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PIXE Analysis in Uninvolved Skin of Atopic Patients and in Aged Skin

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PIXE (proton-induced X-ray emission) analysis was used to determine the elemental distribution in normal-appearing skin of patients suffering from atopic eczema and in the skin of elderly people. With this technique, elements with atomic numbers ≥ 14 can be detected simultaneously in cryosections of skin biopsies down to a concentration of 1 ppm.

Compared with a control group, the epidermal concentrations of Zn and Cu, which are constituent parts of a variety of enzymes, were increased in uninvolved skin of patients with atopic eczema. An increased concentration of these two metals might indicate that even in the epidermis of clinically normal skin of atopic patients, the content of certain enzymes is increased.

In the epidermis of elderly people the level of K was lower and that of Ca was higher than in the epidermis of a younger age group. The decreased K level may reflect a reduction of the intracellular volume in the epidermis of aged skin. As high Ca concentrations inhibit the proliferation of and promote the differentiation of keratinocytes, elevated Ca levels may be of importance for the age-associated decrease in epidermal turnover rate. ***Key words: X-ray fluorescence; Elemental distribution; Skin sections.***

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PIXE (proton-induced X-ray emission) analysis is a relatively new method with which to solve analytical problems in biological tissues. The principle of this method is the irradiation of a tissue sample with accelerated protons. If the protons transfer their energy to electrons of the sample atom, these can be ionized by creating voids in their atomic shells. When the voids are filled with electrons from outer shells, characteristic X-rays are emitted. As each element has a specific X-ray pattern, their amounts in the sample can be determined by the intensity of their X-ray patterns (1).

It is the main advantage of PIXE analysis that all elements with atomic numbers ≥ 14 can be determined simultaneously. Since detection sensitivity is in the order of 1 ppm, even trace elements such as Fe, Cu and Zn can be determined (2). For a single measurement an area of only some μm^2 of a cryosection is required. Therefore, not only mean elemental concentrations but also the elemental distribution within microscopical structures can be examined.

Because of the acuracy of the method and the small amount of tissue required, PIXE analysis has been employed in several dermatologic problems. Forslind et al. (3–4), Mahrok el al. (5) and Hong-Kou & Akselsson (6) reviewed the elemental distribution in normal human skin and in cross sections of hair shafts, respectively. Enderer et al. (7) were also able to identify gold deposits in the skin of 2 patients after gold therapy and Kurz et al. (8) described increased contents of Cu and Zn in the hair shaft periphery of a patient suffering from 'green hair syndrome'.

The elemental distribution in psoriatic skin has been investigated both with PIXE analysis (9) and with other X-ray fluorescence techniques (10). Kurz et al. (9), for example, found increased P and K concentrations in psoriatic epidermis and increased Zn levels in pinpoint lesions.

In this study, PIXE analysis was performed in normal-appearing skin of atopic patients and in aged skin, representing two common skin conditions in which, to our knowledge, elemental distribution has never been examined.

MATERIAL AND METHODS

Three groups of patients were included in the study, the first consisting of 4 patients (3 females, 1 male, age 19–46 years, mean age 31.5 years) who had a history of atopic eczema but were completely free from symptoms at the time of the study. The second group consisted of 5 patients (3 females, 2 males) aged at least 60 (64–83 years, mean age 73.6 years). These patients had been hospitalized in the University of Cologne, Department of Dermatology, for operations on skin cancer and were free from other dermatologic disorders.

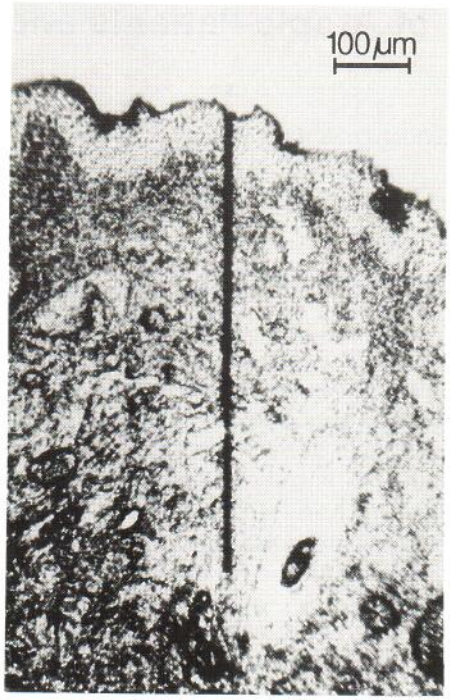

Fig. 1. Microphoto of a cryosection of uninvolved atopic skin after PIXE analysis. The dark line is composed of 60 spots which have been irradiated with the proton beam.

The control group consisted of 4 healthy young volunteers (2 females, 2 males, age 23–27 years, mean age 25.0 years). None of the patients or volunteers was using any external therapy at the time of the study.

Skin biopsies were taken from areas on the trunk, or the proximal parts of the extremities which had not been exposed to sunlight. In previous experiments, skin from the trunk and the extremities did not show any differences in elemental distribution. For local anaesthesia, 1% xylocain was used. Care was taken to inject the anaesthetic deep into the subcutaneous tissue to prevent it from penetrating into dermis and epidermis. Biopsies were immediately frozen and stored in liquid nitrogen (-196°C).

The further processing of the biopsy material and the determination of elemental distributions with PIXE analysis have been described in detail elsewhere (9). Briefly, cryosections 10 µm thick were cut and fixed to Formvar foils where they were allowed to thaw and to dry. Elemental distributions were determined with the PIXE method in combination with the Bochum proton microprobe (11). A beam current of 1 nA and a proton energy of 3 MeV were used. In each cryosection the amounts of P, S, Cl, K, Ca, Fe, Cu, and Zn were measured for 60 different spots which lay on a line perpendicular to the skin surface (Fig. 1).

The mass of the tissue which was irradiated for each measurement was determined from the intensity of the secondary electron *bremsstrahlung*. The lateral resolution ranged about 5×5 µm^2. Data were processed with a PDP 11/44/CAMAC system (12). After irradiation, microphotos of the cryosections were taken to determine the position of the *dermal-epidermal* junction.

Mean values of elemental concentrations were calculated for epidermis and dermis. For statistical analysis, Student's *t*-test was used.

RESULTS

Fig. 2 shows concentration profiles of K and Ca for 3 representative patients. From these plots, mean elemental concentrations in normal skin, normal-ap-

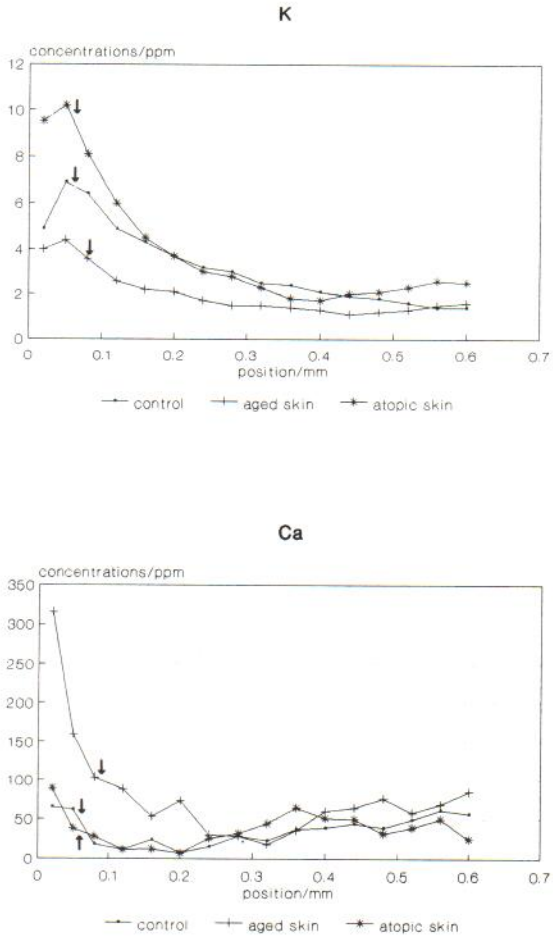

Fig. 2. Elemental concentrations of potassium (K) and calcium (Ca) in the skin of 3 representative patients. The X-axis starts at the stratum corneum of the skin biopsies. Black arrows mark the position of the *dermal-epidermal* junction.

Table I. *Elemental concentrations in the epidermis of normal, uninvolved atopic, and aged skin.* Values represent mean concentrations in μg/g dry weight; values in parentheses represent standard errors of the mean.

	P	S	Cl	K	Ca	Fe	Cu	Zn
Normal skin	1,600 (600)	4,300 (1,000)	9,300 (1,000)	6,800 (1,600)	43 (29)	710 (910)	7.3 (5.2)	42 (21)
Uninvolved atopic skin	2,100 (1,000)	4,500 (900)	8,400 (1,100)	7,900 (2,900)	37 (18)	370 (380)	16.0* (7.6)	92*** (8)
Aged skin	1,500 (600)	4,400 (1,200)	11,700 (3,800)	4,500** (1,100)	136** (49)	97 (34)	22.4 (23.7)	88 (71)

*$p < 0.10$, **$p < 0.05$, ***$p < 0.01$.

pearing skin of atopic patients and aged skin were calculated for epidermis and dermis and are summarized in Tables I and II, respectively.

In uninvolved atopic skin the epidermis showed significantly higher concentrations of Zn and Cu compared with those in normal skin. In the dermis of uninvolved atopic skin, no significant differences from the dermis of normal skin were found.

In aged skin, epidermis revealed reduced K and increased Ca levels. In the dermis of aged skin the concentration of Cl was found to be elevated; those of K and Fe were decreased. The differences in Cl, K and Fe concentrations, however, had only a low significance level.

The concentrations of the other elements determined in this study, viz. P and S, were not affected in epidermis or dermis of uninvolved atopic or aged skin.

DISCUSSION

The plots shown in Fig. 2 demonstrate that the elements are not evenly distributed over epidermis and dermis, but they do show characteristic concentration profiles. This observation has already been reported by other authors (3/4). In uninvolved atopic and aged skin the mean levels of several elements showed differences, compared with normal skin. The course of the concentration profiles, however, did not show striking differences. Therefore, subsequent discussion will deal with mean elemental levels of epidermis and dermis in different skin conditions.

In uninvolved atopic skin, alterations in elemental concentrations were found only in the epidermis. Here, the levels of Zn – and, to a lower extent, Cu – were increased. Both Zn and Cu are known to be constituent parts of a great variety of enzymes (13/14), for example DNA- and RNA-polymerases and cytochrome oxidase. Elevated Zn and Cu levels may therefore indicate increased amounts of these enzymes in the epidermis of uninvolved atopic skin. Zn (but not Cu) concentrations have already been reported to be elevated in some inflammatory skin disorders, for instance psoriasis (9/15) and prurigo nodularis (16). In the latter two dermatoses, however, Zn concentrations seem to be augmented only

Table II. *Elemental concentrations in the dermis of normal, uninvolved atopic, and aged skin.* Values represent mean concentrations in μg/g dry weight; values in parentheses represent standard errors of the mean.

	P	S	Cl	K	Ca	Fe	Cu	Zn
Normal skin	1,100 (900)	4,000 (1,200)	9,500 (2,200)	4,200 (1,700)	65 (13)	135 (107)	7.3 (3.3)	49 (39)
Uninvolved atopic skin	900 (500)	3,400 (500)	8,800 (1,500)	4,000 (1,400)	66 (12)	126 (37)	8.0 (2.7)	42 (24)
Aged skin	600 (200)	3,400 (700)	13.500* (3,600)	2,500* (400)	81 (18)	45* (7)	9.8 (2.9)	25 (4)

*$p < 0.10$.

in the active phases of the disease. Kurz el al. (9), for example, found elevated Zn levels only in pinpoint lesions, Molin & Wester (15) in untreated psoriatic lesions. Neither authors detected alterations in epidermal Zn content in uninvolved psoriatic skin. In prurigo nodularis, too, Zn concentrations are correlated with the clinical severity of the lesions (16). The results of the present study show that, unlike psoriasis and prurigo nodularis, epidermal Zn levels are increased in the skin of atopic patients free from clinical symptoms at the time of the investigation. This could mean that the amount of certain Zn – dependent enzymes is increased in atopic skin, even during disease – free intervals.

In both epidermis and dermis of aged skin, K concentrations were found to be reduced. This may reflect a decrease in the K-rich intracellular volume compared with the K-poor extracellular volume in aged skin. The increased Ca concentration in the epidermis of aged skin may be of importance for epidermal proliferation. In vitro experiments with human (17) and mouse keratinocytes (18) showed that reduced Ca concentrations accelerate the proliferation of keratinocytes, whereas increased Ca concentrations promote their differentiation. By this mechanism the increased Ca level may be one of the reasons for the reduced epidermal turnover rate in aged skin (19).

The present study shows that alterations in element distribution are not dependent on visible disorders, but can also, for example in atopic eczema, be found in skin which lacks clinical symptoms at the time of the investigation. This observation supports the thesis that variations in elemental concentrations are not only epiphenomenons of skin diseases, but may even play a role in their pathogenesis.

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