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GRENZ RAY THERAPY IN DERMATOLOGY. AN EXPERIMENTAL, CLINICAL AND EPIDEMIOLOGICAL STUDY.

By

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To

Christina, Ellinor and Hillevi

This thesis is based on the following papers, which will be referred to by their Roman numerals.

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Photodermatology 2:367-371, 1985.

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ABBREVIATIONS AND DEFINITIONS

ACD - Allergic contact dermatitis

$D_{\frac{1}{2}}$ - The half value depth. Is the depth in tissue at which the dose is 50% of the surface dose.

Gy - Gray = Absorbed dose of ionizing radiation. 1 Gy = 1 Joule/kg.

HVL - Half value layer. Is defined as that thickness of a given filter material (in dermatology usually aluminium) which reduces the intensity of the ray beam to 50% of the original incident radiation.

LC - Langerhans' cell/cells.

R - The R is a measure of ionizing in air. "The quantity of X- or gamma radiation such that the associated corpuscular emission per 0.001293 g of air produces, in air, ions carrying one electrostatic unit of quantity of electricity of either sign".

rad - The rad is the unit of the absorbed dose in tissue. One rad is equivalent to the absorption of 0.01 Joule of energy per kg of absorbing material. This corresponds to 0.01 Gy (1 Gy=100 rad).

SDT - Serial dilution patch test.

SLS - Sodium lauryl sulphate.

ABSTRACT

Grenz rays (ultrasoft X-rays, Bucky rays) have been used in the treatment of benign skin disorders for more than 60 years. The mechanism of action, the clinical effect, and the potential carcinogenic effect have been mainly unknown, and many of the reported studies are obsolete today. In the present work these concepts have been studied by means of immuno-histological methods, transmission electron microscopy, patch testing, clinical evaluation and epidemiological methods. The results can be summarized as follows.

The number of Langerhans' cells (OKT-6 positive cells) decreases in human epidermis after grenz ray radiation and this reflects a true disappearance of the Langerhans' cells as shown by electron microscopy.

Grenz ray treatment was found to
- suppress the expression of nickel allergy in sensitive individuals.
- have a tendency to suppress irritant skin reactions.
- have a very good effect on psoriasis of the scalp.

Grenz ray therapy cannot be excluded as a risk factor in the development of non-melanoma skin tumors, but this risk factor is small, if any, when certain therapy recommendations are followed.

Keywords: Ionizing radiation, X-rays, allergic contact dermatitis, Langerhans´ cell, electron microscopy, psoriasis, cancer.

INTRODUCTION

Technically produced electromagnetic radiation has been successfully used in dermatological therapy since the beginning of this century. Initially ultraviolet radiation was favoured, but during the 1920´s ultrasoft X-rays (synonyms: grenz rays, Bucky rays) were developed and gained considerable appreciation owing to obvious success in treating a variety of skin conditions. With an increasing awareness of the risk associated with X-rays, grenz ray treatment was considered by many to be obsolete without any clear-cut scientific foundations for the hesitation involved. The present work was undertaken to find reasons for the beneficial effects of grenz rays, to investigate clinical effects and to elucidate the risk involved in this type of therapy.

ULTRAVIOLET RADIATION

The foundations of modern day ultraviolet phototherapy began with the work of Niels Finsen who was awarded the Nobel Prize for Medicine in 1903 for his successful treatment of tuberculosis of the skin. Following this pioneering work, the beneficial and adverse effects of non-ionizing radiation in the treatment of certain skin diseases have been investigated and phototherapy has expanded rapidly. Several treatment systems involving UV radiation have been used. The most commonly used are: 1) Goeckerman regimen (Goeckerman, 1925) which involves exposure to UV radiation and application of crude coal tar to the skin. 2) UVB phototherapy, 3) PUVA therapy (Parrish et al, 1974) which involves a photosensitizer, 8-methoxypsoralen, followed by exposure to UVA radiation. The long-term side effects of these treatments are mainly skin ageing and the production of cutaneous carcinomas (Forbes, 1982).

GRENZ RAY THERAPY

1. Historical aspects

In parallel with ultraviolet radiation, ionizing radiation has played an important role in the treatment of skin diseases since Röntgen described his X-rays in 1885. The effects of radiation on the skin became apparent quickly and radiation-induced dermatitis, epilation and pigmentation first led to recognition of the biologic effects of these rays. Some workers tried soft X-rays early in an effort to prevent severe sequelae but the idea of using soft X-rays in treating skin conditions lay dormant until 1923 when Gustav Bucky succeeded in devising an apparatus which produced ultrasoft X-rays (Bucky, 1929). He used a hot-cathode vacuum tube with a Lindemann window and obtained X-rays in clinically usable amounts at tensions as low as 5 or 6 kV. Because he believed that the biologic effects of these very soft rays resembled those of conventional X-rays in some ways and those of ultraviolet rays in others, Bucky called the new rays grenz rays. The name "grenz" means "border" in German. Since then many dermatologists have used these rays. The equipment has been modernized, particularly the tubes with mica-beryllium windows, which permit very precise radiation.

Over the last few decades, ionizing radiation therapy has played an increasingly smaller role in dermatologic practice. This decrease in the use of X-ray radiation therapy is probably due to the introduction of PUVA and more effective topical and systemic drugs, particularly corticosteroids. An increasing public awareness of the biologic effects of ionizing radiation and its potential hazards may also be of importance (Johnsson, 1986).

2. The nature of grenz rays

Grenz rays are very soft X-rays. They are closely related to those of conventional X-rays but the penetration is considerably smaller. The physical effects produced by both grenz rays and conventional X-rays are qualitatively identical, but differ considerably as to degree.

Both grenz rays and conventional X-rays are part of the vast range of electromagnetic radiation which reaches from long electrical waves to the minute waves of cosmic rays. In the electromagnetic spectrum, the grenz rays are bordering on the long-wave side on ultraviolet radiation and on the short-wave side on the conventional X-rays. Grenz rays have wave lengths between 0.07 to 0.4 nm. They are easily absorbed, but on the other hand they have enough energy to produce ionization and therewith biologic effects.

3. The production of grenz rays

A grenz ray machine is essentially a smaller type of X-ray machine, using electric potentials of the order of 8-17 kV for the accelerations of the electrons. The source of electrons is a wire filament heated by an electric current. This forms the negative electrode, the cathode, in the grenz ray tube. The target, the anode, at which the electrons are stopped is made of heavy metal, usually tungsten. This grenz ray tube is highly evacuated, so that no air molecules can interfere with the passage of the electrons. When the electrons generated and driven across this evacuated gap are stopped suddenly by impingement upon the target (the anode), grenz rays are produced. A window will permit the emergence of grenz rays in clinically usable amounts. For grenz rays this window must be made of a material that absorbs the rays to a minimum degree. It can be either a thin sheet of glass or a metal of very low atomic weight, such as beryllium or aluminium.

Windows of lithium glass or of Lindeman glass were formerly used, but were abandoned because of their low textural strength and high sensitivity to humidity. The tube is usually water-cooled to prevent overheating and melting of the anode. Adjustable step-down transformers are used to furnish the current to heat the filament and to regulate the primary voltage for the acceleration of the electrons.

4. Measurement of grenz rays

Owing to the almost endless variations recommended by different authors, the selection of physical factors in dermatologic radiation therapy was difficult in the past. Modern standardization of technical factors has reduced these variations to a minimum.

Quality of radiation

The quality of X-rays is defined as their penetrating ability. The most frequently used definition for various X-ray qualities is the half value layer (H.V.L.). It is defined as that thickness of a given filter material (in dermatology usually aluminium) which reduces the intensity to 50% of the original incident radiation. For grenz rays, they will be referred to as soft (H.V.L. up to 0.02 mm Al), medium (H.V.L. 0.023 to 0.029 mm Al) and hard grenz rays (H.V.L. 0.030 to 0.036 mm Al). The half value layer is influenced by multiple factors but for practical purposes only two of them are important: kilovoltage and additional filtration. An X-ray beam produced by higher kilovoltage has shorter wavelengths and greater penetrating power, and by placing a filter in the X-ray beam the quality is changed in such a way that the higher the atomic number of the filter, the greater the reduction in beam intensity.

Quantity of radiation

The intensity or dose rate of radiation is influenced by: kilovoltage, milliamperage, filter, exposure time and target skin distance. It increases when the kV and mA are increased. It decreases as the distance is increased, approximately in inverse square proportion, and it is also reduced as the thickness and atomic number of the filter are increased. The radiation dose is directly proportional to the exposure time if all other factors remain constant.

The X-ray dose in roentgen (R) specifies the exposure to a certain quantity of radiation, based on its ability to ionize air. It is not directly indicating the observed dose in the tissue, which is expressed in rads. The unit of the absorbed dose (tissue dose) used today is Gray (Gy) according to SI standards where 1 Gy = 100 rad.

5. The biological effects of grenz rays

Grenz rays are absorbed predominantly through the photoelectric effect. Since their energy is small at the outset, the path of the photoelectron is short, so that its entire quantum of energy is absorbed within one cell. Thousands of collisions occur, however, along that short path. This produces ions, and excited atoms and molecules which are able to enter into chemical combinations with free radicals or other molecules to form new molecules of unpredictable effect on the tissue (Hollander, 1968).

6. Grenz ray therapy in skin diseases

Goldschmidt (1975 a) has pointed out that most inflammatory dermatoses have their pathology in the first millimeter of skin and many of the rest, in the

first three millimeters of skin. For this reason there are indications and expectations of good effect from grenz ray therapy because 50% of grenz ray radiation given is absorbed by the first 0.5 mm of the skin. This form of radiation is extremely suitable if one considers the sparing effect of hair roots, sebaceus and sweat glands, eyes and gonads.

A survey in 1973 showed that many dermatologists in the United States and Canada considered dermatologic radiotherapy to be necessitous or worth-while part of dermatologic practice (Goldschmidt, 1975 b). 44% of 2400 dermatologists answering a questionnaire stated that they used superficial X-ray therapy or grenz ray therapy regularly. A follow-up survey in 1985 of dermatologic training centers in the United States and Canada disclosed that only 15% used grenz ray therapy (Kingery, 1986). However there is no doubt that grenz rays even today are used in a high percentage in certain clinics where this form of treatment is based on local and national traditions.

The list of conditions for which grenz ray therapy has been reported to be effective could be made very long, but much of the treatment which has been recorded in literature is obsolete today. For a full account of grenz ray therapy the monographs of Bucky and Combes (1954) and Hollander (1968) should be consulted.

The list of conditions for which grenz ray therapy today is indicated and effective is according to C.T. Janssen, (1978), as follows:

1) Lichen simplex chronicus
2) Pruritus ani et vulvae
3) Seborrhoic dermatitis
4) Nummular eczema
5) Dyshidrosis
6) Nonspecific, persistent eczematous conditions
7) Psoriasis
8) Lichen planus

Neurodermatitis

It is in lichenified dermatitis that grenz rays have been most uniformly effective (Jungmann, 1939; Giraudeau, 1940; Ryan, 1942; Jambor et al, 1959; Lynne-Davies, 1975). Recommended doses varied widely.

Psoriasis

Grenz ray therapy should be considered as one of the most useful external therapeutic agents in psoriasis (Arouete, 1968). A large group of observers have reported good response to grenz rays but some of them also emphasized the frequence and/or promptness of recurrence (White, 1937; Giraudeau, 1940; Kalz, 1941; Ryan, 1942; Leitner, 1943; Sagher, 1943; Bluefarb, 1944; Reymann, 1951; Kopp and Reymann, 1957; Harber, 1958; Frain-Bell et al, 1959; 1957; Frain-Bell et al, 1960-61; Stewart, 1975 a; Brodersen and Reymann, 1981; Wiskemann, 1977,1981). Some of them also warned against the production of erythema as possibly leading to a Koebner reaction (Kalz, 1941; Leitner, 1943; Sagher, 1943).

Lichen planus

Good results have been obtained by many workers (Ryan, 1942; Kopp and Reymann, 1956;) especially that of lichen planus of the oral mucosa (Koltai, 1939; Bucky and Combes, 1954; Garretts, 1975). However, also for lichen planus patients, there is a risk for an acute flare up, possibly because of a Koebner reaction (Brodkin and Bleiberg, 1965).

Pruritus ani, scroti and vulva

Grenz ray therapy may be helpful in these conditions (Jungmann, 1939;

Giraudeau, 1940; Reyes Garcia, 1940; Kalz, 1941; Ryan, 1942, Leitner, 1943; Bluefarb, 1944). Cipollaro and Crossland (1967) claim that grenz rays should not have any effect on sperm production.

Seborrhoic dermatitis

Good response to moderate doses has been reported (Sagher, 1943; Bluefarb, 1944; Jambor et al, 1959).

Darier's disease

There are several reports of improvement or complete clearance of lesions of Darier's disease after grenz ray therapy (Blank, 1952; Shelley et al, 1959; Hollander, 1968; Wiskemann, 1969; Cipollaro and Shaps, 1979).

Hailey-Hailey disease

Familial benign chronic pemphigus is said to respond quite well to grenz ray therapy. (Sarkaney, 1959; Hollander, 1968).

Recurrent herpes simplex

Grenz ray therapy has been reported to be effectively used to prevent recurrent episodes of herpes simplex (Reyes Garcia, 1940; Ryan, 1942; Knight, 1972).

Verruccae

Many workers have reported good results in treating common warts with grenz rays (Ryan, 1942; Leitner, 1943; Sagher, 1943; Reymann, 1950; Klem, 1951;

Knudsen et al, 1955; Hollander, 1968). Poor results have also been reported (Jungmann, 1939; Anker, 1951).

Apart from these reviewed conditions in which the therapeutic result of grenz rays has been fairly well documented, there is an almost endless list of conditions reported to respond well to grenz ray therapy: Parapsoriasis, granuloma annulare, urticaria pigmentosa, dermatitis herpetiformis, pernio, pyodermas, stasis dermatitis and ulcers, scleroderma, poikiloderma, vitiligo, pigmented nevi, keloids, seborrhoic keratoses, pyogenic granuloma, lupus vulgaris, tuberculosis verrucosa cutis, erythema induratum, scrofuloderma, sarcoid, leprosy, fungous diseases, acne vulgaris, alopecia areata and port wine stains.

Indirect therapy has also been reported. General body irrdiation was reported to give specific results in polycytemia (Bucky, 1929; Bucky et al, 1939), alopecia areata and vitiligo (Bucky, 1929; Hanfling, 1948).

Apart from these benign skin disorders there are certain malignant and premalignant skin diseases which respond well to grenz ray therapy. Actinic lesions of Bowen's disease respond well to grenz ray therapy (Hollander, 1978). Also Mycosis fungoides (Jungmann, 1939; Kalz, 1941; Bluefarb, 1944; Dostrovsky et al, 1945) has been reported to respond well. However, the doses given in these malignant conditions are considerably larger than for the benign conditions reviewed.

7. Adverse effects of grenz ray therapy.

Possible effects of grenz ray therapy are qualitatively identical to those of conventional X-rays. The principal adverse effects are erythema and pigmentation. Epilation, radiodermatitis and cancer development have also

been described, mainly owing to overdoses.

Erythema

Grenz ray erythema is relatively asymptomatic, and its latent period is shorter than that of conventional X-ray erythema. It is not followed ordinarily by sequelae other than pigmentation (Hollander, 1968). The intensity of this cutaneo-vascular reaction varies greatly, not only among different individuals, but also among different body regions of the same individual (Kalz, 1959).

Pigmentation

Pigmentation results from grenz ray therapy and close shielding should be avoided in order not to produce a sharp line at the edge of the treated area. Pigmentation so induced varies with race, age and body region, but is never permanent (Rowell, 1978). Large doses can give rise uncommonly to a peculiar pigment displacement, a spotty hyperpigmentation instead of to uniform hyperpigmentation (Jungmann, 1939; Kalz, 1941; Hollander; 1968).

Epilation

The risk of epilation caused by grenz ray therapy must be very small in humans, because of the fact that the penetration of the grenz rays is not deep enough to reach the hair roots in sufficient doses. Early investigators did, however, report temporarily epilation both in experimental animals and in man (Kissmeyer, 1932; Carrie, 1933).

Radiodermatitis

Atrophy, teleangiectasia and depigmentation of the skin have been reported (Beller, 1936; Sagher, 1944; Hollander, 1957). It has been suggested that tissue damage occurs only after high individual doses, while the cumulative effect of small doses appears to be remarkably low (Kalz, 1959).

Cancer development

In 1959, the first case of a squamous cell carcinoma resulting from an extreme overdosage of grenz rays was described (Kalz, 1959). After that a total of 10 cases has been reported, Table Ia. The first two reported cases were dermatologists. In both a squamous cell carcinoma developed on the distal phalanx of an index finger, due to the fact that the physicians had been careless about exposing their hands to the direct beam (Kalz, 1959; Sagher, 1962). The carcinogenic effect of grenz rays has also been studied in experimental animals, Table Ib. It has been shown that grenz rays can produce metastatic squamous cell carcinoma under certain experimental conditions (Wulf and Hou-Jensen, 1979), and that the pattern of tumor development, like ultraviolet light, was that of carcinogenic summation (Epstein, 1970).

Table Ia. Cutaneous neoplasms in humans produced by grenz rays, reviewed in the literature.

Case	Source, year	Type of neoplasm	Localisation	Dose of grenz rays	Latency period	Additional therapy	Comments
1	Kalz, 1959	Squamous cell carcinoma	Finger	Overdose[a]	6 yr	Not known	Physician
2	Sagher, 1962	- " -	Thumb	600-700 Gy	14 yr	- " -	Physician
3	Ohkido and Horiuchi, 1965	- " -	Groins	100 Gy[a]	1 yr	UV-light	Eczema
4	Cipollaro and Crossland, 1967	Basal cell carcinoma	Lumbar region	a)	23 yr	Not known	Treated with grenz rays for a low back pain
5	- " -	Squamous cell carcinoma and basal cell carcinoma	Face	a)	10-11 yr	- " -	Treated for nevus flammeus
6	Lagerholm and Skog, 1968	Squamous cell carcinoma	Penis	> 100 Gy[a]	Not known	- " -	Psoriasis
7	Brodkin and Bleiberg, 1968	- " -	Buttock	130 Gy	7 yr	U.V., Tar, Superficial X-rays, mercury	Psoriasis
8	Volden and Larsen, 1977	- " -	Neck	132 Gy	3 yr	Soft X-rays	Mycosis fungoides
9	- " -	- " -	Neck	136 Gy	10 yr	Soft X-rays	- " -
10	Dabski and Stoll, 1986	- " -	Extremities	30 Gy	16 yr	Methotrexate	Psoriasis

[a]The amount of exposure could not be determined.

Table Ib. Cutaneous neoplasms in experimental animals produced by grenz rays, reviewed in the literature.

Source, year	Type of neoplasm	Dose of Grenz rays	Animal	Comments
Shapiro et al, 1961	Squamous cell carcinoma	0.5-1 Gy/day Total dose: 300 Gy	Mouse	Total dose of 200 Gy did not produce any squamous cell carcinoma
Zackheim et al, 1964	Squamous cell carcinoma and basal cell carcinoma	Single dose 50-90 or 3-6 Gy/weekly Total dose: 78-264 Gy	Rat	
Epstein, 1970	Skin tumors	1 Gy 3 times/week Total dose: 140-205 Gy	Mouse	The pattern of tumor development was that of carcinogenic summation
Wulf and Hou-Jensen, 1979	Squamous cell carcinoma	2-3 Gy 4 times/week Total dose:652-945 Gy	Guinea pig	1 Gy 4 times/week, total dose: 326 Gy did not produce any squamous cell carcinoma

OTHER X-RAY THERAPIES USED IN DERMATOLOGY

Apart from grenz ray therapy, a number of X-ray therapy methods have been used in dermatology both for benign skin disorders and for skin cancers. The methods used for benign disorders are summarized in table II.

Table II. Radiation methods for benign skin disorders.

Therapy	Sources and synonyms	KV	Wavelength (nm) (average)	Half value layer (aluminium)	Half value depth (tissue)
Superficial X-ray	Low voltage standard X-ray Pyrex window	60-100	0.05	0.7-2 mm	7-10 mm
Soft X-ray	Beryllium window	20-100	0.015	0.1-2 mm	1-20 mm
Grenz ray	Ultrasoft Supersoft Bucky rays	5-20	0.2	0.03 mm	0.2-0.8 mm

AIMS OF THE STUDY

A. TO STUDY MECHANISMS OF ACTION OF GRENZ RAY THERAPY (I-IV)

B. TO STUDY CLINICAL EFFECTS OF GRENZ RAY THERAPY IN CERTAIN INDICATIONS (II, IV, V)

C. TO STUDY SIDE EFFECTS OF GRENZ RAY THERAPY (VI)

PATIENTS AND METHODS

A. THE MECHANISM OF ACTION OF GRENZ RAY THERAPY (I-IV).

1. Patients

Paper I, III, IV: The patients were 22 healthy volunteers of both sexes, age range 20-69 years, without clinical signs of dermatological diseases. Paper II: Six patients with nickel sensitivity, confirmed by patch tests with 2.5% nickel sulphate in water. All were untanned white women, 20-47 years of age. All patients had given their informed consent.

2. Grenz ray machine factors

Paper I: 10 kV, 10 mA, half-value layer 0.03 mm Al, focus-skin distance 10 cm. One single dose of 4 Gy was given. Paper III: 10 kV, 10 mA, half-value layer 0.03 mm Al, focus-skin distance 10 cm, 3 Gy was given 3 times with 1 week interval. Paper II, IV: 11 kV, 20 mA, half-value layer 0.03 mm Al, focus-skin distance 20 cm, 3 Gy was given 3 times with 1 week interval.

3. Patch testing

Paper II: Patch tests were performed with Al test patches fixed to the skin with scanpore tape. The contact allergen used was nickel sulphate in aqueous solution. Eight different concentrations, 1.25-0.0098%, were tested with H_2O as a control. The test patches were removed at 48 h, and reading was done 72 h after application of the test. The sites were scored by the scoring system recommended by the International Contact Dermatitis Research Group. Paper IV: Irritant reactions were produced by patch testing (Finn chambers) with

different concentrations of sodium lauryl sulphate (SLS). In a first group (3 subjects) we used 0.5, 1.0 and 2% SLS solution and in a second group (8 subjects) we applied 0.5, 1.0, 2 and 5% SLS solutions. The test patches were left in place for 48 h and the reading of the test reactions was done 72 h after the application of the tests. In two subjects in the first group the corresponding times were 24 and 48 h.

4. Experimental procedure

Paper I: Each subject was exposed to grenz rays in a circular area, 5 cm in diameter, of buttock skin (untanned). Punch biopsies were taken from the buttock site before irradiation and from the irradiated site, 30 min, 6 h, 24 h, 1 week and 3 weeks after grenz ray therapy. Paper II: Each patient was first exposed to grenz rays in one and the same area of 15 x 15 cm on the right side of the back. Twenty-four hours after the last treatment one serial dilution nickel patch test (SDT) was carried out on the site of the grenz ray treatment and one SDT on the untreated side of the back. Skin biopsy specimens were taken at the 72 h reading from (1) grenz-ray-treated area, not nickel-tested; (2) grenz-ray-treated area, nickel-tested; (3) untreated area, not nickel-tested; (4) untreated area, strongest positive test reaction to nickel; and (5) H_2O control. Thus, a total of five punch specimens were obtained from each patient. Paper III: Each subject was exposed to grenz rays in a circular area 1 cm in diameter of buttock skin (untanned). Punch biopsies were obtained from the buttock before irradiation and from the irradiated site 24 h after the last treatment. Paper IV: Each subject was first exposed to grenz rays in the same way as in (II). Twenty-four hours after the last treatment identical SLS patch tests were applied on the site of the grenz ray treatment and symmetrically on the untreated side of the back. At the time of reading of the test reactions 2 biopsies were taken from each subject. One biopsy was taken from the strongest positive

test reaction both in the untreated and in the grenz ray treated skin.

In all the studies, punch biopsies (diameter 3 mm) were taken after anesthesia with lidocain without epinephrine. Each specimen was immediately frozen on solid carbon dioxide and stored at -80°C until used. The specimens were cryostat sectioned at 6-8 µm and mounted on cooled glass slides (I, II, IV). For transmission electron microscopy (III) the biopsies were divided into 4 pieces cut perpendicularly to the skin surface and immediately fixed in 2.5 % glutaraldehyde in an 0.13 mosm phosphate buffer and subsequently processed according to a standard protocol.

5. Immunohistological method (I, II, IV).

Paper I: The slides were incubated in OKT-6, a monoclonal antibody (Ortho Pharmaceutical Corp.). OKT-6 reacts with the majority of thymocytes and with Langerhans' cells (Murphy, 1982). Subsequently a highly sensitive immunoperoxidase technique was employed (Vectastain ABC Kit, Vector Laboratories). Briefly, tissue sections were layered with a biotin-labelled secondary antibody and this introduced biotinyl residues into the section at the location of the primary antibody. An avidin-biotinylated horseradish peroxidase complex was then added and the tissue antigen was localised by incubation in a peroxidase substrate (amino-aethyl carbazol). The slides were then examined at a magnification of 500X using an ocular square grid. From each biopsy a total of 40 grid fields were examined. This represented 10 mm of skin surface length. The samples were counted blind. Paper II: The following Leu (Becton-Dickinson) OK (Ortho) and B1 (Coulter Immunology) mouse anti-human monoclonal antibodies were used: Leu 2 (cytotoxic/supressor T-cellsubset), Leu 3 (inducer/helper T-cell subset), Leu 4 (all T-cells), Leu 7 (killer cells), Leu MI (monocytes and null cells), and B1 (all B cells). The production of these antibodies and their specificities have been reviewed previously (Kung et al, 1983).

The frozen sections were overlaid with the monoclonal antibody at a dilution of 1:100. After that Vectastain ABC kit was employed as described in (I). From each biopsy specimen from all 6 patients, sections were stained separately with haematoxylin-eosin and with the monoclonal antibody indicated above. Paper IV: The antibodies employed were the same as in (II). The sections were rinsed in phosphate buffered saline solution, incubated in normal swine serum and then overlaid with the monoclonal antibody at a dilution of 1:200 (Leu M1), 1:100 (OKT-6 and BI), 1:40 (Leu 2 and 7), and 1:20 (Leu 3 and 4). Secondary incubation was performed with horse radish peroxidase-conjugated rabbit anti-mouse immunoglobulin antibody (Dako). Peroxidase activity was visualized by incubation with 3-amino-9-ethylcarbazol, AEC, and finally the sections were counterstained with Mayers haemalum.

In I, II, IV the primary antibody was omitted in control sections for each reaction. In paper IV we used tonsils as positive controls. The proportion of infiltrating cells was estimated semiquantitatively at x500 magnification (II, IV). In case of OKT-6, only dendritic cells with visible nucleus were counted as positive, and isolated dendrites were not scored.

6. Transmission electron microscopy

Twenty-four hours after fixation the biopsies were postfixed in osmium tetroxide, dehydrated in graded series of ethanol, transferred to propylene oxide and embedded in Epon. Uranyl acetate and lead citrate were used for contrast enhancement. From each of the control and test biopsies, 1 block with optimal orientation was chosen for ultrathin sectioning (40-60 nm) using LKB Ultratome. The sections included dermis and all the epidermal cell layers. To obtain a satisfactory length of epidermis, 2 series of sections

were prepared from each block. At least 50 µm were trimmed away between each series of sections. Thus, different volumes of epidermis in the block were sectioned each time.

The sections were systematically surveyed in a Philips EM 301 electron microscope operated at 80 kV, and whenever a cell or a cell element contained LC granules it was defined as an LC. In a few cases, only a dendritic process of an LC contained the LC granules. Such a process was defined as an LC only if no other LC was present within a radius of at least 10 consecutive keratinocytes. Whenever it was difficult to identify LC granules in cells suspected to be LC, the microscope goniometer was employed and the sections tilted and/or serial sections studied. LC were counted in each section, length of epidermis ranging from 0.071-0.181 mm. A quantitative assessment of the overall morphology of the LC and keratinocytes was made before and after grenz ray therapy.

B. CLINICAL EFFECT OF GRENZ RAY THERAPY. (II, IV, V)

1. Patients

Paper V: Sixteen patients with symmetrical psoriasis lesions of the scalp took part in the study. All lesions were located in hair-bearing areas. Age range was 27-71 yr. Duration of disease was 1-44 yr. The patients had been untreated for at least 4 wk before the start of the study. Paper II, IV (see page 22).

2. Grenz ray regimen

Paper V: The grenz ray machine factors were: 11 kV, 20 mA, half-value layer 0.03 mm Al, focus-skin distance 20 cm, beryllium window. The design of the

study was that of a double-blind trial. Each patient received 4 Gy of grenz rays given on 6 occasions at an interval of 1 wk. One side of the scalp received active treatment, the other side was treated with placebo. This was done by allowing the apparatus to "hum" without irradiation. The nurse treating the patient gave the active radiation or placebo treatment according to a randomized predetermined code. Neither the patients nor the evaluating doctors knew which side had received active grenz ray therapy. Clinical evaluation was performed before the grenz ray treatment and 1 wk after the 6th treatment. The observer made a graded assessment of each side of the scalp. The symptoms assessed were erythema, scaling, itching and distribution of the psoriatic lesions. A 5-grade scale was employed, where 0 denoted absence of symptoms and 4 denoted very severe symptoms. When the patient and the nurse giving the treatment noted a pronounced difference between the two sides, a clinical evaluation was made. In all patients a score difference of at least 50% was noted between the 2 sides and, at this point, the nurse started to give active treatment to the whole scalp. During the trial, no treatment other than topical oils containing salicylic acid was permitted. When the trial was completed, the irradiated side was identified and the results were analysed sequentially. After the last treatment each patient was told to contact the authors when relapse occurred on the first healed side. Relapse was defined as a need for treatment more active than the usual scalp and hair wash twice a week. For this purpose the patients received an inquiry letter giving the definition of relapse, to be returned to the authors. All patients were interviewed 3 months after the last treatment. Five patients who were healed 3 months after treatment and who had not contacted the authors by letter were interviewed after 6 months. Paper II, IV (see page 22).

C. SIDE EFFECTS OF GRENZ RAY THERAPY

1. Patients

During the years 1949 to 1975, 14,237 patients received therapeutic doses of grenz rays at the Department of Dermatology, Karolinska Hospital, for the treatment of benign skin disorders such as chronic eczema, psoriasis, warts etc. Only 35 patients were lost because of insufficient information about name or date of birth and 62 patients were lost to follow up because of errors in the computation of the records. Thus 14,140 patients (99.3%) were available as subjects for the epidemiologic study. The patient population consisted of 6,525 females and 7,615 males in the age range of 1-90 years, with a mean age of 40.8 years at the first treatment. The information obtained from the patients' records were: name, date of birth, sex, date of first treatment, dose of grenz rays, site of treatment and number of treatments. The records contained drawings showing in detail where the grenz ray therapy had been given for each session.

2. Grenz ray regimen

The grenz ray machine factors have been 10 kV, 10 mA, focus-skin distance 10 cm or 11 kV, 20 mA, focus-skin distance 20 cm, beryllium window. Those factors have been unchanged during the period. One and the same nurse has given the treatment and also kept the records. The standard regimen followed in most cases at the department was:

1. No area of skin should be subjected to more than 100 Gy in a lifetime.

2. The dose must be fractionated. (One treatment a week for 4 to 6 weeks is recommended). At least half a year should elapse between such courses.

3. The dose of grenz rays must be adapted to the skin disease in question and to the treated area. 0.5 Gy x 4-6 for lichen sclerosis et atrophicus (vulva) and 4 Gy x 4-6 for scalp psoriasis are examples of doses used.

However, many patients had received considerably higher doses than recommended. In a random sample based on 274 patients the accumulated dose of grenz rays given to the patients in one and the same area ranged from 1 Gy to 290 Gy with an average of 25.0 and a S.D. of 39.8. 481 patients had received a total high dose of grenz rays ($\geq$100 Gy) on one and the same area. Table III. The diagnoses for those high dose treated patients are shown in Table IV. The maximum dose given to any patient on the same area was for warts: 1470 Gy, psoriasis: 388.5 Gy and for eczema: 231.5 Gy.

3. Swedish Cancer Registry

Information from the Swedish Cancer Registry (1958-1981) and the 14,140 patients was linked in order to identify individuals with malignant skin tumors occurring after the initial grenz ray treatment. Nationwide information on the cancer incidence in Sweden is available from 1958, when a compulsory registration was begun. The registry collects diagnosed cancers not only from physicians but also from pathologists. Thus, most diagnosed cases are reported by two sources. Basal cell carcinoma is not registered. Each patient is characterized by a unique identification number, used in all population statistics in Sweden. This number is composed of 6 digits based on year, month and day of birth, supplemented with a registration number (3 digits) and a check digit. The identification numbers are therefore not affected by possible changes in names. The completeness of registration in the Swedish Cancer Registry has been found to be 96-97% for all cancers. There is an even higher degree of completeness for skin cancers owing to the high frequence of histologic diagnoses of these tumors (Mattsson, 1984).

Statistics

The expected number of malignant skin tumors was estimated on the basis of incidence data from the Swedish Cancer Registry. The age and sex specific cancer incidence was calculated as a national average for 1970. Regional differences were disregarded. With few exceptions malignant tumors of the skin are slow-growing, and there will be an interval between exposure and the first occurrence of the tumor. Thus, in the calculation of person-years at risk, the first 5 years of the observation period were disregarded. The period at risk therefore started 5 years after the first grenz ray treatment. To allow for deaths occurring during the observation period, we made deductions on the basis of life tables for the whole Swedish population. The number of person-years at risk was calculated separately for each sex and amounted to 174,000. After the ratio between observed and expected number of malignancies had been estimated, significance and confidence interval analysis was performed using the Poisson distribution.

Table III.

Patients who had received $\geq$100 Gy of grenz rays on the same area, distribution with respect to dose.

Dose (Gy)	N:o of patients
100 - 200	385
201 - 300	65
301 - 400	23
$\geq$ 401	8
Total	481

Table IV.

Patients who had received ≥100 Gy of grenz rays on the same area, distribution with respect to diagnosis.

Diagnosis	N:o of patients
Warts	431
Psoriasis, scalp	29
Psoriasis, body	10
Neurodermatitis	10
Acne	1
Total	481

RESULTS

A. MECHANISM OF ACTION OF GRENZ RAY THERAPY

1. Effect on Langerhans' cells (I, II)

The results from the immunological study (I) are summarized in Table V. In the majority of the counted sections the distribution of OKT-6 positive cells was regular and the cells easily identified. After 30 min we found a slight reduction ($p < 0.05$) of OKT-6 positive cells regarded as LC and after 1 and 3 weeks the reduction was highly statistically significant ($p < 0.001$, paired t-test). The skin exposed to grenz rays did not show any erythemal reaction or other signs of inflammation.

Table V. Number of OKT-6 positive cells/10 mm of skin surface length in sections of human epidermis after a single dose of 4 Gy of grenz rays.

		Time interval between exposure and biopsy					
Subj.	Age/Sex	Before	30 min	6 h	24 h	1 w	3 w
1	38/M	154	147	159	157	84	62
2	36/F	188	145	127	149	67	43
3	64/F	168	118	137	145	52	48
4	69/F	146	112	97	116	52	45
5	63/F	134	107	76	53	22	34
	Mean	158	125.8	119.2	124	55.4	46.4
	±SD	20.8	18.3	32.9	42.6	22.9	10.2
	$p<$		0.05	0.05	NS	0.001	0.001

The results of the electron microscopy study (II) are summarized in Table VI. The dose of grenz rays employed did not produce erythema. Only a slight pigmentation was observed in 2/6 subjects. Quantification was performed by assessing the frequency with which LC were found in the grenz-ray-exposed skin and comparing this to the non-exposed control skin.

In each of the control biopsies we could readily distinguish LC by their convoluted nucleus, lack of desmosomes and clear, ample cytoplasm containing LC granules. When we systematically surveyed the biopsies taken from the grenz ray treated area, we were not able to identify any LC in 3 of our subjects and only a few LC in the other three (Table VI). This reduction was statistically significant ($P < 0.05$, Wilcoxons' Signed Rank Test, two tailed). In the treated biopsies, the few remaining LC appeared morphologically unaltered and there was no clear evidence of death of LC in situ. The majority of the remaining LC were located at the basal lamina. In the control biopsies numerous LC were found in a suprabasal position. In the grenz-ray-treated skin, not only LC were rare but also "invading" non-keratinocytes were sparse compared to normal skin.

There appeared to be no consistent changes in keratinocyte morphology in the grenz-ray-treated area, as judged by nuclear size, cytoplasmic dense bodies and vacuolization. There were no conspicious changes of the intercellular spaces.

Table VI. Electron microscopic quantitation of Langerhans´cells in sections of human epidermis, before and after grenz ray therapy (3 Gy x 3, 1 wk interval).

Subject	Age/Sex	Before grenz rays			After grenz rays		
		Section N:o	Length (mm)	N:o of LC	Section N:o	Length (mm)	N:o of LC
1	38/M	1	0.106	2	1	0.106	0
		2	0.129	2	2	0.144	1
2	20/M	1	0.106	2	1	0.091	0
		2	0.106	3	2	0.121	1
3	39/F	1	0.106	2	1	0.100	0
		2	0.098	2	2	0.100	0
4	50/M	1	0.071	1	1	0.100	0
		2	0.125	2	2	0.125	0
5	37/M	1	0.159	5	1	0.136	0
		2	0.121	2	2	0.181	0
6	36/F	1	0.136	1	1	0.144	1
		2	0.117	1	2	0.129	0

$P < 0.05$ Wilcoxons' Signed Rank Test (two-tailed).

2. Effect on ACD. Immunohistologic characterization (II)

The results are summarized in Table VII. In unchallenged and nickel-challenged skin from the grenz ray treated area, the cell populations positive for Leu 2, 3, 4, 7, Leu M1 and B1 occurred in about the same proportions as in untreated skin. The number of OKT-6 positive cells, regarded as LC, was greatly reduced in the epidermis in the grenz ray treated skin as compared with untreated control skin. In the positive patch test to nickel in untreated control skin, the lymphoid infiltrating cells in dermis were mainly of Leu 2, 3 and 4 phenotype, with Leu 3 more prevalent than Leu 2. The cell infiltrative also contained a small number of Leu 7, Leu M1, B1, and OKT-6 positive cells.

Table VII. Histologic and immunohistologic characterization of dermal cell populations in nickel patch tests and adjacent control skin from an area treated with grenz rays (3 Gy x 3, 1-week interval) and from untreated skin, in six nickel-sensitive patients.

Site of biopsy	Density of cellular infiltrate (haematoxylin-eosin)	Leu 2 (T suppr./cytotox)	Leu 3 (T help./induc)	Leu 4 (pan T)	Leu 7 (NK cells)	B1 (pan B)	Leu M1 (monocytes)	OKT-6 (Langerhans cells, thymocytes)	
								Epidermis	Dermis
Grenz-ray-treated area									
Nickel patch test	Sparse	0–1	1	1	0–1	0–1	0–1	1	0–1
Control	Sparse	0–1	0–1	1	0–1	0–1	0–1	1	0–1
Untreated skin									
Nickel patch test	Heavy	3	3	3	1–3	0–2	2–3	3	0–1
Control	Sparse	1	0–1	1	1	0–1	1	3	0–1
H_2O patch test	Sparse	0–1	1	1	0–1	0–1	0–1	3	0–1

The occurrence of OKT6-positive cells was also assessed in epidermis.
The figures indicate labelling of numbers of cells as follows: 0=no cells; 1=few, scattered; 2=moderate number; and 3=large number of cells.

3. Effect on irritative skin reactions. Immunohistologic characterization (IV)

The results are summarized in Table VIII. The cell populations of Leu 2, Leu 3, Leu 4, Leu 7, Leu M1 and Leu B1 occurred in about the same proportions in the cellular infiltrates found in the grenz ray treated skin as in the untreated skin. Compared with the positive reactions in the untreated skin the number of epidermal OKT-6 positive cells was greatly reduced in the reactions in the grenz ray treated skin. The dermal cell infiltrate consisted mainly of T-lymphocytes (Leu 4) with T-helper/inducer cells (Leu 3) more prevalent than T-suppressor/cytotoxic cells (Leu 2). In the cell infiltrate we also identified mononuclear/null cells (Leu M1) and occasional NK-cells (Leu 7), B-lymphocytes (B1) and OKT-6 positive cells.

In the light microscope it was not possible to reveal any difference in the thickness of stratum corneum in the patch tests in grenz ray treated skin compared with untreated skin.

Table VIII. Immunohistologic characterization of the cellular infiltrate in irritant reactions in grenz ray treated and in untreated skin in eleven subjects.

0=no cells, 1=few, scattered, 2=moderate number, 3=large number of cells

Phenotype	Irritant reactions in	
	Grenz ray treated skin	Untreated skin
Leu 2 (T suppr/cytotox)	1–2	1–2
Leu 3 (T helper/induc)	2–3	2–3
Leu 4 (pan T)	1–3	2–3
Leu 7 (NK cells	0–1	0–1
B1 (pan B)	0–1	0–1
Leu M1 (monocytes)	1–3	2–3
OKT6 (Langerhans' cells, thymocytes)		
Epidermis	1	3
Dermis	0–1	0–1

B. CLINICAL EFFECT OF GRENZ RAY THERAPY (II, IV, V)

1. Effect on ACD (II)

The results of patch testing are summarized in Table IX. In the grenz ray treated area we found a total suppression of the patch test response to nickel in 5 of the 6 patients. In the sixth patient (no 6), who was strongly sensitive to nickel, there were 2 follicular papules at the site of the 1.25% nickel sulphate test on the grenz ray treated area, but no oedema, redness or vesicles (Fig 1).

The strength of the test responses to nickel in the control area before and after this experiment did not differ in 5 of 6 patients. In one patient (no 2) we noted a +++ reaction before the experiment, but only a + reaction after. The patient had a common cold with fever and perspiration during patch testing. In one patient (no 1) we performed a nickel patch test 6 weeks after grenz ray treatment in the treated area and found that the response now was equal to that of the control site.

Table IX. Patch test results in nickel-sensitive patients. Nickel patch test applied on the grenz-ray-treated area (3 Gy x 3 , 1 wk interval) and on the control area.

Patient	Age/sex	Confirming nickel patch test* before experiment	Nickel patch test† on control area (strongest reaction)	Nickel patch test† on grenz-ray-treated area
1	23/F	++	++	–
2	47/F	+++	+	–
3	42/F	++	++	–
4	38/F	++	++	–
5	20/F	++	++	–
6	32/F‡	+++	+++	2 Papules (no erythema, oedema, or vesicles)

–, Negative reaction; +, weak (non-vesicular) reaction; ++, strong (oedematous or vesicular) reaction; and +++, extreme (bullous or ulcerative) reaction.
*2.5% nickel sulphate in aqueous solution.
†1.25–0.0098% nickel sulphate in aqueous solution.
‡See Fig. 1.

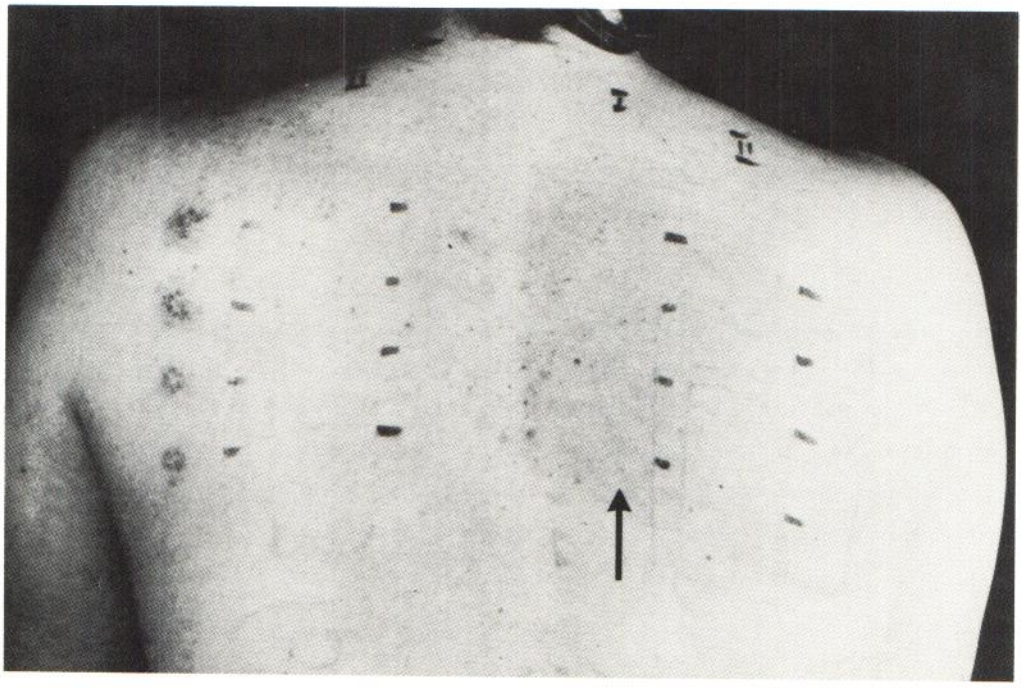

Fig 1. Result of patch testing in a nickel-sensitive patient (no. 6). Nickel sulphate of different concentration applied on the right side of the back on a recently grenz-ray-treated area (slightly pigmented) and on an untreated control site on the left side of the back. To the far left four strongly positive reactions are seen. The corresponding test in the grenz-ray-treated skin is seen below I, immediately to the right of the spine (arrow).

2. Effect on irritative skin reactions (IV)

The results of patch testing are summarized in Table X. In the grenz ray treated areas there was a slight decrease of the test responses as compared with the tests in the untreated skin. However, this decrease was not statistically significant ($P > 0.05$). The grenz ray treatment resulted in a slight pigmentation of the skin in all volunteers.

Table X. Irritant reactions produced by sodium lauryl sulphate in patch tests applied on grenz-ray-treated skin (GR) and on untreated control skin (CONTR).

−=negative reaction, +=erythema, ++=oedema or vesiculation, +++=bullous or ulcerative reaction. ND=not done

			Sodium lauryl sulphate patch tests			
Subject	Age/sex	Treatment	5%	2%	1%	0.5%
1	31/F	CONTR	ND	+	+	+
		GR	ND	(+)	−	−
2	39/M	CONTR	ND	+	+	+
		GR	ND	(+)	−	−
3	32/M	CONTR	ND	+	+	−
		GR	ND	(+)	(+)	−
4	29/M	CONTR	++	+	+	−
		GR	++	+	+	−
5	33/M	CONTR	++	+	+	+
		GR	++	+	+	−
6	28/M	CONTR	+++	+	+	+
		GR	+++	+	+	+
7	27/F	CONTR	++	+	+	+
		GR	−	−	+	−
8	37/F	CONTR	+	+	(+)	−
		GR	+	−	−	−
9	56/F	CONTR	++	+	+	(+)
		GR	++	+	+	−
10	27/M	CONTR	++	+	−	−
		GR	+	−	−	−
11	31/F	CONTR	++	(+)	+	+
		GR	−	+	−	+

3. Effect on psoriasis of the scalp (V)

The assessment made after grenz ray therapy showed an improvement on the half of the scalp receiving active treatment in all 16 patients. Fourteen patients healed completely (87.5%). One patient showed a marked improvement, but because of persistent itching the patient was not satisfied with the treatment. One patient showed only a slight improvement (Table XI). No one showed a side difference favoring placebo. Sequential analysis showed that treatment with grenz rays was significantly better than placebo ($P < 0.0001$) The initial placebo-treated side showed the same response to grenz ray therapy as the 1st treated side, but was delayed in time owing to the sequential starting of active therapy. Nine of 14 healed patients were still free from relapse 3 months after treatment. After that, several relapses occurred (Fig 2) and only 3 patients remained healed 6 months after treatment.

Severity score	Pre-treatment patients	Post-treatment patients
0– 2	0	14
3– 5	0	1
6– 8	3	0
9–11	5	1
12–14	8	0

Table XI. Pre- and post- treatment scores for 16 patients with psoriasis of the scalp, treated with grenz rays (4 Gy x 6, 1 wk interval).

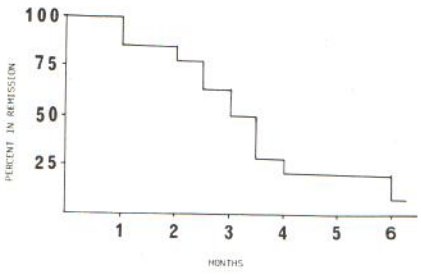

Fig 2. Remission diagram of 14 patients initially showing total clearance after grenz ray therapy for psoriasis of the scalp.

C. SIDE EFFECTS OF GRENZ RAY THERAPY

1. Incidence of malignant skin tumors in patients after grenz ray therapy (IV)

In 58 patients a malignant skin tumor was diagnosed more than five years after the grenz ray therapy first had been given. 19 patients had malignant melanoma and 39 had other malignant skin tumors. The expected number of melanomas was 17.8 and that of other malignant skin tumors was 26.9 (Table XII). In the case of malignant melanomas, there was no excess risk, but for other malignant skin tumors the risk was significantly elevated. This risk was particularly high for the lower legs, (42% of the patients had received grenz ray treatment on lower legs in a randomized sample of 270 patients). None of the patients with malignant melanomas but eight of the patients with other malignant skin tumors had received grenz ray therapy at the site of the tumor. The histological diagnosis and site of the skin tumors (excl. melanomas) are summarized in Tables XIII and XIV. Clinical and histopathological findings of the eight malignant skin tumors found on grenz ray treated areas are summarized in Table XV. Three of the patients had been exposed to arsenic, one had received conventional X-rays and one had been treated with methotrexate and hydroxyurea. Five of the patients had also been treated with tars at the site of the tumor. No malignancies were found on those 481 patients who had received a total high dose of grenz rays ($\geq$100 Gy) on one and the same area, Table III. The diagnoses of those high dose treated patients are shown in Table IV. Only one of those patients had developed a cancer in a non-irradiated area.

Table XII.

Observed and expected number of malignant skin tumors in 14,140 patients after grenz ray therapy for benign dermatoses. (The first five years after first grenz ray treatment are excluded).

	Observed N:o	Expected N:o	Ratio between observed and expected numbers
Melanomas	19	17.8	1.07
Skin tumors (excl. melanoma)	39	26.9	1.45*

* A significant excess risk at the 5% significance level.
95% confidence interval: (1.03,1.98)

Table XIII.

Histological diagnosis of malignant skin tumors (excl. melanoma) in 14,140 patients after grenz ray therapy for benign dermatoses.

Histological diagnosis	N:o of patients
Squamous cell carcinoma	32
Basalioma "type mixed"	5
Sarcoma Kaposi	2

Table XIV.

Cases of malignant skin tumors (excl. melanoma) by site in 14,140 patients after grenz ray therapy for benign dermatoses.

Site of skin tumor	Observed N:o
Ear	4
Face	11
Scalp and neck	2
Trunk	1
Superior extremities	5
Inferior extremities	10*
Multiple	2
Not specified	4

* The expected number is 1.6 and the ratio between observed and expected numbers is 6. The ratio is highly significantly greater than 1 ($p<0.0001$). 95% confidence interval for the ratio: (3,11).

Table XV. Clinical and pathologic findings in eight patients with squamous cell carcinomas appearing in areas treated with grenz rays.

Sex	Skin Disorder	Other Possible Risk Factors	Grenz-Ray Therapy		Site of Squamous Cell Carcinoma	Interval Between First Grenz Ray and Tumor, y
			Site	Dose Administered to the Site of Tumor, Rad (Gy)		
M	Neurodermatitis	Conventional roentgen rays, tars	Right knee flexure	4600 (46)	Right knee flexure	12
F	Neurodermatitis	Not known	Anus, vulva, left lower leg	2700 (27)	Left lower leg	22
F	Lichen planus	Arsenic	Lower legs, back, fingers	1550 (15.5)	Finger	14
M	Psoriasis	Arsenic, ultraviolet light	Lower legs, scalp, trunk, hands and arms	1600 (46)	Lower leg	17
F	Psoriasis	Methotrexate, hydroxyurea, tars	Legs, hips	3600 (36)	Lower leg	21
M	Neurodermatitis	Not known	Lower legs	1800 (18)	Lower leg	10
F	Psoriasis	Tars	Lower arms, lower legs, dorsal hands, dorsal feet	3700 (37)	Lower arm	6
M	Psoriasis	Arsenic, tars	Whole body	5950 (59.5)	Lower leg	9

DISCUSSION

A. MECHANISM OF ACTION OF GRENZ RAY THERAPY (I-IV)

The results of this study show that grenz rays reduce the number of LC (OKT-6 positive cells) in human epidermis and that this reflects a true disappearance of the LC as shown by electron microscopy. Grenz ray treatment was also found to suppress the expression of nickel allergy in sensitive individuals.

The effector phase of allergic contact dermatitis has been studied by means of grenz rays previously. In 1934, Samek (Samek 1934 a,b) reported that temporary desensitization with involution of the dermatitis in a treated area was obtained in ACD in man by treatment with 10 x 200 R daily. The desensitization persisted for several months, while other parts of the skin remained sensitive. In 1941, Kalz (Kalz, 1941) concluded that true desensitization could, at times, occur after grenz ray therapy with 3 x 250 R. He believed that the desensitation was due to the thickening of the stratum corneum and the pigmentation following irradiation, with resultant protection against external irritation.

ACD is a special type of delayed, cell-mediated hypersensitivity. Whereas ample information exists about cellular interactions in the sensitization phase, less is known about the mechanisms operative in the effector phase. In the sensitization phase, LC are considered to be crucial. They have been the target of much work and are now thought to be responsible for the antigen presentation during the sensitization phase (Wolff and Stingl, 1983). This antigen presentation could be modulated by the action of ultraviolet radiation (Toews et al, 1980) which causes a decrease in the density of epidermal LC. PUVA (Friedman, 1981), glucocorticosteroids (Belsito et al, 1982) and X-rays such as soft X-rays (Groh et al, 1984) and 250 kV X-rays

(Cole, 1986) also cause a decrease in epidermal LC. In contrast, gamma radiation (750 R) had no effect on epidermal LC (Belsito et al, 1984). UVB (Haniszki and Suskind, 1963, Morison et al, 1981) and PUVA (Austad and Mork, 1981, Meyer et al, 1980) can also partially inhibit expression of contact hypersensitivity in guinea pigs. In 2 patients sensitive to nickel, PUVA has been shown to diminish or abolish ACD to nickel, respectively (Thorvaldsen and Volden,1980). The patients were treated twice a week, until the total number of exposures was 18-20.

The early data on the effect of grenz rays on ACD have never been explained. The data presented in this study make it probable that this irradiation works by affecting the LC. Thus, these cells might be necessary not only in the sensitization phase but also in the effector phase of the ACD. Another possible explanation for the effect of grenz ray treatment on ACD could be that the treatment causes a non-specific suppression of the inflammatory response. This hypothesis has been tested and it was found that pre-treatment with grenz rays does cause a certain reduction of the expression of irritant reactions. This reduction was not found to be statistically significant in this material. In contrast, the reduction of ACD was almost total. Such a difference can be explained by the effect on the epidermal LC. The irritant reaction is a non-specific response to a harmful stimulus elicited by the direct toxic effect on the cells and tissues of the skin and does not involve the immunological system (Prottey, 1978). The elicitation of ACD is instead a strictly regulated and antigen specific event requiring the presence of functional antigen presenting cells. The number of LC had been reduced in epidermis by the same dose of grenz rays when studying both the ACD and the irritative reaction. This suggests that the LC are required for the elicitation of ACD but not to the same degree for the elicitation of irritant reactions. These data do not exclude the possibility that grenz rays may have an effect on already developed inflammatory processes via an influence on other levels than the antigen presenting cells.

The hypothesis that grenz rays may act by an influence on mediators of inflammation has also been tested. It was found that grenz rays have no effect on the biologic action of IL-1, when tested under clinical circumstances (Lindelöf and Söder, 1987). It has been demonstrated (Thorvaldsen and Volden, 1980) that PUVA treatment causes a non-specific reduction of the skin response to irritant stimuli and this effect was considered to be due to an increased thickness of stratum corneum with a reduced penetration of the irritant substance. It cannot be excluded that the penetration barrier is altered by the grenz ray treatment, but no morphological differences of the stratum corneum in the grenz ray treated skin compared to the control skin have been detected.

After UV and PUVA treatment, evidence of cellular damage of the LC has been described ultrastructurally, such as cytoplasmic vacuoles (Gilchrest et al, 1982), condensation (Friedmann, 1981), reduction of granules (Ree, 1982, Koulo et al, 1984). The doses of UV and PUVA employed in these studies were able to cause an erythemal reaction and/or pigmentation. It is important to note that the dose of grenz rays employed in our electron microscope study did not cause erythema, only a slight pigmentation in 2 of 6 cases. This might explain why we were not able to find any damaged LC or keratinocytes by electron microscopy.

The fate of the disappearing LC persists as an enigma. This study does not provide any clues as to what happens to the LC after grenz ray therapy.

In an attempt to detect possible differences between control skin and grenz ray treated skin, as well as between ACD and irritative reactions, we used a panel of monoclonal antibodies. The characterization of cell populations

in situ by use of monoclonal antibodies has become widely used very rapidly in recent years. According to present knowledge they are highly specific and sensitive (Kung et al, 1983). However the final status of the antibodies is still to be clarified. Precise quantitative assessment of the cell populations was not attempted in this study. Previous immunohistological studies (Reitamo et al, 1981; Ranki et al, 1983; Kanerva et al, 1984; Scheynius et al, 1984) have shown that there are no major differences between the dermal cellular infiltrate in irritant and allergic contact reactions. These results are confirmed in this study (II, IV).

In grenz ray treated skin a pronounced reduction of the number of LC in the epidermis, were found. No other differences between grenz ray treated and control skin were observed by the immunohistological method used.

B. CLINICAL EFFECT OF GRENZ RAY THERAPY (II, IV, V)

In the present study it is clearly shown that psoriasis of the scalp responds well to grenz ray therapy and that the healing rate is at least as high as for topical steroids or dithranol (Suurmond, 1968; Rogers et al, 1979; Hillström et al, 1982).

Previously, the efficiency of grenz rays has been shown for psoriasis lesions on trunk and extremities only in a few well-controlled paired comparison studies (Kopp and Reymann, 1957; Harber, 1958; Brodersen and Reymann, 1981). In the present study 50% of the healed patients were still free from lesions 3 months after the end of treatment. In another similar study (Johannesson and Lindelöf, 1987) it has been shown that this remission time could be increased by an additional treatment with topical steroid. In that case more than 50% of the patients were still free from lesions 6 months after the end of treatment.

Scales and hair absorb a very important quantity of the dose. In clinical practice it has been suggested that the thickness of the hair should be assessed and the normal skin dose multiplied by 1.5 for patients with a thin hair layer, by 2 in case of medium, and by 3 in case of a thick hair layer (Wulf and Brodthagen, 1977).

Grenz ray therapy should be considered as a useful therapeutic agent of psoriasis of the scalp. Apart form the results obtained, it outclasses most other local therapies by its convenience in application.

The effect of grenz rays on ACD and on irritative skin reactions shown in this study is important in clinical practice. Grenz ray treatment has for a long time been a convenient method to treat eczema of the hands and it has been shown, in a double blind manner, that grenz rays have a significant effect in chronic eczema of the hands. (Lindelöf et al, 1987) and also on experimental pruritus (Fjellner et al, 1987).

C. SIDE EFFECTS OF GRENZ RAY THERAPY (VI)

After the introduction of grenz rays by Gustav Bucky in 1923 it was not until 1959 that the first instance of carcinoma of the skin was reported by Kalz, (Kalz, 1959). His patient, a dermatologist, had been notoriously careless about exposing his hands to the direct beam. It is impossible to estimate the total magnitude of such casual exposure. After 15 years, a squamous cell carcinoma developed on the distal phalanx of the index finger. The second case reported by Sagher, (Sagher, 1962) was also a dermatologist, who developed a squamous cell carcinoma of an index finger owing to overexposure to grenz rays. After these initial cases, only 8 cases of carcinoma of the skin in patients have been reported after grenz ray therapy, Table Ia.

In the present study the grenz ray therapy had been applied following strict

rules, respecting time intervals between the different series and honouring the dose for each session. The follow-up period was long - more than 15 years on an average - and the number of patients was large. The records had been kept very carefully by one and the same nurse, who had also given the treatment. According to Stern (1986) few other centers have this unique data source.

It is possible that grenz rays could induce basal cell carcinomas, but the cases reported in the literature had been mainly squamous cell carcinomas. Also, we cannot exclude an indirect effect of grenz rays on the development of skin malignancies, but the fact that only one of the high dose treated patients developed a cancer on a non-irradiated area does not support this hypothesis.

In the population studied a total of 8 malignancies appeared on grenz ray treated areas more than five years after the first treatment. None of these areas was high-dose treated. Six of the eight patients with malignancies on grenz ray treated areas had also received treatment with arsenic, conventional x-rays, UV-light and tars, which are known to be carcinogenic (Saunderson 1976, Albert 1976, Götz, 1976). Three of the patients had - apart from grenz rays - also received two known carcinogens on the site of the tumor. All of the patients had chronic irritative skin diseases, thus one can speculate whether the treated skin lesion itself may be a risk factor for cancer development. The frequency of other risk factors such as arsenic and ultraviolet radiation in this population has also made it difficult to verify whether grenz rays are a carcinogenic factor or not, especially as no dose-response relationship for grenz rays was found. However, the anatomical distribution on the lower legs of the squamous cell carcinomas in the study population suggests that grenz ray therapy cannot be excluded as a risk factor in the development of non-melanoma skin tumors. However, as many as 42% of the patients had received treatment on the lower legs and thus had their skin diseases located in this area, a circumstance which also may contribute to the carcinogenesis.

The study shows that grenz rays cannot be excluded as a risk factor in the development of non-melanoma skin tumors. However, this risk factor is small, if any, in case the above mentioned recommendations are followed, which are based on the standard regime used in our department.

107 patients who had been treated with grenz rays 20 years before examination, have also been investigated. The patients were examined for signs of atrophy, teleangiectasia, ulcers, malignancies, epilation and pigmentation at the site of the previous irradiation. In one patient a pigmentation of the scrotum could be caused by the grenz ray irradiation but no other changes were found on the irradiated sites (Lindelöf, 1987), compared to the corresponding control areas.

The mutagenic response of Ames Salmonella tester strain (TA 100) to grenz rays has also been investigated. Grenz rays were not mutagenic in that test-system, in contrast to UVA (Lindelöf et al, 1987).

GENERAL SUMMARY

The number of LC (OKT-6 positive cells) in human epidermis decreases after grenz therapy. It was shown that after a single dose of 4 Gy there was a slight reduction after 30 min and a very pronounced reduction of LC 1 and 3 weeks after irradiation. This reduction of LC reflected a true disappearance of the LC as shown by transmission electron microscopy. The few remaining LC appeared morphologically unaltered and there was no clear evidence of death of LC in situ. There appeared to be no consistent changes in keratinocyte morphology in the grenz ray treated area, as judged by nuclear size, cytoplasmic dense bodies and vacuolization. There were no conspicious changes of the intercellular spaces.

Grenz ray treatment was found to suppress the expression of nickel allergy totally in 5 out of 6 sensitive individuals. By using a panel of monoclonal antibodies (Leu 2, 3, 4, 7, Leu M1, B1, OKT-6) it was shown that in the non reactive grenz-ray-treated skin there was a pronounced reduction of LC, but no other differences were observed between control and grenz ray treated skin. This suggests that LC might be required also for the effector phase of the ACD.

In contrast to the strong suppression in ACD it was shown that grenz ray treatment only has a so far non-verified tendency to suppress irritant skin reactions in eleven healthy volunteers provoked by sodium lauryl sulphate. By using the same panel of monoclonal antibodies as in the study of ACD it was shown, however, that the number of LC had been reduced in epidermis to the same extent as in the study of ACD.

Sixteen patients with symmetrical psoriasis lesions of the scalp were treated with grenz rays. In a double blind fashion, one side of the scalp was irradiated with 4 Gy of grenz rays applied on 6 occasions at intervals of 1 week, and the other side of the scalp was given placebo treatment. The patients were seen before and after X-ray therapy. A significantly ($P < 0.0001$) better therapeutic result was recorded on the side of the scalp which had received active grenz ray therapy. In 14 of the 16 patients there was complete healing on the grenz ray-treated side after 6 wk of treatment. Nine patients were still free of lesions of the scalp 3 months after the start of the grenz ray therapy.

The records of 14,140 patients who have received grenz rays for the treatment of benign skin disorders formed the basis for an epidemiologic study of the incidence of skin malignancies in this population. The follow-up time was 15 years on the average. Information from the Swedish Cancer Registry (1958-1981) and the 14,140 patients was linked to identify individuals with malignant skin tumors occurring after the initial grenz ray treatment. In 58 patients, a malignant skin tumor was diagnosed more than 5 years after grenz ray therapy first had been administred. Nineteen patients had malignant melanomas and 39 patients had other malignant skin tumors. The expected number of melanomas was 17.8 and that of other malignant skin tumors was 26.9. None of the patients with melanomas and only 8 of the patients with other malignant skin tumors had received grenz ray therapy at the site of the tumor. Six of these 8 patients had also been exposed to other known carcinogens. Four hundred eighty-one patients had received an accumulated high dose of grenz rays ($\geq$100 Gy) on one and the same area. No malignancies were found on those areas. It is concluded that grenz ray therapy cannot be excluded as a risk factor in the development of non-melanoma skin malignancies, but this risk, if any, is small if recommendations for therapy are followed.

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