated. To permit comparison with previous work a single dose of 3 000 rad was chosen. It was given to the neck, chest, or upper abdomen, and particular consideration was given to the effects on the oesophagus, trachea, lungs and stomach. In a following paper the problem of fractionation, in the case of lung irradiation, will be considered separately (ENGFELDT et coll.).

Material and Methods

Irradiation was performed with the external 185 MeV proton beam from the 230 cm synchrocyclotron under the general conditions described by LARSSON (1961). The absorbed dose was in all cases 3 000 rad and single. It was determined with parallel-plate ionization chambers at an estimated uncertainty of less than ± 5 %. The fluency homogeneity was in the range ± 5 %.

Three separate experiments were performed, each on nine rabbits. The period

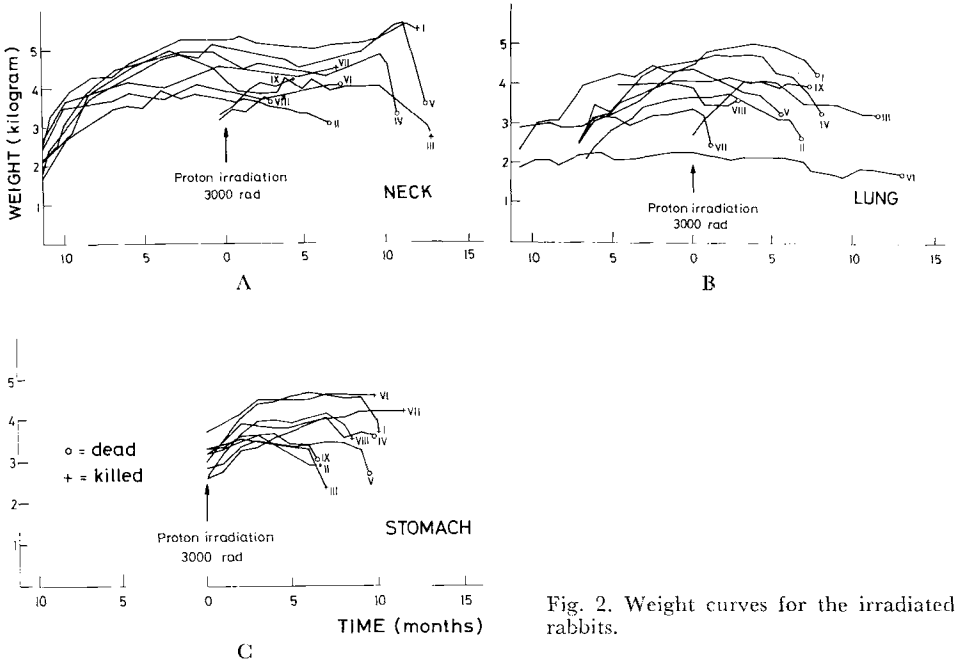


Fig. 2. Weight curves for the irradiated rabbits.

during which the animals were kept at the animal cages before irradiation is seen from the weight diagrams in Fig. 2.

Trachea and oesophagus. Rabbits A-I to A-IX were used for this experiment. The rabbits were anaesthetized with 20 % nembutal solution (Abbot, 1 ml/kg body weight) injected into an aural vein through a polythene cateter (size 50). The catheter was kept in situ throughout the treatment so that, when necessary, additional injection could easily be given. The rabbit was strapped in the supine position to a Lucite stand, which was then placed with the frontal plane of the rabbit at right angles to the axis of the beam and with the front of the animal facing the incoming beam. The stand with the rabbit lay with its longitudinal axis horizontal, so that the radiation field of 4 cm × 6 cm covered the neck, its longest side being perpendicular to the longitudinal axis of the animal. A ridge filter (LARSSON 1961) and absorbers were placed in the beam so that a 5 cm plateau of the depth-dose curve contained the thickness of the neck. The dose rate in the plateau varied between 50 and 100 rad/minute.

The rabbits were weighed once weekly and their general condition was observed. White blood cell and haemoglobin determinations were made before and after the treatment.

Lungs. In rabbits B-I to B-IX the entire right lung was irradiated. The position of the lung was determined by a roentgen technique ensuring that the radiation field, which measured 4 cm × 6 cm, covered the right half of the chest. The radiation technique was in other respects the same as described above.

In the course of the investigation the animals were kept under observation and weighed approximately once weekly. White blood cell and haemoglobin determinations were made both before and after irradiation.

Stomach. Rabbits C-I to C-IX were all irradiated by a field of radiation, 3 cm × 4 cm, so arranged that the center of the beam met the animal 0.5 cm to the left of the midline and 1.5 cm below the base of the lung (Fig. 1). For this group Lucite absorbers and the ridge filter described by KARLSSON (1964) were used, so arranged that the thickness of the animal was contained in the plateau region. The dose rate in the plateau varied between 50 and 100 rad/minute. A photographic film irradiated in front of the Lucite plates on the side facing the incoming beam showed a pattern of regular stripes after development, while a film between the absorber and the rabbit was homogeneously darkened. The estimation was made that the relative dose variation in the animal due to the structure of the filter was in the range ± 5 %. These rabbits were usually not anaesthetized but they nevertheless lay still during irradiation, as was ascertained by television.

The general condition of the rabbits was observed and recorded in the course of the investigation, and their weights were registered once weekly.

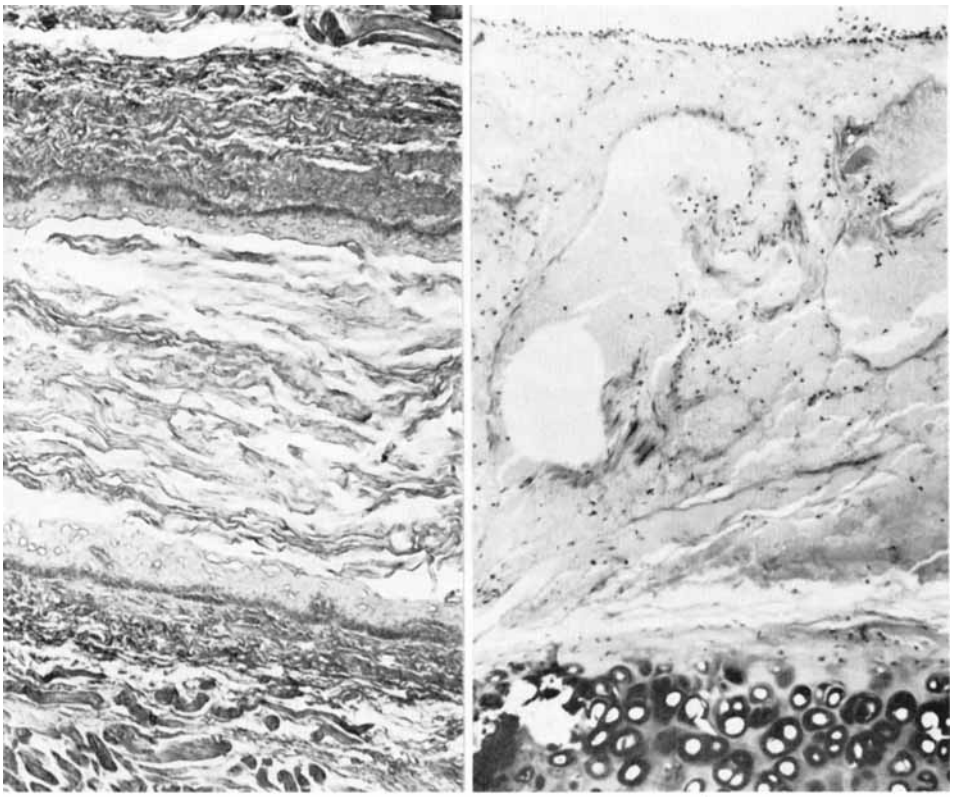
Results

The experimental observations will now be reported separately for the three experiments.

Trachea and oesophagus. Seven of these rabbits (A-I to A-VII which were in the stable one year before irradiation had normal weight increase during this time. After irradiation, the increase was only negligible, or the weight remained unchanged, except in three cases (A-III, A-IV, and A-V) in which the weight decreased considerably just before death (in bilateral bronchopneumonia). The general condition of the animals is thus well reflected in the weight diagram (Fig. 2).

Animals A-VIII and A-IX on the other hand, which had been bought immediately before irradiation, increased distinctly in weight during the first few months after treatment. Their weights then remained more or less unchanged.

More or less severe epilation was seen on the front of the neck 1 to 2 months after irradiation, and in two rabbits slight dermatitis. On the dorsal side of the



a b

Fig. 3. Sections from the neck organs in rabbit A-I about one year after irradiation. a) Oesophagus. b) Trachea.

neck, where the proton beam had emerged, almost complete epilation and dermatitis were seen in all cases, and in seven animals ulceration that did not heal for several months.

One rabbit, A-III, displayed disturbed balance and deflection of the head to one side a few months after irradiation, and this condition persisted for some time. This rabbit had severe scurf on both ears.

Blood tests before proton irradiation gave the following values: haemoglobin 62—70 %, mean value 67 %; white blood cells 3 900—7 660/mm^3, mean value 6 200/mm^3 with 28 % mononuclear cells. Two months after irradiation somewhat higher values were obtained: haemoglobin 60—74 %, mean value 72 %; white blood cells 7 900—13 700/mm^{-3}, mean value 8 700/mm^{-3}, with 32 % mononuclear cells.

The period of observation and the time of autopsy are seen in Fig. 2. Autopsy

revealed bilateral bronchopneumonic infiltration in A-VIII (2 1/2 months) and in A-VII (7 months) and also in four rabbits, A-I, A-III, A-IV and A-V, who died or were killed approximately one year after irradiation. No obvious changes were observed on macroscopic examination of the neck organs at autopsy.

Microscopic examination of the oesophagus showed nothing pathologic in any of the animals (Fig. 3a). In microscopic preparations from the trachea, oedema and dilated blood-filled vessels and also round-cell infiltration in the mucosa were seen in A-I to A-VII (Fig. 3b). In A-II, parts of the mucosa were also eroded, and towards the lumen some detritus masses were seen. In A-V, even the regions surrounding the trachea displayed oedema and lymphocytic infiltration. In A-IX, who was killed after five months, the trachea showed further changes in the form of poor basophilia, with a suggestion of necrosis of the cartilage, and also desquamated mucosal epithelium, which was partly eroded. In A-VIII, who died in bronchopneumonia 2 1/2 months after irradiation, the changes in the tracheal mucosa were even more accentuated, with haemorrhages and necrosis in superficial regions and also profuse inflammatory cell infiltration. In addition, the cartilage had in parts lost its stainability and showed signs of degeneration in small areas.

Lungs. One rabbit died 26 days before irradiation and autopsy revealed bilateral bronchopneumonia. This rabbit was replaced by another (B-IX). The general condition of the irradiated rabbits is reflected in the weight diagram (Fig. 2). Rabbits B-I to B-VIII who had been observed for a long period before irradiation mostly had little weight increase or reduction during a long period after irradiation. The general condition deteriorated shortly before death, when also a dramatic weight decrease was seen. The weight curve for rabbit B-IX was different; the weight of this animal increased by 50 % during the three months immediately following treatment.

In all animals, epilation and slight dermatitis were observed on the right ventral side of the chest between one and two months after the irradiation. No ulceration occurred and the skin conditions regressed gradually. On the right side of the dorsal region considerably more severe changes, with complete epilation and dermatitis, and, in six cases, ulceration, were observed.

The period of observation and the time of autopsy are given in Fig. 2. At autopsy, oedema and emphysema in both lungs were present in B-I and B-IV, who died 7 1/2 months after irradiation. In addition, there was regressively changed cartilage in the right lung of B-I. No inflammatory infiltration was observed in these rabbits. The remaining rabbits, in addition to signs of pulmonary oedema and emphysema, had bronchopneumonic infiltration. In B-IX, dead seven months after irradiation, such infiltration occurred only in the right

lung where pleuritis and leucocytic exudate in the bronchi were also seen. In this rabbit nothing abnormal, apart from oedema, was seen in the left lung. The remaining rabbits had pneumonic changes in both lungs, fibrosis and degeneration, and fusion of the alveoli. The changes were in the main similar in both lungs but in two rabbits (B-VI and B-VIII) the inflammatory infiltration was more marked in the right lung. Profuse inflammatory exudate was also seen in the bronchi. In B-III, pleuritic changes were present in both lungs although they were more extensive on the right side.

Stomach. The general condition showed good agreement with the weight curves. These rabbits had a fairly constant weight increase during the first two months after irradiation (Fig. 2) and with the exception of C-II, which had an upper respiratory infection during the third month, the weight continued to increase slightly even later. During the period immediately before death the weight suddenly decreased except in C-VI and C-VII who were killed ten months after irradiation. The animals had a good general condition during the main observation period and deteriorated only immediately before death. Two rabbits (C-III and C-IX) showed signs of weakness of the hind limbs during their last few weeks.

During the second month, all animals exhibited epilation on the irradiated skin area. It was less severe ventrally than on the back where the proton beam had emerged. There the epilation was generally complete and in C-I, C-V and C-VII ulceration had occurred. The period of observation and the time of autopsy are seen in Fig. 2. All rabbits were still alive six months after irradiation. During the seventh month C-IX died and C-II and C-III were killed. The remaining rabbits lived until the ninth to eleventh months when two died and four were killed.

In C-II with an upper respiratory infection, autopsy revealed right-sided pleural empyema and an abscess in the right lung. In the left lung moderately extensive infiltration and slight oedema were observed. In the liver, marked degeneration of the parenchyma was seen, with extreme stasis. In histologic sections from the stomach the mucosa was seen to be of normal height and there were no definite changes. In C-III and C-IX, who had had paralysis of the hind limbs and who were killed or died after seven months, autopsy revealed oedema in the lungs and fusion of the alveoli and inflammatory exudate in the bronchi. The gastric mucosa was of normal height in both cases, with no definite changes. In C-V, who died during the tenth month, autopsy revealed an abscess in the thoracic cavity, and microscopic examination of the lungs showed oedema and, in places, inflammatory infiltration. In C-VI also, who was killed after ten months, extensive broncho-pneumonic lesions were seen, which in some places

were undergoing organization. Sections from these two rabbits showed a gastric mucosa of normal architectonics and no pathologic changes. In C-I, C-IV, C-VII and C-VIII essentially the same autopsy findings were made. There were oedema and emphysema in the lungs and, in places, fibrosis and hyaline areas. Sections from these animals showed microscopically a gastric mucosa of normal architectonics and height.

Discussion

The results indicate that a single proton dose of 3 000 rad did not cause severe damage of the inner organs studied, i.e. the trachea, oesophagus, lungs and stomach. Most animals were in a good condition throughout the period of observation and their weights either increased or remained at a high level. Unfortunately, a relatively high incidence of bronchopneumonia was observed in all three series. It appears, however, that the site of these changes does not necessarily conform with the irradiated lung region. Thus, at autopsy, six animals were found to have bilateral pneumonia after irradiation of the neck region or the stomach. However, one rabbit in the lung group died in bilateral bronchopneumonia some time before irradiation.

After proton irradiation of the neck, no or only moderate pathologic changes were seen in the irradiated region. Macro- and microscopic examination of the oesophagus disclosed nothing abnormal in any rabbit. In the trachea, although no macroscopic changes were found, the microscopic examination revealed oedema and dilated vessels and sometimes round-cell infiltrations in the mucosa. There was also in one rabbit a desquamated and partly eroded mucosal epithelium, poor basophilia and a suggestion of necrosis. This damage was not directly attributed to the effect of irradiation.

In most lung-irradiated rabbits more or less marked bronchopneumonic infiltration and inflammatory exudate in the bronchi were observed. Approximately similar changes were present in both lungs in all these rabbits, except one. In this latter rabbit who died seven months after irradiation, autopsy revealed, in the right lung, bronchopneumonic infiltration, oedema, pleuritis and leucocytic exudate in the bronchi, while oedema was present only in the left lung. This localization of the inflammatory changes to the right lung may conceivably have been connected with the proton irradiation seven months previously. Autopsy in two rabbits showed only oedema and emphysema. In one of them, regressive cartilaginous changes were seen in some of the bronchi of the right lung. This seemed to be the only marked alteration in the lung caused by irradiation.

After irradiation of the stomach, no macroscopic or microscopic changes were

observed at the time of examination. The gastric mucosa was of normal height and of normal architectonics.

It may be concluded that no pathologic changes in the oesophagus, trachea, lung or stomach could be safely ascribed to the radiation treatment, and no constant signs of a decreased general condition of the rabbit were noted throughout the main observation period. The findings are in conformity with previous observations after abdominal irradiation (NAESLUND et coll. 1959, DANIELSSON et coll. 1968).

SUMMARY

Proton irradiation of the neck region, the lung, or stomach in the rabbit, with 3 000 rad in a single dose, did not cause any obvious pathologic changes.

ZUSAMMENFASSUNG

Protonen-Bestrahlung der Nackenregion, der Lunge oder des Magens des Kaninchens mit 3 000 rad in einer Dosis führte zu keinen augenscheinlichen pathologischen Veränderungen.

RÉSUMÉ

L'irradiation par des protons à une dose unique de 3 000 rad sur la région cervicale, le poumon ou l'estomac du lapin, n'entraîne aucune modification pathologique évidente.

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