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CELL MIGRATION FOLLOWING IRRADIATION OF THE SKIN IN MICE

Effect of shielding minute areas

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In the acute radiation reaction of skin it is well known that a hyperplasia may develop in the irradiated skin along the border of a sharply defined field. It has generally been assumed that the hyperplasia is due to migration of non-irradiated epidermal cells into the irradiated epithelium (GLÜCKSMANN 1951, VON ESSEN 1969). Following experiments with shielding of narrow strips of epithelium, migration was questioned by DEVIK (1957), mainly because it would have to occur in the epithelium without loss of cells. Experiments in general pathology have indicated that complete loss of epithelium is a prerequisite for migration of epithelial cells. DEVIK concluded that the cells injured by radiation received some factor, or factors, from the shielded cells of importance to recovery, without specifying whether it might be diffusible substances, or parts of cells.

In 1969, BARENDSEN in a discussion on the genesis of malignant cells suggested that irradiated cells with defective genomes might fuse, or that a defective cell might be restored by taking up part of the genetic material from another cell.

The present experiments were designed as an attempt to detect the origin of the cells responsible for the border hyperplasia. To provide data for analysis, narrow

Submitted for publication 8 November 1976.

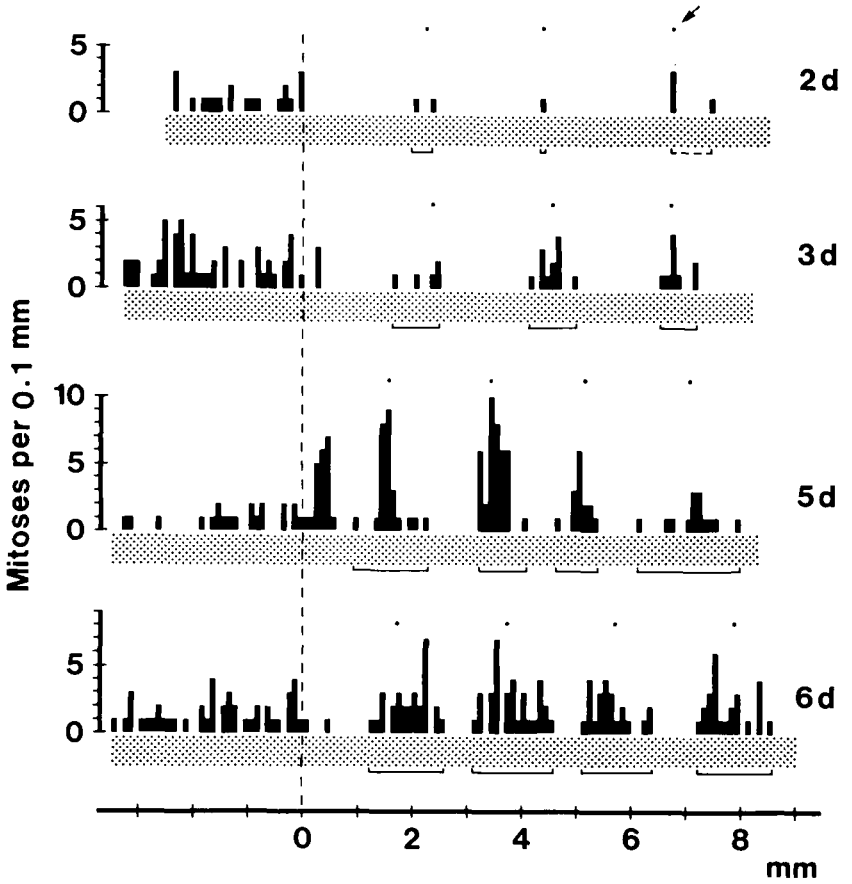


Fig. 1. Semi-diagrammatic illustration of the spreading of mitoses in the epidermis from the parts shielded by 0.08 mm tungsten wires, spaced 2 mm apart. The cross-section of the wires is drawn to scale, and their position is indicated above the skin. (During the irradiation with 10 800 R they were in contact with the epidermis.) To the left of the vertical broken line, the non-irradiated skin. Mitoses were recorded for each 0.1 mm along the skin. In the irradiated part, the mitoses appear in clusters around the shielded strips.

strips of skin were shielded during irradiation, and the location and number of mitoses in and around the shielded cells were recorded at several intervals until the acute reaction became marked.

Material and Methods

Female mice of the hairless strain (rh, rh), 2 to 3 months old, were used. During Nembutal anesthesia (0.04 ml of a 5% solution per 20 g mouse subcutaneously) a flap of the skin on the back was temporarily hooked on to a frame above the mouse in a geometrically well-defined position, and a field of 9 mm × 14 mm of double skin

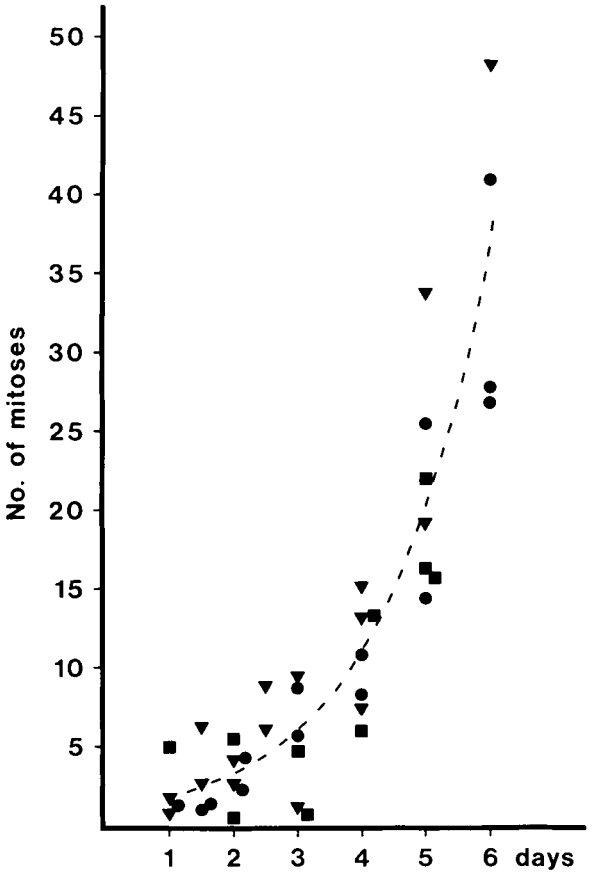


Fig. 2. Number of mitoses recorded under and close to one shielded area (corresponding to the brackets below the skin in Fig. 1), as a function of time. The exposure was 10 800 R. Each point represents the average score from one mouse. The different symbols refer to mice with 80 μm wires spaced 1 (▼), 2 (●) or 3 (■) mm apart, respectively.

above the body of the mouse was irradiated. The thickness of the double skin was 0.5 to 0.7 mm. The arrangement assured a good immobilization of the flap during irradiation. The blood circulation of the flap was not affected during irradiation, as estimated by the natural pink colour, without cyanosis, nor paleness.

The mouse holder was placed in a fixed position in front of the roentgen tube with a beryllium window of a Dermamobil 100 unit. With unfiltered radiation, 15 kV, 25 mA, an exposure rate of 5 400 R/min was measured at the site of the flap, 75 mm from the anode.

An additional frame with thin tungsten wires of 0.08 mm diameter across the opening provided shielding of narrow strips, 1, 2 or 3 mm apart. For identification of the irradiated area the corners were marked permanently with Indian ink.

At 50 kV irradiation, as was used in the experiments of 1957, the amount of scattered radiation and its penetration are such that the shielded cells would be injured at higher doses. At 15 kV irradiation the scattered radiation has a very short range, and at an exposure of 10 800 R satisfactory shielding was obtained with gold wires

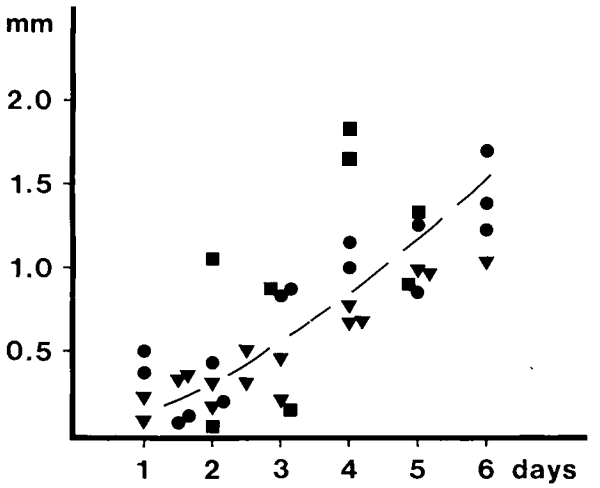


Fig. 3. The distance between the mitoses furthest apart in the cluster around the shielded area, as a function of time. Same symbols as in Fig. 2.

of only 0.04 mm diameter. However, such thin wires are difficult to handle, and for practical mechanical reasons tungsten wire of 0.08 mm diameter was therefore used.

Unfiltered 15 kV radiation has little penetration. The depth dose in tissue at 14 kV, 10 cm SSD and a field area of 2 to 20 cm^2 has been calculated by JENNINGS (1972) as follows: 78 % at 0.2 mm, 61 % at 0.4 mm, and 40 % at 0.8 mm.

After a number of preliminary experiments 98 mice were used. The exposure was 10 800 R to 65 mice, of which 43 had 0.08 mm wires located 1, 2 or 3 mm from each other across the field of irradiation. Thirty-three mice received 2 700 R. The mice were killed by neck luxation 1, 1 1/2, 2, 2 1/2, 3, 4, 5 or 6 days after irradiation.

Three and a half hours before killing, the mice were given an intraperitoneal injection of 0.15 mg Colcemid. After neck luxation the skin was stretched on thick paper and fixed in Bouin's fluid, and after embedding in Histowax, sections were cut across the shielded strips and stained with haematoxylin and with eosin or azophloxin-safranin. Mitoses were counted and recorded for each 100 μm along the skin.

At the beginning, counting of labelled cells after injection of tritiated thymidine was performed but was discontinued as it was found to give less information than colcemid-arrested mitoses.

Results

A semidiagrammatic illustration of the distribution of mitoses in the epidermis and its appendages in skin sections taken on four different days appears in Fig. 1. In each section the number of mitoses (indicated by the brackets in the figure) was recorded, and the average for each animal is plotted in Fig. 2. This figure shows that the variations between the individual values were considerable, but a clear trend exists; thus the exponential curve with a doubling time of 1.15 days seems well-grounded.

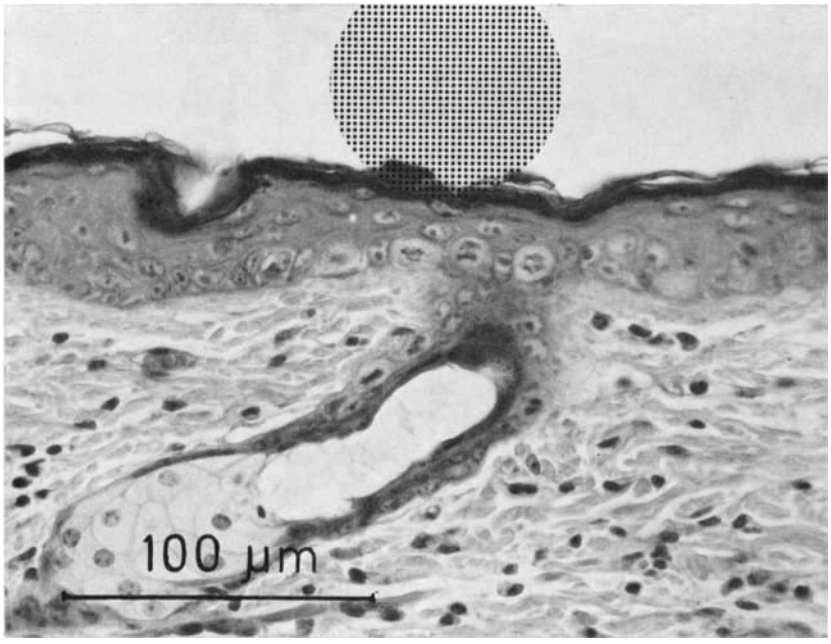


Fig. 4. Section of skin 36 hours after exposure to 10 800 R. Shielding by 80 μm tungsten wires one mm apart. The size of the cross-section of one wire is indicated above four normal-looking mitoses.

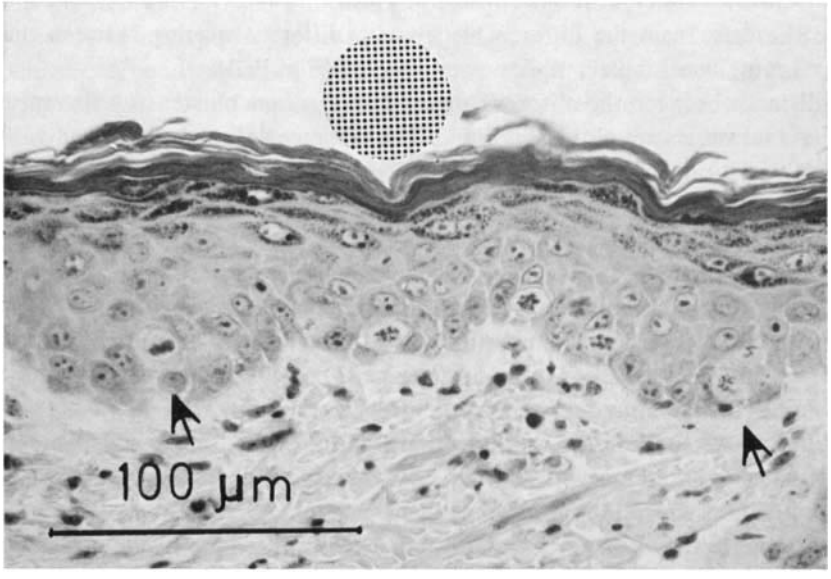


Fig. 5. Section of skin 3 days after exposure to 10 800 R. Shielding by 40 μm gold wires one mm apart. The size of the cross-section of one wire is indicated above the epidermis. Two arrows point to two mitoses about 180 μm apart.

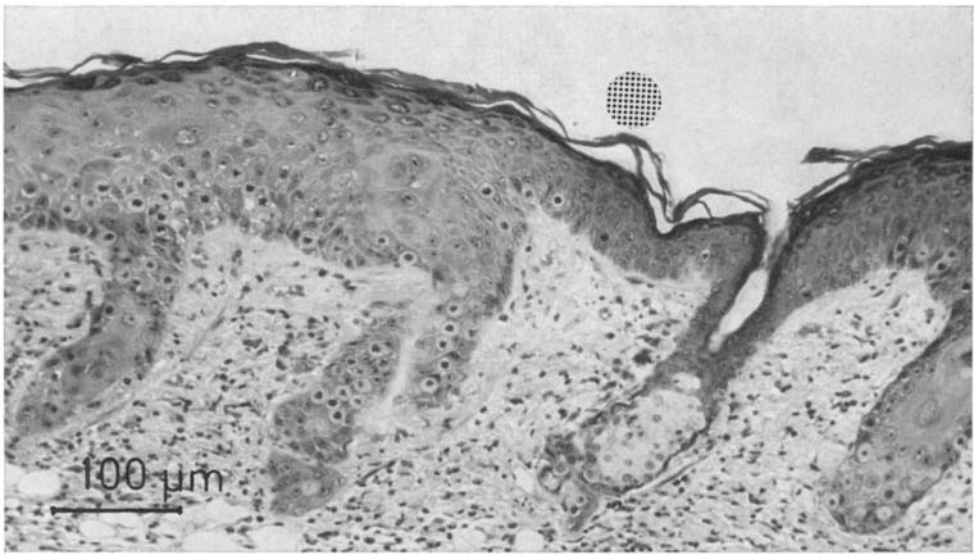


Fig. 6. Section of skin 6 days after exposure to 10 800 R. Shielding by 40 μm gold wires one mm apart. The size of the cross-section of one wire is indicated above a remaining sebaceous gland; the latter indicates that the skin was shielded at this site. Numerous mitoses in the basal layer of the epidermis, and in the hypertrophic remnants of sebaceous glands and hair sheaths extending downwards in the dermis.

Since the data from the different series with different spacing between the wires (1, 2 or 3 mm) were similar, all data were pooled (Figs 2, 3).

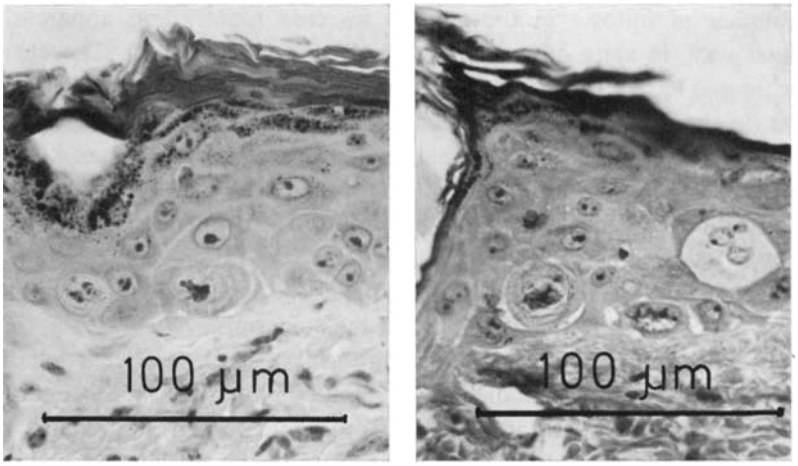
The distance between the mitoses furthest apart in each cluster was also measured; the individual values are plotted in Fig. 3. The distance definitely increased with time, but great variations existed. The increase appeared to be about 1/3 mm per day.

The skin of 22 mice was exposed to 10 800 R without any wire-shielding. In this material normal mitoses or clusters of mitoses were not observed in any section taken from one to 6 days after irradiation.

The results from the smaller series of experiments exposed with 2 700 R are not documented. However, they were similar to those with 10 800 R, except that the number of mitoses seemed to have a somewhat longer doubling time, 1.4 days.

The mitoses were found in the basal layer of the epidermis (Figs 4 to 6). The epithelium above the mitoses was hyperplastic, and many of the cells were swollen and edematous, but there was little or no evidence of decrease in number of cells in the epithelium where the mitoses occurred.

The mitoses in the clusters looked normal at photomicrography. This is in contrast to the occasional mitoses found in the irradiated epidermis; they were enlarged, often poorly stained, and frequently rather irregular with grossly visible chromosome fragmentation, bridging and clumping (Fig. 7).



a b

Fig. 7. Abnormal mitoses in irradiated epidermis, a) 3 days, and b) 4 days after exposure to 10 800 R.

Discussion

Mitoses accumulated over several hours due to the stathmokinetic effect of Colcemid provide good visual indicators of cell proliferation (Figs 4 to 6).

The clusters of mitoses in the irradiated field (Fig. 1) develop from the shielded strips, which can be identified in the section after about 4 days. Irradiated sebaceous glands then disappear, and remaining glands indicate the site of the strips. Furthermore, accumulations of mitoses are found at intervals equal to the intervals between the wires.

An exposure of 10 800 R means a sterilizing dose, which certainly causes an extended mitotic depression. In the hairless mouse, the number of epidermal cells in an area measuring 0.9 cm × 1.4 cm is of the order of 10^7. The high radiation dose used could hardly leave any cell capable of proliferation, assuming a D_0 of 1.35 Gy (135 rad) (BARENDSEN, WITHERS 1967). Even if the 15 kV depth-dose curve falls steeply, the mouse epidermis is so thin that it may be disregarded.

Cell divisions corresponding to the shielded parts are observed in the sections taken one day and one day and a half after irradiation; they look normal. Heavily irradiated epidermal cells cannot produce normal-looking mitoses at this time, and if and when mitoses later appear, they are grossly abnormal (Fig. 7 a and b). Therefore, it is concluded that the mitoses in the clusters are derived from the shielded cells. The observations do not exclude the possibility that injured cells may fuse with genetic material from other cells, but this hypothesis is not necessary to explain the results.

The number of mitoses in the clusters increase rapidly and apparently in an exponential way, in spite of great individual variations (Fig. 2). The curve has a doubling time of 1.15 days. This is a short generation time compared to the normal 3.5 to 4 days (in the hairless mouse). But it is well known that cell cycling may be accelerated when required, and in the hairless mouse times as short as 8 hours have been found in the regenerating epidermis (DEVIK 1962).

The new mitoses are located in the basal layer of the irradiated epidermis, and they migrate well below the surface of the epidermis, before any increased cell loss is visible. Migration of epidermal cells along the basal membrane is therefore demonstrated without a break in the continuity of the epidermis.

The rate of migration is not rapid during the first six days after irradiation. The line drawn in Fig. 3 indicates an increase of about 1/3 mm per day. Since this includes both sides of the shielded strip it corresponds to a rate of migration of about 1/6 mm per day on each side, possibly increasing towards the end of the observation period. From the classical work of CARREL & HARTMANN (1916) on cicatrization of wounds it may be inferred that epithelialization of a wound in man proceeds about 0.5 mm per day. HELL & CRUICKSHANK (1963) found the same rate of migration in wounds in guinea-pigs.

The question arises how cell proliferation is induced without cell loss. One possibility is that the irradiation decreases the chalone production of the differentiated cells (IVERSEN 1973). Experiments to throw light on this problem are in progress.

Most of the cells in the area examined had received a very high dose of radiation. The narrow shielded fraction of cells seemed to be unaffected despite the close proximity to the radiation-injured cells, as evidenced by their rapid start of proliferation. This suggests that irradiated cells on the whole do not contain, or at least not produce, many substances of direct toxic effect to other cells, at least not before cell degeneration and cell death begin.

Acknowledgements

The author is indebted to Miss Linda Kvalsund and Mrs Vivi Svejkovsky for technical assistance and to Mr Finn Welde and Mr Steinar Backe for the exposure measurements. Thanks are also due to Philips Norsk A/S, who kindly placed thin tungsten wires at the author's disposal.

SUMMARY

In hairless mice, dorsal skin flaps were exposed to 15 kV roentgen radiation. Thin metal wires were stretched across the field. At eight different intervals, up to 6 days, and following Colcemid injection, the number and location of mitoses in the epidermis were recorded. The regeneration observed in the irradiated parts of the epidermis seems to be due to migration of cells from shielded areas, the rate during the first 6 days being about 1/6 mm per day. Migration takes place along the basal membrane of the epidermis before any increased loss of cells in superficial layers has occurred.

ZUSAMMENFASSUNG

Bei haarlosen Mäusen wurden Hautlappen des Rückens mit 15 kV Röntgen bestrahlt. Dünne Metalldrähte wurden über das Feld ausgebreitet. Zu 8 verschiedenen Zeitpunkten bis zu 6 Tagen und im Anschluss an eine Colcemidinjektion wurden die Zahl und die Lokalisation der Mitosen in der Epidermis festgestellt. Die Regeneration, die in den bestrahlten Teilen der Epidermis beobachtet wurde, scheint darauf zu beruhen, dass Zellen aus den geschützten Abschnitten auswandern mit einer Geschwindigkeit von etwa 1/6 mm pro Tag während den ersten 6 Tage. Die Wanderung geschieht längs des Basalmembrans der Epidermis bevor ein gesteigerter Verlust von Zellen in den oberflächlichen Schichten auftritt.

RÉSUMÉ

Sur des souris sans poils des lambeaux cutanés dorsaux ont été exposés à l'irradiation de rayons roentgen de 15 kV. De fins fils métalliques ont été tendus en travers du champ. Le nombre et la localisation des mitoses dans l'épiderme ont été notés à huit intervalles différents, jusqu'à 6 jours, et après injection de Colcemid. La régénération observée dans les parties irradiées de l'épiderme semble être dûe à la migration de cellules à partir des aires protégées, la vitesse de régénération pendant les premiers 6 jours étant d'environ 1/6 mm par jour. La migration a lieu le long de la membrane basale de l'épiderme avant que se soit produite une perte de cellules dans les couches superficielles.

REFERENCES

BARENDSEN G. W.: Tumour induction in rat skin by 300-kV X-rays and 15-MeV neutrons, *In*: Radiation-induced cancer, p. 413. IAEA, Vienna 1969.

CARREL A. and HARTMANN A.: Cicatrization of wounds. I. The relation between the size of a wound and the rate of its cicatrization. J. exp. Med. 24 (1916), 429.

DEVIK F.: Modification of the X-ray reaction in the skin of mice by shielding of minute areas of the skin. *In*: Advances in radiobiology, p. 226. Oliver and Boyd, London 1957.

— Studies on the duration of DNA-synthesis and mitosis in irradiated and regenerating epidermis cells in mice, by means of tritium-labelled thymidine. Int. J. Radiat. Biol. 5 (1962), 59.

VON ESSEN C. F.: Radiation tolerance of the skin. Acta radiol. Ther. Phys. Biol. 8 (1969), 311.

GLÜCKSMANN A.: The histogenesis of radiation-induced and of benzpyrene-induced epidermal tumours in the mouse. J. Path. Bact. 63 (1951), 176.

HELL E. A. and CRUICKSHANK C. N. D.: The effect of injury upon the uptake of ^{3}H-thymidine by guinea-pig epidermis. Exp. Cell Res. 31 (1963), 128.

IVERSEN O. H.: Summary from chalone conference. *In*: Chalones: Concepts and current researches. Edited by J. Houck. J. nat. Cancer Inst. Monogr. 38 (1973), 225.

JENNINGS W. A.: X-rays: Half-value thickness range 0.01–8 mm of Al. (approx. 6–150 kVp). Brit. J. Radiol. (1972) Suppl. No. 11, p. 1.

WITHERS H. R.: The effect of oxygen and anaesthesia on radiosensitivity in vivo of epithelial cells of mouse skin. Brit. J. Radiol. 40 (1967), 335.