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PHYSIOLOGICALLY IMPORTANT TRACE ELEMENTS OF PARALESIONAL PSORIATIC SKIN

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Abstract

The elemental distributions over epidermal skin cross sections as revealed by proton probe analysis on cryo-sections from human skin provides new insight into the physiology of skin. The present investigation shows that in comparison with skin from non-afflicted individuals non-lesional skin from psoriatics reveals abnormality (higher than normal levels) in calcium (Ca), iron (Fe) and zinc (Zn). In addition, the abnormality is not only present in the quantity recorded but also in the particular distribution of these elements. Thus, the stratum corneum contains appreciable amounts of Fe and Zn in the skin of psoriatics, a finding never recorded in normal skin. The increased Ca levels may be related to the phenomenon of "programmed cell death" which will be the end point of the differentiation process in the epidermis. The Fe and Zn findings may be interpreted as defects in the retaining mechanisms for these valuable trace elements. The recorded defects in trace element distributions can be regarded as new additions to the already known spectrum of abnormality in the genetics expressed as the disorder of psoriasis.

Key Words: Psoriasis, trace elements, Ca^{2+}, Zn^{2+}, Fe^{2+}, X-ray microanalysis, particle probe analysis, PIXE (proton induced X-ray emission).

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Introduction

At a basic level, the fundamental physiological factors involved in the pathogenesis of psoriasis are still unknown. A great deal of research has been focused on the immunological events coupled to the evolving psoriatic lesion. Thus, it has been demonstrated that in very early lesions a chemotactic attraction of T-cells (CD 4+) is operating directed towards the epidermis. These T-cells originate from the blood stream. A sequel inflammatory response is then stimulated by cytokines from different kinds of cells which results in a proliferation of keratinocytes in the basal region [14]. The (patho-)physiological process resulting in this activation of the T-cells is not known.

A direct physiological approach to the study and understanding of the disease has been impeded by the physical dimensions of the epidermis, e.g., the cellular part of the skin which is only approximately 120 μm thick [7, 23]. The situation is further complicated by the fact that the epidermis contains layers of cells at different stages of differentiation. Thus, bulk methods, which sample the entire cross section of the epidermis as one unit, do not make it possible to differentiate between the epidermal strata [12]. Consequently, bulk methods provide analysis results which do not allow a functional analysis based on elemental contents. Conventional physiological capillary probes generally cannot be located with any satisfactory precision, within a specified cell of a specified skin layer in normal or in pathological conditions. Other means of exploration are therefore necessary for the study of skin physiology. Particle probe analysis on quench-frozen and freeze-sectioned, freeze-dried material provides such a practical approach that allows a "snap-shot" view on the physiological status of the tissue [5, 16, 24].

Particle probe analysis allows only the detection of elements but does not provide information on the chemical status of the elements [5]. However, biochemistry provides information that allows elemental distribution data to be interpreted in terms of ion distributions and physiological function of the tissue. The elemental distributions over the cross section of psoriatic skin has been studied using X-ray microanalysis (XRMA) in the electron microscope [3, 11]

and using proton induced X-ray emission (PIXE) analysis [15, 24], in most cases in the spot mode. Whereas XRMA allows a high spatial resolution, it has comparatively low sensitivity, i.e., ~200 ppm. In contrast, PIXE has a high sensitivity, around 1 ppm, and therefore allows determination of trace elements such as Ca, Fe, and Zn. The spatial resolution of PIXE is generally taken to be lower than that of XRMA in order to achieve sufficient signal-to-noise ratio.

Trace elements have been subject of special interest in recent years. Calcium (Ca^{2+}) and zinc (Zn^{2+}) has proven to be a very important signaling substance in a great variety of cellular systems [1]. The Ca distribution over the skin cross section has been studied in the transmission electron microscope (TEM) using histochemical methods [18] in order to ascertain its role in the differentiation process of the epidermis. Additional properties of this particular ion are currently the subject of intensive studies in general cell biology as well as in experimental dermatology. Thus, the importance of Ca^{2+} to "programmed cell death," has been suggested [2]. Obviously, the final step of differentiation between the stratum granulosum level and the stratum corneum represents a particular aspect of "programmed cell death."

Concerning other trace elements, there are old suggestions of an inference of the skin's dependence on appropriate availability of zinc (Zn^{2+}) for normal function and at wound healing [10, 21, 22, 25]. Finally, there are the findings of substantial iron (Fe)-losses via psoriatic lesions [19]. For such reasons, we have pursued PIXE studies of normal human skin and skin disorders, including psoriasis and atopic dermatitis, to provide an understanding of the formation of a mature stratum corneum with a functional barrier [4, 6, 7, 9] as reflected in physiological/biochemical mechanisms controlled by trace elements, e.g., Ca^{2+}, Fe^{2+} and Zn^{2+}.

In the present study, our aim has been to investigate physiological parameters represented by the elemental and trace elemental distributions over cross sections of paralesional psoriatic skin, and as controls, skin from normal, non-afflicted individuals. We sought to find very early changes in the psoriatic skin as expressed by physiological/biochemical mechanisms active before the immunological system has been alerted.

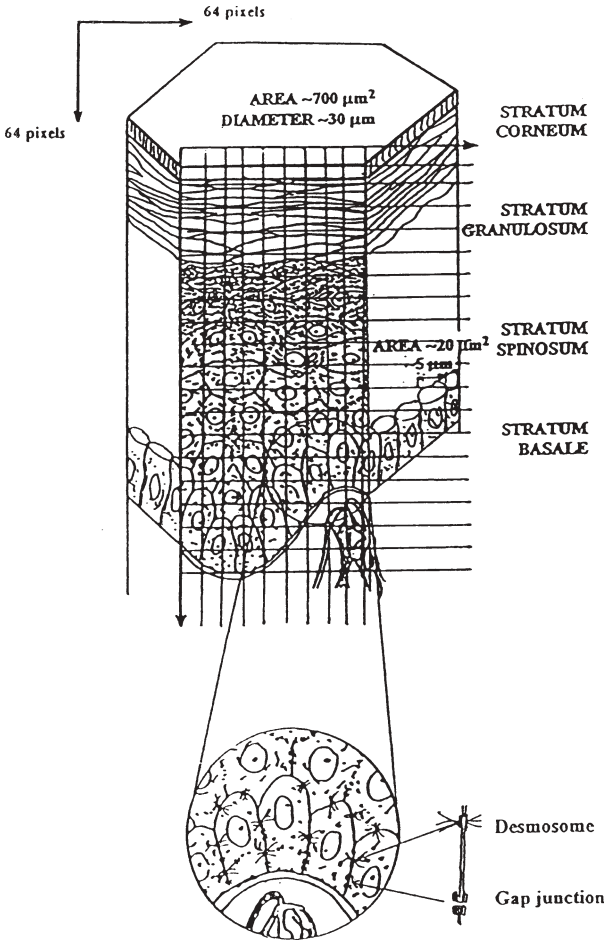

Figure 1. Anatomy of a skin cross section including the principle of pixel mapping. A pattern of pixels, usually 64 x 64, is laid out over a skin cross section. From each pixel, intensity data can be retrieved on all elements included within the volume covered by the pixel.

Materials and Methods

After subcutaneous infiltration of the chosen area with 10 mg/ml lidocaine in 5 mg/ml adrenaline using 4 mm punches, skin biopsies were immediately obtained from the lower back in 3 psoriatics at least 3 cm from lesions or lesional rests. Biopsies for control were taken from a corresponding area of 3 volunteers with no records of skin disorder. The biopsies were immediately quench-frozen in liquid nitrogen and stored in a deep freezer at -20°C. Sectioning was performed at -20°C in a conventional cryostat with a nominal section thickness of 16 µm. The sections were transferred to specimen support rings covered with Kimfol® (Kimberley Clark, Neenah, WI) foil, which gives virtually no contribution to the particle induced X-ray spectrum. The complete specimen support sandwich was stored in sealed vessels with drying material to prevent water uptake until analysis.

The new Lund Nuclear Scanning Microprobe facility provided a 2.5 MeV proton beam for irradiation of the specimens. The proton beam was focused by quadropole electromagnets to a nominal beam cross section of 5 µm x 5 µm, and the beam current on the specimen was 0.5 - 1 nA. The rectangular beam scan was located over a selected area

on each skin section so as to cover a cross section of epidermis and dermis down to the reticular dermis (Fig. 1), which corresponds to a total depth of about 200 µm. To minimize the thermal load on the specimen, a scanning mode for element excitation and data acquisition was used. Thus, the probe irradiates each specimen volume (pixel) on the average 5 ms (milliseconds) in an iterate process. The pixel size was chosen so as to avoid overlap of the beam penumbra (8 µm in the X- and Y-directions) and typical acquisition times for a pixel map (64 x 64 pixels) was between 30 minutes to 2 hours. The beam charge was collected in a Faraday cup positioned after, and in line with the sample. The induced X-ray radiation (PIXE) was detected and quantitated by a Kevex (Kevex Instruments, Scotts Valley, CA) energy dispersive system, and the mass cross section at a point (i.e., specimen sub-volume) of analysis was determined by the backscatter (BS) which was subsequently used for mass normalization of the samples [26]. Beam scanning control and data collection was performed using a VME bus computer [17] (Motorola, Schaumburg, IL) while storage and sorting was done on a DEC 3000 Alpha AXP work station (Digital Equipment Corp., Hudson, MA).

During specimen irradiation with the scanning proton beam in an iterative way, the data acquisition system sorted the produced information into elemental pixel maps (Fig. 2). The complete X-ray data (the relative elemental content) and BS (the mass cross section) data for corresponding pixels were merged into a number of pixel spectra evaluated by computer fitting using HEX [13]. In addition, from the elemental maps produced, data from pixel arrays representing a cross section of the skin are presented as cross section distributions of mass and elements (Fig. 3). In a corresponding manner, pixel arrays representing similar structures (e.g., parallel band in the epidermis) were chosen for presentation of the data on the quantitative elemental content of a specified cellular layer [e.g., a horizontal layer: stratum basale, stratum spinosum or stratum corneum (Fig. 4)].

Results

The sensitivity of the PIXE method is to a certain extent dependent on the acquisition time at analysis. In the present experiments, the time frame used has not allowed detection of copper (Cu) and magnesium (Mg) above their respective thresholds in most of the individual experiments. We have, therefore, chosen not to indicate the spurious presence of these elements.

To illustrate the fact that there is a considerable variation (i.e., ranges of content) in trace elemental content in different strata and also within a particular stratum due to the actual metabolic status of particular cells, we have compiled data in Table 1.

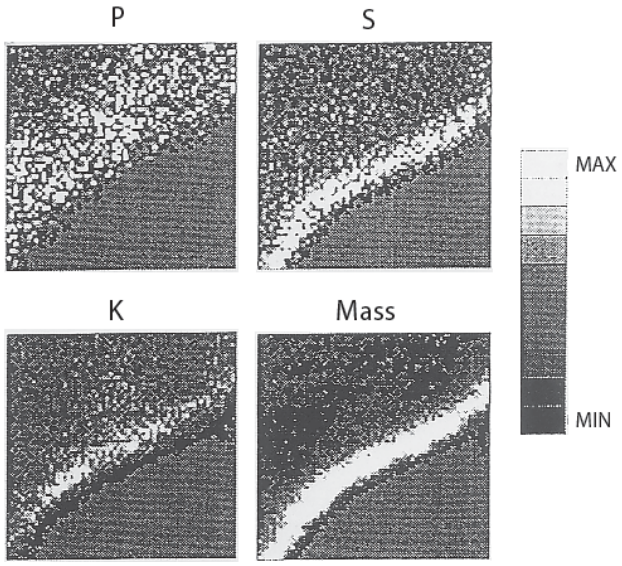

Figure 2. Pixel map of P, S, K, and mass density distribution laid out over a skin cross section from clinically normal paralesional skin of a psoriatic patient.

Cross section elemental distributions

Normal skin The mass distribution curves peak in the stratum corneum region and flatten out in the basal cell region, again to rise in the dermis. The sulfur (S) distribution curves follow the mass curves in concert (Fig. 3a).

The phosphorus (P) distributions peak somewhat 50 µm below the maximum mass peak at a region corresponding to the upper stratum spinosum.

Chlorine (Cl) has a weak minimum approximately where the P distribution has its peak (Fig. 3a). There is a conspicuous drop in the Cl content in the vicinity of stratum corneum. Conversely, the Cl content rises in the dermis compared to the epidermal content. Potassium (K) reaches the highest levels in the Malpighian layers, drops nil in the stratum corneum and shows relatively low values in the dermis, i.e., the extracellular compartment.

Iron (Fe) has a high peak value in the basal cell region and drops to values less than half the peak value in the uppermost epidermal layers and is not detectable in the stratum corneum region (Fig. 3b). Zinc (Zn) shows a comparatively stable level over the Malpighian epidermis and disappears coincidentally with Fe in the stratum corneum region. Copper (Cu) is just barely detectable in the Malpighian layers, but no quantification is possible with the data available in these experiments.

Calcium (Ca) maintains a relatively constant level up to the stratum corneum and lingers somewhat closer to the skin surface than the trace elements Fe and Zn, although

Table 1. Elemental content of normal and psoriatic epidermis, PIXE analysis. Data in g/g dry weight; DL: detection limit.

	Normal, basal layer		Normal, str. spinosum		Normal, str. granulosum/corneum	
	Mean ± SD	**Range**	**Mean ± SD**	**Range**	**Mean ± SD**	**Range**
Ca	320 ± 77	160 - 455	234 ± 120	43 - 352	351 ± 107	252 - 582
Fe	105.0 ± 53.7	12.2 - 228.8	35.0 ± 16.0	DL - 51.6	16.6 ± 10.6	116 - 30.6
Zn	23.1 ± 27.7	DL - 86.4	45.3 ± 43.1	DL - 113.2	28.5 ± 13.1	15.2 - 61.2
	Psoriasis, basal layer		**Psoriasis, str. spinosum**		**Psoriasis, str. granulosum/corneum**	
	Mean ± SD	**Range**	**Mean ± SD**	**Range**	**Mean ± SD**	**Range**
Ca	1073.9 ± 553.6	267.7 - 2187	727 ± 183	92.0 ± 1075	841.0 ± 156.5	449.6 ± 928.4
Fe	161.9 ± 83.6	26.8 - 270.4	128.5 ± 78.0	55.4 ± 279.7	45.8 ± 22.5	16.6 ± 81.7
Zn	35.4 ± 56.5	DL - 176.7	50 ± 50	DL - 174.9	97.0 ± 40.4	DL - 180.9

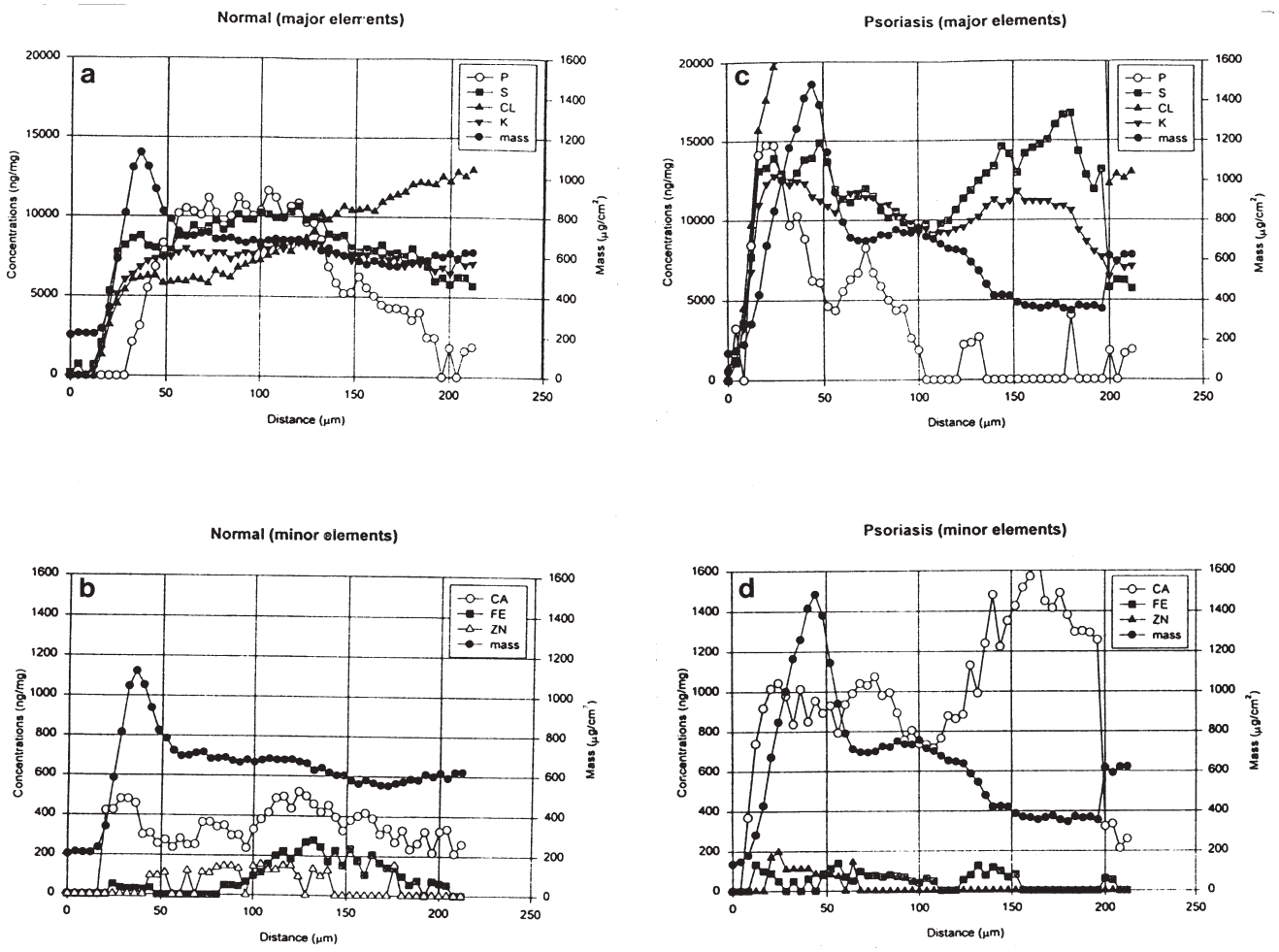

Figure 3. (**a**) Mass distribution, element; (**b**) trace element data extracted from a pixel map of a normal skin cross section; (**c**) mass distribution, element; and (**d**) trace element data extracted from a pixel map of a clinically normal skin cross section from a case of psoriasis.

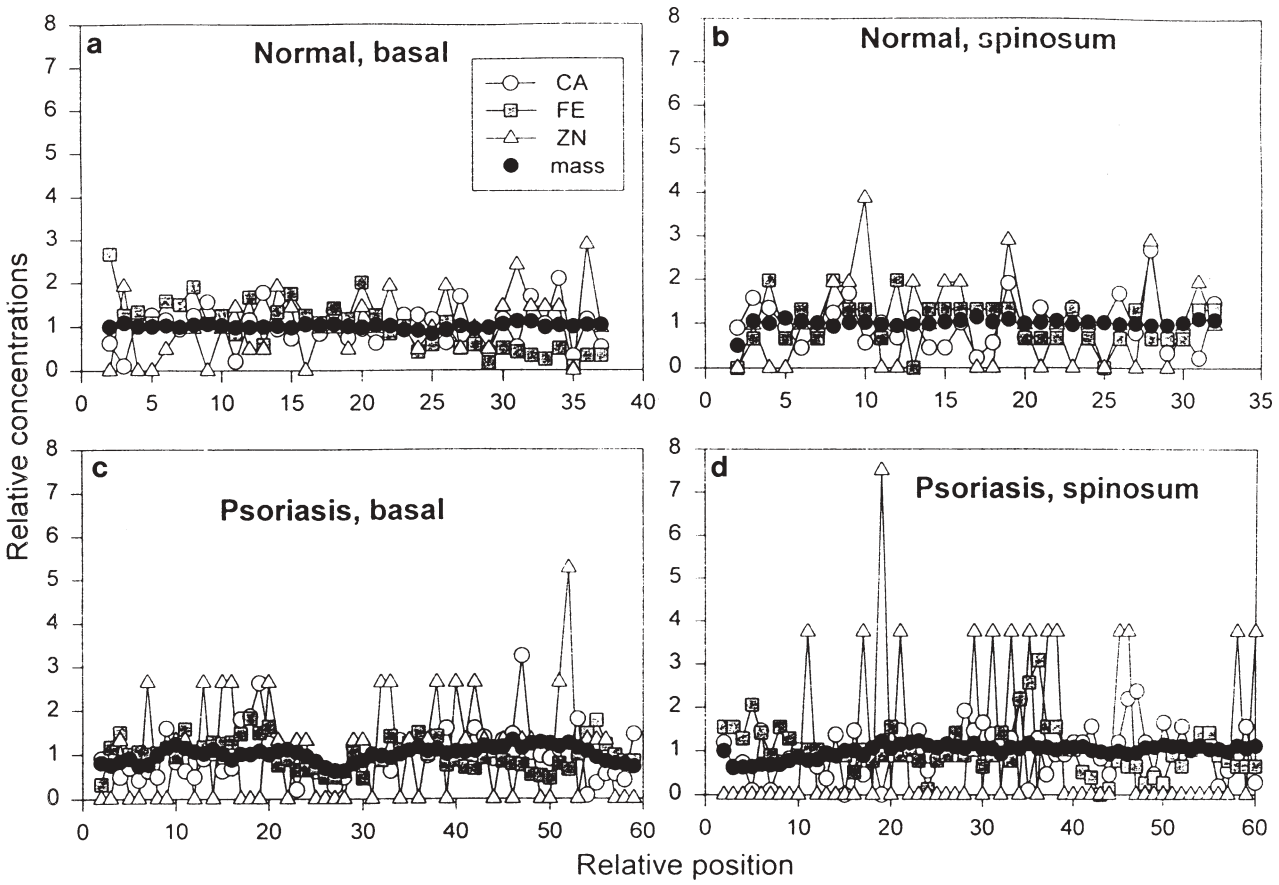

Figure 4. A horizontal channel extracted from a pixel map of normal (**a**, **b**) and paralesional psoriatic (**c**, **d**) skin, representing the basal (a and c) and stratum spinosum (b and d) regions.

close to the detection limit.

Looking at the elemental distribution patterns from one section to another and from one subject to another, there are slight but obvious variations in concentration values, although a general trend can clearly be discerned. Thus, the Fe distribution in a few cases has its center of gravity in the stratum spinosum/stratum granulosum area rather than in the basal cell region. In contrast, Zn is confined to the basal layer.

Paralesional normal looking skin from cases of psoriasis The mass distribution has the same general features as that of the normal skin although generally at a lower level (absolute mass content). The P and S distributions are not conspicuously different from those of normal skin (Fig. 3c).

In contrast to the Ca distribution in normal skin, that of psoriasis follows the mass distribution more closely (Fig. 3d). Ca shows a two-fold or even higher increase in stratum granulosum region compared to normal skin. In many sections, there is an additional Ca peak in the vicinity of the basal cell layer.

The main Fe peak appears closer to the mass distribution peak than in the normal skin (Fig. 3d). There are obvious variations in the Fe content of different strata (cell layers), and the lowermost values are consistently at least twice as high as in normal skin. The Zn content is increased especially in the stratum spinosum, except in one single section where the Zn follows suit with Fe distribution.

Although not shown here, it may be stated that the Cu concentrations are, as a rule below the detection threshold. Cu is therefore not considered in this context.

Horizontal elemental distributions

Normal skin The mass distribution along the basal cell layer varies minimally along the horizontal scan (Fig. 4a) and remains fairly constant at the upper level (the level of the stratum spinosum/granulosum). Potassium (K) and chlorine (Cl) are covariant with mass in the basal region but more independent in the upper region. Sulfur (S) co-varies to a great extent with the mass distribution. In both regions (basal and upper), more conspicuous variations in relation to mass are seen in the phosphorus (P) distribution.

Table 2. Elemental content of skin. Data compiled from ref. [12].

Element	Content range (in ppm)	Method
Ca	0.034 - 20	AES/XRF
Cl	2654 -	SAS
Cu	6.9 - 143	AAS/AES
Fe	34 - 1900	AAS/AES
K	0.846 - 118	AES/FES
Na	1016 - 4968	FES/misc.
P	0.3 - 40	AES/SAS
S	1577 - 1850	SAS/misc.
Zn #1 (whole skin)	6.1 - 1000	AAS/NAA
Zn #2 (epidermis)	39 - 83	NAA
Zn #3 (dermis)	13 - 23	NAA

AAS: atomic absorption spectrometry; CAT: catalytic method; FES: flame emission spectrometry; NAA: neutron activation analysis; SAS: solution absorption spectrometry; XRF: X-ray fluorescence spectrometry; misc.: miscellaneous.

Iron (Fe) and zinc (Zn) distributions vary extensively in the basal region (Fig. 4b). The Fe content is close to or below the detection limit in the upper region, the Zn content shows some peaks above the detection limit. Single off-limit values in these trace elements were seen. Interestingly, calcium (Ca) appears to stay rather constant within each horizontal scanned band.

Psoriatics normal-looking skin The mass distribution pattern essentially follows that seen in the normal control skin with somewhat more variability in the upper layer (Fig. 4c).

In comparison to the control skin, there are high mean values and prominent variations in the trace elements, notably Fe and Zn in the upper level of the epidermis (Fig. 4d). In comparison to the control skin, where copper (Cu) was a spurious finding, there were more spots of Cu above the detection limit in the psoriatic skin sections both in the basal and the upper level (not shown).

Discussion

A full understanding of the influence of trace element content on the differentiation process of human skin has not yet been reached. In the past, the attempts to analyse the skin content of physiologically important elements and trace elements have essentially been restricted to bulk methods [12]. Compilations of such data show such great ranges of elemental contents, and it appears that the ranges are more a matter of the manner in which the material has been collected than related to the sensitivity and precision of the methods used (Table 2). A biological interpretation of such data is impossible.

The skin's dependence on appropriate availability of Zn^{2+} for normal function [10, 22, 25] is a subject which has not been resolved. The importance of Ca^{2+} to promote and Zn^{2+} to inhibit "programmed cell death" has also emerged as a topic of interest [2]. Facts such as these have prompted us to study normal-looking skin from psoriatics as well as skin from non-afflicted individuals for controls. The ultimate aim of our studies is to provide an understanding of the physiological/biochemical mechanisms behind the properties of changed skin in patients afflicted with skin disorders of genetic and constitutional origin. To our knowledge, this investigation is the first to present elemental distribution maps of skin cross sections from both normal and pathological skins.

The new scanning nuclear microprobe in Lund which collects data in a format of pixel maps provides extensive possibilities for subsequent analysis of the primary data. The two-dimensional pattern of data constituting a map of elemental distributions (Fig. 2) can be directly compared with the mass distribution map which represents the morphological structures from which data are derived. The data used for the elemental profiles (Fig. 3) have been extracted from the elemental maps by choosing a "channel" of pixels representing an interfollicular area free from hair follicles. In a corresponding way, we have assessed the horizontal variation of different elements in a specified layer of the differentiating epidermis by selecting pixel "channels" parallel to the basal lamina and the stratum corneum representing the basal cell layer and the stratum spinosum, respectively (Fig. 4).

The number of specimens investigated in a PIXE study may appear small in comparison with corresponding numbers used in different types of light microscopic investigations or biochemical studies. It must be realized that the typical acquisition times for a pixel map from a single section is generally at least 2 hours. The costs/benefit aspects of running an experiment will require optimization of data acquisition for evaluation. This is a limiting factor for the number of items that can be analysed. For a number of data, that would allow statistical analysis by the algorithm used, the time allowed was not sufficient in this present study. Consequently, a trace element such as Cu which was only occasionally detected above the threshold of sensitivity in our experiments had to be excluded from the present study. However, the interesting indications of higher than normal content of Cu in the paralesional psoriatic skin

Table 3. Elemental content of psoriatic epidermis, nuclear activation analysis. Data in g/g dry weight compiled from refs. [19] and [20]. SD = standard deviation.

	Normal		Psoriatic uninvolved		Psoriatic involved	
	Mean ± SD	Range	Mean ± SD	Range	Mean ± SD	Range
Fe	26.53 ± 6.7	16.5 - 30.5	30.62 ± 12.4	17.0 - 46.4	57.51 ± 26.3	27.5-97.8
Cu	3.76 ± 0.83	2.6 - 4.6	4.35 ± 0.57	3.7 - 5.1	4,27 ± 1.22	3.0-7.4
Zn	39.3 ± 12.4	28.7 - 59.2	41.0 ± 7.11	34.1 - 48.3	90.8 ± 48.2	46.6-198.7

suggest that Cu is an interesting element to explore in future studies.

The nominal probe size used in the experiments reported here was 5 µm x 5 µm, to which must be added a halo region of approximately 2 µm. This means that the spatial resolution of the PIXE analysis system is low when compared with the resolution obtainable with an XRMA system, which allows subcellular compartment (< 1 µm) analysis. The PIXE analysis thus superposes the intra- and extracellular compartment data during analysis, depending both on the comparatively low spatial resolution of the measuring system and on the thickness of the sections (~16 µm) which of necessity must contain both compartments in a cross section. These facts relate to the considerable smothering of curves describing the elemental and mass distribution over the cellular layers of a differentiated epidermis (Fig. 3). The effect is perhaps most conspicuous in the very narrow stratum corneum region (a total width in a section of approximately 10 µm) where the mass curve is rather wide in these experiments. The variation in the mass distribution in the basal cells corresponds to the fact that these cells are involved in the cell division cycle. In the present study, we did not employ a spatial resolution that allowed identification of single cells separated from the intercellular space or neighboring cells. Details related to the different stages of the cell division cycle are therefore not possible to give. It is to be expected that once the progeny has entered the stratum spinosum, a higher degree of synchronization of the physiological events, including protein and lipid synthesis should be at hand.

The mass distribution curves peak in the stratum corneum region, subsequently flatten out in the basal cell region, and finally rise somewhat again in the dermis. This form of the mass distribution curves is well related to the morphology of human skin. The basal cells have comparatively low mass content, but as the protein (keratin) synthesis progresses, there is a continuous increase in cellular mass that reaches a peak in the lower part of the stratum corneum. Here, the cellular content is compacted and contains a smaller amount of water [27]. Going from the basal cell layer downwards into the dermis, we find that the papillary dermis is a relatively open compartment dominated by ground substance with a low content of fibrous (collagen and elastin) material. Approaching the reticular dermis, we find that the density of fibrous proteins increases, corresponding to the mass increment seen in our curves.

Bearing in mind that the spatial resolution does not allow discrimination between the intra- and extracellular compartments, our data nevertheless reveal some crucial points concerning the physiology of normal appearing psoriatic skin, as contrasted to the skin of non-afflicted individuals. Of special interest is the Ca distribution profile, which in the normal skin remains at an almost constant level over the skin cross section, but in certain specimens, shows a slight increase in the stratum granulosum region. The present findings differ somewhat from our earlier findings in a preliminary study [8] based on selected point measurements in different strata of skin sections. However, with the new information obtained from the elemental maps such a variation is likely to occur as an expression of the continuous changes occurring *in vivo*. These continuous changes are represented in studies like this using quench frozen specimens by a "snap-shot," depicting momentarily what are actually transient processes in the metabolically active tissue.

A salient feature of biological tissues is variation in morphological and physiological features within the range that are characteristically considered to be normal. One conspicuous feature of our maps is the variation in the distribution of elements and trace elements seen in the different sections. This is a finding occurring both in the normal skin as well as in the clinically normal psoriatic skin. Variations in the distribution of elements and especially trace elements, may be interpreted to indicate that there are obvious differences in the detailed cellular physiology of the differentiating keratinocytes. This interpretation is underlined by our previous experience from XRMA studies of contact allergic reactions which show that the correlation between the morphological image and the quantitative elemental data is not a direct one [5, 16]. Thus, in certain stratum cells that morphologically appear to be similar, their different physiological status in the patterns of elemental distributions are revealed, as suggested by the elemental maps and the horizontal distribution spectra (Fig. 4). Future

investigations using, e.g., immunological techniques to tag different markers of differentiation in parallel with elemental analysis, should provide an additional detailed insight into the keratinization process programmed to develop a complete stratum corneum with a functional barrier.

In the past, only occasionally has interest been focused onto the elemental content of epidermis. The pioneering study on psoriasis by Molin and Wester [19, 20] using neutron activation analysis represents one such attempt. Since the material analysed had its origin in suction blister roofs, these data relate to the bulk content of the main part of epidermis including the stratum corneum (Table 3). Hence, gradients of the elements cannot be detected. Analysis performed with particle probes on skin cross sections allows recognition of elemental (ion) distributions in contrast to these earlier investigations performed on bulk specimens. It is interesting to note that our present data to a great extent are compatible with those given by this previous work [19].

The previously reported finding that psoriatic patients lose iron (Fe) through the shedding of stratum corneum cells in lesional areas [19] was given an interesting corollary in a recent preliminary PIXE investigation where clinically normal skin in psoriatic patients were shown to contain higher than normal amounts of Fe [24]. We have been able to verify this previous finding in our present investigation (Fig. 3d).

In a previous XRMA study [11], it was shown that the concentrations of Mg, P and K were higher in the stratum germinativum, spinosum and granulosum compared to corresponding strata in uninvolved psoriatic skin. Concerning stratum germinativum, no conspicuous difference was noted between either non-involved or involved psoriatic skin as compared to non-psoriatic control skin. In comparison to uninvolved psoriatic skin, the various strata of involved psoriatic skin showed a pattern of elemental distributions typical for highly proliferative neoplastic cells. Special notice may be taken of an increased Ca level in the upper strata of the epidermis of uninvolved psoriatic and an unusually high P level of the stratum corneum in the lesional psoriatic skin. The high P values may be correlated to the presence of nuclear material in the diseased stratum corneum.

An interesting aspect of the Ca and Zn distributions is related to recent findings in lymphoid tumor cell lines which have been shown to suffer apoptosis as a result of increased Ca levels [2]. However, this effect was counteracted when the Zn levels were increased. The ratio of Ca/Zn in stratum corneum of paralesional psoriatic skin is approximately 8:1 compared to 12:1 in normal skin. This suggests that the balance of Ca and Zn is shifted more towards favoring Zn, and that the differentiation process in the paralesional psoriatic skin may actually be an example of disturbed "programmed cell death," hence the finding of sporadic acanthotic cells in this skin. Though it is still speculation that this effect is directly coupled to increased cellular activity in the germinative pool, the actual high levels of Fe compared to normal skin suggest such an increased activity. Kurtz *et al*. [15] in their PIXE study of psoriasis showed that Zn levels were elevated in pinpoint lesions but remained at normal levels in the control and old plaque specimens as compared to the control. No Fe distributions were given, and the bulk data indicates a high degree of variation in the controls and lower Fe values in the afflicted skin compared to uninvolved psoriatic skin. Obviously, more detailed particle probe analyses, preferably coordinated with biochemical/immunological analysis, of the tissues are required before the questions concerning levels of Fe and Zn content as related to cellular activity and elemental distributions can be settled.

It is obvious that these initial experiments call for analysis of a larger population of psoriatics. Not only the clinically normal skin, but also lesional skin merits such investigations. Future studies should help to relate the variations in elemental content of specific strata to the anabolic and catabolic processes that are the metabolic expressions of cellular differentiation, e.g., apoptosis.

Conclusions

The Lund microprobe scanning system allows PIXE analysis that produces a wealth of data allowing functional analysis of the tissue physiology. A determination of quantitative elemental content of the skin cross section, e.g., from stratum corneum down into the dermis, is possible to extract from the maps choosing interfollicular areas (Fig. 3). Horizontal scans taken from the different strata of the epidermis skin, i.e., parallel to the basal lamina (which is the border between the cellular epidermis and the essentially non-cellular connective tissue of the dermis; Fig. 4), or the stratum corneum gives an opportunity to study different stages in the differentiation process. The dynamics of the differentiating epidermis expressed as redistributions of ions thus provides a more detailed image of the cellular events than morphology has hitherto given.

Normal-looking psoriatic skin harbors a number of defects which in response to (a) crucial trigger(s) causes an outbreak of a psoriatic lesion. Using the Lund scanning microprobe system, we have been able to present elemental data which reflect this basic disturbance as abnormal trace element distributions. PIXE thus represents a new method for obtaining physiological information about skin disease that should complement existing biochemical and immunological approaches in the study of genetic dermatoses.

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Discussion with Reviewers

J. Trevithick: Does the prepsoriatic or psoriatic skin increase

in thickness? Could such a possibility exist, and if so how would it affect the results?
Authors: Our material is too small for a statistical analysis of the thickness on normal-looking skin from psoriatic individuals as compared to normal skin, but our impression is that there is no such difference. If there is no specific indication of an accelerated cell division cycle, we expect no significant deviation from the data presented here.

J. Trevithick: Could part of the increased iron concentration be a result of an erythema-like reddening of the skin which would not be visually perceptible? How might you control for this possibility?
Authors: If the kind of phenomenon you suggest would be at hand, we would expect an increase in the iron content of the dermis due to an increased presence of hemoglobin or derivatives of that in the connective tissue. This we do not see in our maps of skin cross sections which cover as broad a band of the dermis as the epidermis is wide.