LOCALIZED SCLERODERMA (MORPHOEA)

Clinical, physiological, biochemical and ultrastructural studies with particular reference to quantitation of scleroderma

by

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III Serup J. (1984) Decreased skin thickness of pigmented spots appearing in localized scleroderma (morphoea). Measurement of skin thickness by 15 MHz pulsed ultrasound. Archives of Dermatological Research 276:135-137.

IV Serup J. & Northeved A. (1985) Skin elasticity in localized scleroderma (morphoea). Introduction of a biaxial in vivo method, and measurement of tensile distensibility, hysteresis and resilient distension of diseased and normal skin. The Journal of Dermatology (Tokyo) 12:52-62.

V Serup J. & Kristensen J. K. (1984) Blood flow of morphoea plaques as measured by laser-Doppler flowmetry. Archives of Dermatological Research 276:322-325.

VI Serup J. (1984) Localized scleroderma (morphoea): Studies of eccrine sweat gland function and skin microtopography. The Journal of Dermatology (Tokyo) 11:269-276.

VII Serup J., Ammitzbøll T. & Møller R. (1984) Collagen specific amino acids in skin in localized scleroderma. Acta Dermato-Venereologica 64:99-106.

VIII Møller R., Serup J. & Ammitzbøll T. (1985) Glycosaminoglycans in localized scleroderma. Connective Tissue Research 13:227-236.

IX Kobayasi T. & Serup J. (1985) Vascular changes in morphoea. Acta Dermato-Venereologica 65:116-120.

X Kobayasi T. & Serup J. (1983) Nerve changes in morphoea. Acta Dermato-Venereologica 63:321-327.

XI Serup J. & Serup L. (1985) Increased central cornea thickness in localized scleroderma (morphoea). Metabolic, Pediatric and Systemic Ophthalmology 8:11-14.

XII Ammitzbøll T. & Serup J. (1984) Collagen metabolites in urine in localized scleroderma (morphoea). Acta Dermato-Venereologica 64:534-569.

In the survey, the publications will be referred to by their preceding Roman numerals.

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PREFACE

In December 1979 Professor Gustav Asboe-Hansen, Head of the Department of Dermatology H, Rigshospital, Copenhagen extended to me the privilege of studying the localized form of scleroderma. Without hesitation I accepted this invitation to become an active participant in the scientific milieu of this centre of scleroderma and connective tissue research, which he had established. I am very grateful to Professor Asboe-Hansen both for his initiation of the study and for his continuous interest and support of my work.

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Jørgen Serup

CHAPTER 1

Introduction

Scleroderma (Greek *sklēros* = stiff, *derma* = skin) is a group of diseases characterized by a diffuse or circumscribed alteration of the skin with white colour, induration, and an appreciation of increased skin thickness. The histopathological background is accumulation and disorganisation of collagen in the dermis associated with regressive changes of the epidermis and the skin appendices.

Scleroderma is divided into generalized or systemic forms and into localized forms. In *generalized scleroderma (progressive systemic sclerosis)* skin alterations are widespread and diffusely demarcated, in most instances with major affection of acral regions (acrosclerosis) and Raynaud's phenomenon. Internal organs like the esophagus, the lungs, the kidneys, the heart, and the intestines are involved (Kinder & Fleischmajer 1974; Masi et al. 1979). The prognosis with respect to life of generalized scleroderma is serious (Medsger et al. 1971; Bennett et al. 1971; Rowell 1976). In *localized scleroderma* skin affections consist of plaques, lines or guttate lesions with a sharp demarcation to the surrounding, normal appearing skin. Internal organs are not considered diseased, and the prognosis appears favorable with spontaneous remission in most instances (Piper & Helwig 1955; Curtis & Jansen 1958).

Generalized and localized scleroderma are both rare disorders. The localized form appears more frequent, but the exact incidence is unknown.

A causation of scleroderma is not identified. Virus-like particles and acid-fast bacilli have been observed in biopsies, and scleroderma-like skin manifestations are reported in a variety of internal disorders as well as after external exposure to vinyl chloride, perchlorethylene, trichlorethylene, pesticides, epoxy resin and silicium (Rowell 1979). However, the aetiological significance of

these observations remains dubious in the vast majority of patients with scleroderma not having special expositions.

Despite numerous theories, the true pathogenesis of scleroderma is still unknown. The literature was recently reviewed by Fleischmajer, Perlish and Duncan (1983) and by Lee, Anhalt, Voorhees and Diaz (1984). Three predominant theories have emerged. According to the *vascular theory,* the primary event is autoimmune or toxic endothelial cell damage, which under the influence of tissue hypoxia leads to reparative fibrosis with differentiation of endothelial cells or other cells of the vascular wall into fibroblasts and fibroblast-like cells with increased capacity to synthesize collagen. According to the *immune theory,* T-lymphocytes and monocytes sensitized to a cutaneous antigen, perhaps collagen, secrete substances, which stimulate and inhibit fibroblast function (Korn 1980). Observations of a scleroderma-like syndrome (chronic graft-versus-host reaction) in patients who have undergone bone marrow transplantation illustrate that immunological challenge may lead to generalized and excessive fibrosis (Shulman et al. 1980). According to the *abnormality of collagen metabolism theory,* abnormal fibroblasts and fibroblast-like cells in the lower dermis and subcutaneous tissue are in a state of accelerated glycosaminoglycan and collagen synthesis. Active fibroblasts predominate in peripheral parts of affected skin, while fibroblasts of central parts may have a reduced metabolic rate. The three predominant theories are artificial and simplified in many respects, and they need not be independent.

There is obviously a strong demand for effective therapy in generalized scleroderma. A variety of drugs with a theoretical or experimental rationale have been tried. However, methods to quantify cutaneous, metabolic, and internal manifestations of scleroderma have often been inadequate or not systematically practised. The activity of scleroderma and the possible effect of therapy on glycosaminoglycan and collagen metabolism can be determined by measuring the urinary excretions of glycosamino-

glycans, hydroxyproline and hydroxylysine (Blumenkrantz & Asboe-Hansen 1976a; Blumenkrantz & Asboe-Hansen 1977; Blumenkrantz & Asboe-Hansen 1980a). Affection of internal organs can be assessed by radiography, manometry of the esophagus, lung function test, EKG, determination of creatinine clearance, etc. However, objective, non-invasive and accurate methods to quantify skin and subcutaneous tissue involvement are needed.

Generalized scleroderma has as a consequense of its severity gained a major interest and widely overshaded localized scleroderma, which has been rather little studied by new techniques. However, localized scleroderma and particularly morphoea is with the sharp demarcation to normal appearing skin of interest as a study model of scleroderma. In morphoea, regional control examinations representing either normal skin, epiphenomena, or a preclinical state of scleroderma can be obtained in the same individual. With this model, interindividual variations in observations can be eliminated, and alterations characteristic of manifest scleroderma may be identified.

The purposes of the present study of patients with localized scleroderma (morphoea) was

- to evaluate morphoea and its value as a model of scleroderma clinically, physiologically, biochemically, and with respect to ultrastructure with special reference to differences between the sclerotic centre of plaques, the perilesional area and the normal appearing skin of the same body region and the same individual.
- to evaluate whether sclerotic plaques are affected exclusively or if there is a more general involvement of the skin and other connective tissues.
- to develop and test non-invasive methods for quantitation of scleroderma affection of skin. Methods should be relevant for and technically applicable to patients with generalized scleroderma.

Predisposition and onset

Familial localized scleroderma is rare, with only a few instances reported (Wuthrich, Roenigh & Steck 1975; Taj & Ahmad 1977). True morphoea, present at birth, seems not to have been described.

Reports of HLA associations in scleroderma, including B27, A9 and B8 in generalized scleroderma and Bw40 in morphoea need confirmation (Duvic & Goldsmith 1983).

A more frequent occurence amongst the female sex, found previously, was confirmed in this study (O'Leary & Nomland 1930; Christianson et al. 1956; Curtis & Jansen 1958; Stava 1959; I). The gynaecological histories gave, in accordance with a study on generalized scleroderma, no evidence og disturbances of sexual hormones (Serup & Hagdrup 1983). No particular occurrence of previous diseases was noted in patients with localized scleroderma or in their close relatives. The author could not confirm physical trauma as a provocatory factor, as previously suggested (Christianson et al. 1956; Jablonska 1975). In the present series localized scleroderma had its onset throughout life, however, with a tendency for generalized morphoea to appear in the elderly and localized plaques to appear in younger ages including childhood and adolescence.

An association between localized scleroderma and the nervous system is often discussed because of the linear and segmental distribution in some forms. In this study, 67 out of 170 morphoea plaques exhibited linearity either of the sclerotic plaques themselves or of the surrounding pigmentation, always in the transverse direction on the trunk and longitudinally on extremities, see Fig. 1 (I). Of the 170 plaques, 103 exhibited pigmentation and 93 surrounding pigmentation.

Despite many similarities, the regional distribution of localized scleroderma, including morphoea, did not exactly follow the inner-

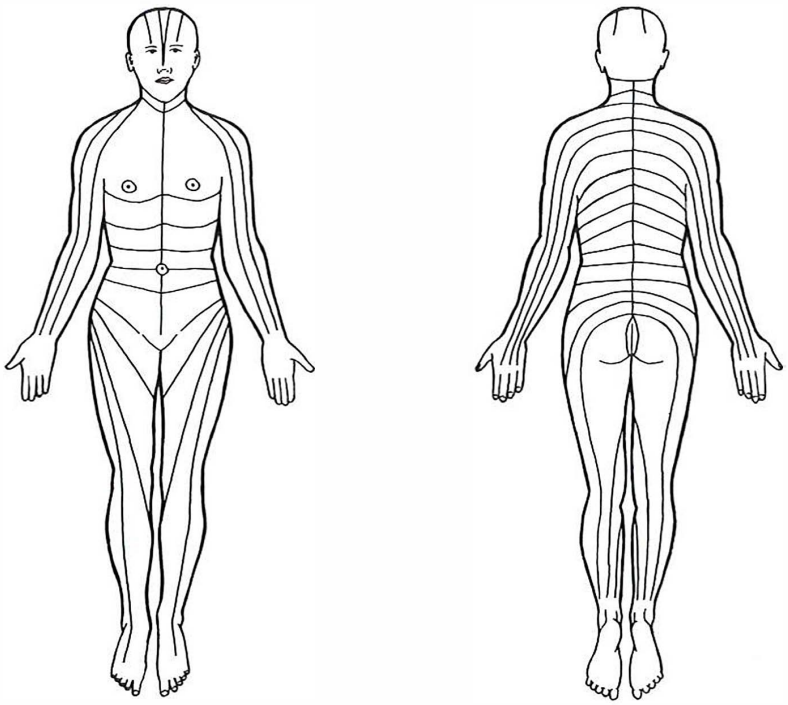


Figure 1. The linear distribution pattern of localized scleroderma including linearity in morphoea.

vation fields of spinal roots as indicated by Foerster (1933) and by Keegan and Garrett (1948) (I). Neither did localized scleroderma exactly follow the distribution lines of linear dermatoses as indicated by Blaschko (1901) (MacDonald & Sims 1969; Jackson 1976). The characteristic en coup de sabre lines of the front not fitting with any anatomical structure may reflect the proliferation and migration of crest-cells in embryonal life with ectodermal cells from the neural crest moving from behind to the face (Johnston 1975). The high frequency of disturbances of pigmentation in localized scleroderma including morphoea may, together with the special linear distribution with lines radiating to the spinal region, point to a predisposing defect in crest-cell migration laid down during embryonal life. It is noteworthy that, in the face, lesions may cross anatomical strucutres and even include the internal eye with affections following the same line (Serup & Alsbirk 1983; Serup, Serup & Sjö 1984).

CHAPTER 3

The Sclerotic Centre of Morphoea Plaques

Clinical observations

Sclerotic plaques in some patients are few and limited in regional distribution while other patients present several plaques larger in size and extensively distributed. Thus, there appear to be two entities of morphoea, i.e. localized morphoea plaque and generalized morphoea. Patients with localized plaques, in comparison with patients with generalized scleroderma, have a more advanced degree of lesional sclerosis as assessed clinically (I).

Quantitation and characterization by means of non-invasive methods

1. Ultrasound measurement of skin thickness

Measurement of skin thickness was previously performed by xeroradiography, skin-fold recordings, and from histological sections (Black 1969; Tanner & Whitehouse 1955; Southwood 1955). The radiographic method needs a larger area of skin, which can be projected free, and it is only used on the forearm. With the skin-fold technique, it cannot be avoided that at least some subcutaneous tissue is included in the fold—and scleroderma skin i difficult to fold. Skin biopsies change in shape during biopsy cutting and histological processing (Dykes & Marks 1977; Serup 1984a).

Ultrasound measurement of the thickness of animal skin, including the layer of fat, with A-mode ultrasound is a standard method in veterinary medicine to assess meat quality of live-stock. Because of

the lower thickness of human skin, higher frequencies of ultrasound are needed to obtain a satisfactory precision. In 1971, Daly & Wheeler introduced a 10 MHz A-mode ultrasound apparatus for measurement of the thickness of the oral mucosa. In 1979, Alexander & Miller developed the first 15 MHz A-mode biometric ruler for measurement of skin thickness in man. Measurements with this ruler compared favorably with the radiographic method. A biometric ruler following the same principles was recently developed (Tan et al. 1981; Tan, Marks & Payne 1981). This apparatus also correlated well with radiography.

Because of the acoustic echo principle of the ultrasound method, the more powerful echoes will be reflected from smooth parts of the dermis-subcutaneous tissue interface between attachments of subcutaneous retinacula, i.e. at the level in depth where the full thickness of the skin is structurally better defined. With A-mode scan, the better interface-echo may be selected immediately during recording. Recently, B-mode ultrasound scanners giving a cross-sectional view of the skin were constructed (Payne et al. 1982; Tikjøb, Kassis & Søndergaard 1984). With B-mode scanners, all echoes from the interface-zone are automatically registered, and the indication of the dermis-subcutaneous tissue interface appears less sharp. B-scan has the advantage that artifacts are easily recognized.

In 1980-81 the author, in cooporation with the Danish Institute of Biomedical Engineering (Academy of Technical Sciences), Glostrup, Denmark, developed a 15 MHz A-mode ultrasound apparatus for skin thickness measurement. This ruler was constructed with particularly small dimensions of the probe and other improvements rendering measurements on the fingers possible (II; Serup 1984b). The accuracy of skin thickness measurement as determined in normal appearing skin and typical morphoea plaques was at the level of 0.1 mm (SD from 0.05-0.09 mm) with coefficients of variation ranging from 2.8-6.8%. It might be feared that structural alterations of the dermis-subcutaneous tissue interface of scleroderma skin

rendered thickness measurements inaccurate, but ultrasound B-scan has recently confirmed that this interface is acoustically properly defined and, moreover, alterations of the subcutaneous tissue could be depicted (Tikjøb, Kassis & Søndergaard 1984).

Ultrasound measurement of morphoea plaques showed the skin thickness to be increased by 13-310%, and plaques with advanced and slight sclerosis clinically could be differentiated (II). The increase in thickness was larger in the normally thinner skin of the extremities in comparison with that of the trunk. The regional differences in thickening of the plaques and degree of sclerosis clinically could not be explained by a different regional affection in localized morphoea plaque and generalized morphoea since these two forms show a similar relation between truncal and peripheral involvement (I). In psoriatic plaques, also evaluated by the ultrasound equipment, thickening was also more pronounced on extremities (Serup 1984c). The thinning of the skin after occlusive application of topical corticosteroids was in absolute terms the same on the forearms and the back (about 0.2 mm after four weeks), i.e. relatively larger on forearms (Serup & Holm 1985). Thus, it may be a general feature that thinning and thickening of the skin in relation to disease and induced by special drugs is comparatively larger on extremities than on the trunk.

The ultrasound method has many potential applications in clinical and experimental dermatology (Serup 1984d; Serup 1984e). The method has been introduced for quantitation of the cutaneous oedema of patch test reactions and histamine weals (Serup, Staberg & Klemp 1984; Serup 1984f). Measurement of the thickness and invasion-depth of malignant melanoma non-invasively and preoperatively may prove very important in the future (Breitbart & Rehpenning 1983).

2. In vivo measurement of skin elasticity

Increased tension is a most pronounced clinical character of scleroderma skin. The stiffening can easily be appreciated by palpa-

tion. A rough quantitative expression may be obtained simply by measuring the height of a skin-fold with an ordinary ruler (Bachman 1961).

Laboratory methods for measuring skin tension based on excised strips of skin are of no clinical interest in relation to monitoring of scleroderma and, moreover, such laboratory measurement of a character like skin rheology is artificial.

The human skin is composite in biochemical constitution and structure and far from ideal for in vivo mechanical testing (Vlasblom 1967; Viidik 1973; Wijn 1980). The living skin is preloaded with a different load in different directions as indicated by the lines of Langer, and the spontaneous tension depends on the position of adjacent joints (Ridge & Wright 1966; Cook 1981). During stress and extension of the skin, the collagen and elastin fibres of the dermis change in position and become aligned parallel to the direction of stress while interstitual fluid is displaced from the fiber network as the fibres are oriented and compacted (Gipson, Kenedi & Craik 1965; Craik & McNeil 1965; Finlay 1969; Brown 1973). During uniaxial stress, the strain is not unequivocally distributed illustrating the anisotrophy of skin (Wan Abas & Barbenel 1982).

Obviously, the rheology of skin not only depends on the supporting fibres and the interstitual fluid of the dermis, but the subcutaneous binding will also be of significance depending on the body region and the area of skin exposed to stress. The epidermis will also have some minor influence depending on hydration, ambient temperature and skin surface lipids (Wolfram, Wolejsza & Laden 1973; Papir, Hsu & Wildnauer 1975; King et al. 1979).

A number of methods to measure skin elasticity in vivo have been introduced (Cook 1981; Marks 1983). Methods may apply an uniaxial or a biaxial tress to the skin. The principles have been tangential, including torsional, and elevating and depressing deformations of the skin with registration of a stress-strain relationship. Most of the methods were used for experimental studies and for evaluation of physiological aspects of skin rheology while studies on skin diseases are limited.

With respect to in vivo measurement of skin elasticity in scleroderme including generalized scleroderma, and with a perspective of monitoring the disease in the clinic, initial considerations may be as follows

- Measurements on the fingers should be possible, and test sites should be defined with respect to extension.
- due to the very small skin areas thus available for testing, strains will be small, and the registration system need be very accurate.
- technique should be non-invasive rendering repeated measurements possible.
- effects of the tension lines of Langer should be minimized or eliminated in practical use.
- measurements should preferably reflect dermal properties.
- registration of initial strain as well as hysteresis is desirable.

In view of these considerations, the author in cooporation with the Danish Institute of Biomedical Engineering (Academy of Technical Sciences), Glostrup, Denmark, developed a method for measuring skin elasticity based on automatically repeated applications of suction to a circular skin area (diameter 1.0 cm, area 0.79 cm^2) and registration of the resultant elevation of the skin and the hysteresis by a special electronic system based on measurement of electrical capacitance calibrated to elevation of surfaces in mm, i.e. with the skin surface representing one electrode and an electrode in the top of the suction chamber being the other (IV). Special adaptation rings gently glued to the skin surface were used. The elevation of the skin after one suction (the tensile distensibility), the resultant elevation of the skin after release of the first suction and resiliency (the resilient distension), and the further increase in registered tensile distension after a number of repeated suctions (the hysteresis) were registered (Fig. 2).

By this automatic and repeated way of recording, including a stress-strain relationship as well as a time axis, a more detailed evaluation of the rheology of skin in vivo was intended with a possi-

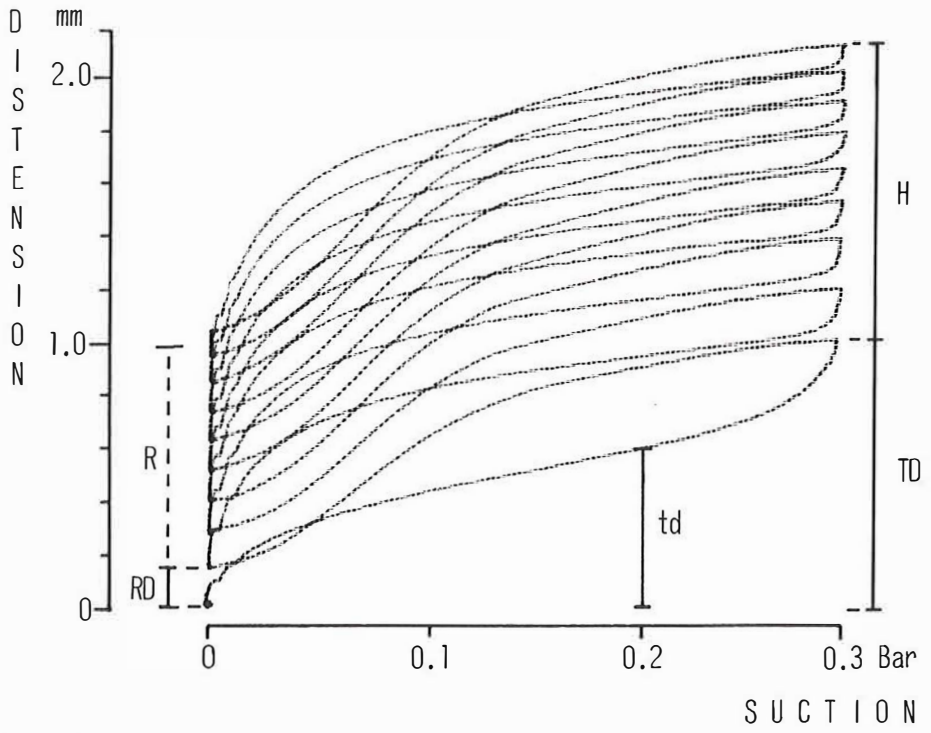


Figure 2. Skin elasticity curve. Distensions (ordinate) after repeated applications of suction (abscissa). TD = tensile distensibility at 0.3 Bar, td = tensile distensibility at 0.2 Bar, R = resiliency, RD = resilient distension, H = hysteresis.

bility of description as well as interpretation and differentiation of clinical conditions and physiological aspects of skin rheology.

The methodology developed proved highly reproducible according to experiments on silicone rubber membrane, but the reproducibility in vivo was, as may be expected, somewhat poorer, including considerable day-to-day and regional variations (IV). Experiments with a conductive cream showed that variations on living skin were not caused by differences in electrical conductance of skin surfaces tested. Thus, because of the very high technical reproducibility of the method, variations as registered in living skin could be considered biological.

Histamine experiments in morphoea patients, in psoriasis patients (experiments including patch test reactions and contact dermatitis), and in healthy skin indicated that the hysteresis mainly re-

flected the viscous component of skin rheology, while the tensile distensibility mainly reflected the structural component (IV; Serup & Northeved 1985). The experiments also illustrated that skin rheology is not a simple and constant character depending on the skin thickness and fibre structure only, but other aspects such as the actual water content and recent mechanical stress may have a significant influence under physiological conditions. The influence of water intake and balance on skin elasticity was already observed by Shade (1912) and by Wiltrup (1924), who used a mechanical method with application of a depressing force.

Sclerotic morphoea plaques presented as a characteristic trait a decreased tensile distensibility which probably was a result of alterations of the collagen fibres and their organisation (IV). Decreased distensibility in morphoea is in accordance with a study on acrosclerosis by Pierard and Lapière (1977) and a study on tight-skin mice by Menton et al. (1978). In the sclerotic state of morphoea the hysteresis was also decreased, but during remission, the hysteresis became temporarily increased. This might reflect a temporary tissue oedema. Sequels of morphoea still showed a decreased tensile distensibility while the hysteresis was normal. Thus, decreased hysteresis of sclerotic plaques turning into increased values might be an indication or predictor of remission. Pigmented spots appearing in morphoea had the same rheology as sequels of sclerotic plaques. A summary of results obtained with the in vivo skin elasticity measurer is presented in Table I.

Previous suction methods for measuring skin elasticity were developed by Vlasblom (1967), by Grahame (1970) and by Cook, Alexander & Cohen (1977). Vlasblom, using a bell-glass covering an area of 9.29 cm^2 suggested that extensions were influenced by subcutaneous binding. Grahame, using a bell-glass covering an area of 3.14 cm^2 and a simple system for registration of extensions was able to describe differences in skin elasticity related to sex, age and pregnancy. Cook, Alexander and Cohen used acrylic cups with a race-track geometry and a special pretension device. In an experi-

Table I. Summary of skin rheology in localized scleroderma (morphoea) and other conditions studied.

	Tensile Distensibility	Hysteresis
Localized Scleroderma		
– sclerotic plaque	Decreased	Decreased
– regressive state	Possibly decreased	Increased
– sequel of plaque	Decreased	Normal
– primarily pigmented spot	Decreased	Normal
Psoriasis		
– psoriatic plaque	Decreased	Increased
Cutaneous Oedema	Increased	Increased
Previous Mechanical Stress	Increased	Decreased

ment performed on the back with their smaller cup covering an area of approximately 6 cm^2, those authors showed that subcutaneous attachments only had a negligible influence. Recording of subcutaneous tissue pressure during suction showed no variations related to the procedure. Experiences with our skin elasticity measurer covering an area of only 0.79 cm^2 showed the method to be widely insensitive to vertical deviations of the probe and, thus, widely independent of subcutaneous tissue attachment (IV). However, at the initial and automatic setting of the zero, attention should be drawn to the fact that the contour of the skin was not deformed.

3. Skin colour and measurement of blood flow by laser-Doppler flowmetry

The initial lesion of morphoea is a reddish or white-coloured spot. The colour of fully developed plaques is white. A few plaques may exhibit a faint redness, which may be interpreted as evidence of inflammatory activity, while more plaques exhibit melanin pigmentation, indicating remission (I).

The colour of living skin can be measured by remittance spectroscopy. However, colour measurement of erythematous skin after UV-A and UV-B radiations with registrations of absorbance at wavelengths of oxy-haemoglobin and haemoglobin show small dif-

ferences between erythema and normal skin for control (Anderson, Hu & Parrish 1981; Feather et al. 1981). The human eye appears very skilled for assessment of small nuances of skin colour, and remittance spectroscopy seems not suited for quantitation of the faint differences in red colour in and around morphoea plaques.

Remittance spectroscopy may quantify melanin pigmentation as measurements in caucasians and negroes and the broad absorption spectrum of melanin indicate (Anderson & Parrish 1981). The author studied different lesions of morphoea with a Perkin-Elmer^{R} spectrophotometer model 555 supplied with an external integrating sphere constructed for surface colour measurements. However, differences in remittance between the white centre of sclerotic plaques, areas with melanin pigmentation, and regional control appeared within the normal range of regional variations and variations in tanning by the sun (Fig. 3).

The white colour of sclerotic plaques leaves an impression af anaemia. Previous studies on blood flow in patients with acrosclerosis by the ^{133}Xenon washout technique and by laser-Doppler flowmetry showed, seemingly contradictorily, a decreased flow in the fingers while the flow on the dorsum of the hand was increased (Kristensen & Wadskov 1978; Kristensen 1980; Kristensen, Engelhart & Nielsen 1983).

Laser-Doppler measurement of cutaneous blood flow was introduced in 1977 by Holloway & Watkins (1977). The method was significantly improved with a higher signal-noise ratio by Tenland (1982). With the Periflux^{R} apparatus, a skin area of about 2 mm^2 is illuminated via an optical fibre, and the reflected light registered by photodetectors. The apparatus is constructed to differentiate Doppler-shifted frequencies, representing small moving particles, i.e. in living skin corpuscles of blood. The penetration depth of the laser light in skin is about 1 mm. The laser-Doppler method correlated favorably with the ^{133}Xenon method (Engelhart & Kristensen 1983). The laser method has the advantage of being very easy in practical use.

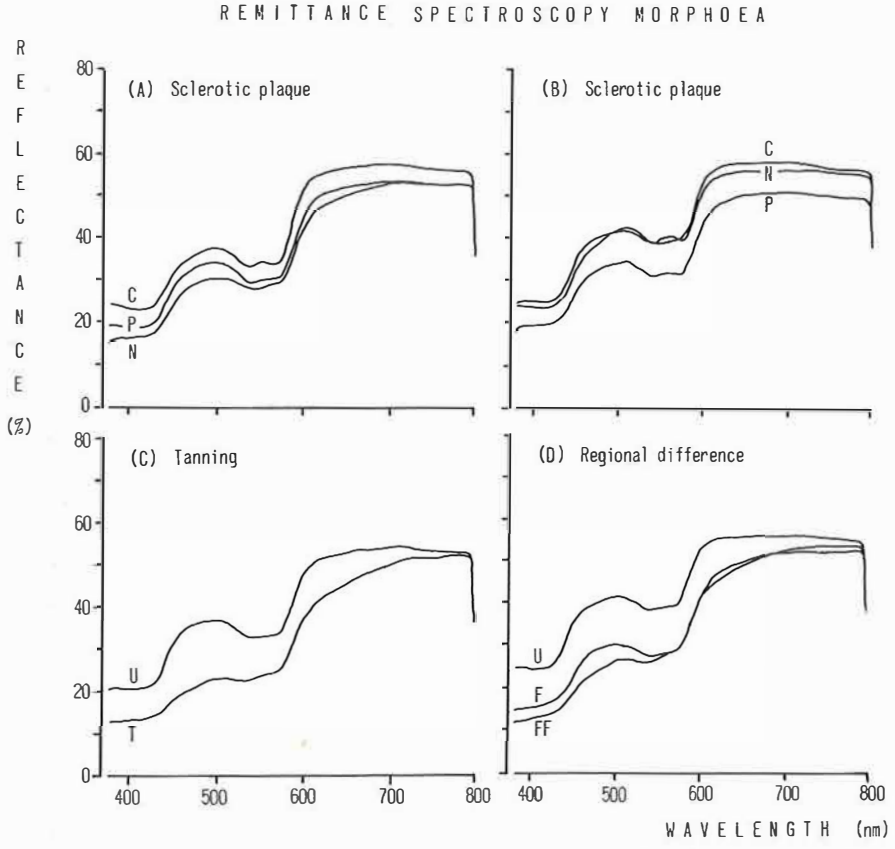


Figure 3. Remittance spectroscopy in a patient with localized morphoea plaques. (A) Sclerotic plaque located to the forearm (C centre, P Perilesional area, N normal appearing skin). (B) Sclerotic plaque with perilesional pigmentation located to the upper arm (C centre, N normal appearing skin, P perilesional area). (C) Tanning of forearm skin, extensor aspect (U unexposed skin under a wrist watch, T tanned skin). (D) Regional difference normal appearing skin (U upper arm, F forearm proximally, FF forearm distally).

In the present study of morphoea, white-coloured and sclerotic plaques showed an increased blood flow as registered by laser-Doppler flowmetry (V). The increase in flow was larger in plaques with an advanced degree of sclerosis clinically. Experiments with proximal arterial compression, measurements of old incisional scars, and comparative measurements with the ^{133}Xenon method showed that registered increases by the laser-Doppler method were not artificial due to changed optical properties of sclerotic and fibrotic skin.

Decreased blood flow on the fingers in contrast to increased flow on the dorsum of the hand in patients with acrosclerosis may, in view of the present study, reflect structural affection of the vascular wall of digital arteries and vasospasm, resulting in a diminished regional blood supply masking the tendency to hyperaemia of scleroderma skin becoming apparent only on the dorsum of the hand.

4. Measurement of sweat gland function and assessment of skin relief

In histological sections, the skin apendices, including the sweat glands, and the epidermis show regressive changes and atrophy (O'Leary, Montgomery & Ragsdale 1957). As a result of epidermal atrophy, the surface of morphoea plaques often looks glossy.

The sweat gland function in scleroderma skin seems not studied previously. In morphoea plaques gravimetric measurement of the secretion of sweat per 30 min after pilocarpine iontophoresis ad modum Gipson and Cooke (1959) showed a marked decrease in accordance with stereomicroscopy of sweat 'pores' in silicone rubber imprints (VI). Moreover, sweat 'pore' counts before and after pilocarpine iontophoresis showed 'gland fatigue' in sclerotic skin. Thus, measurements agreed that the function, stimulated as well as unstimulated, of sweat glands in morphoea plaques is defective. Analyses of the concentrations of sodium and potassium indicated, as might be expected, no specific secretory defect.

Assessment of skin surface relief according to stereomicroscopy of silicone rubber imprints showed smoothing of the surface with total or partial loss of the normal polygonal crease pattern (VI). Extragenital plaques of lichen sclerosus et atrophicus exhibited a different surface with deep furrows and elongated smooth areas together with evidence of disturbed keratinization.

Objective methods for measuring skin relief may be divided into optical methods and stylus methods. The former measures the shadows of crease-impressions as created with incident light. By

the latter a sharp-pointed stylus is moved automatically over surface imprints depicting deviations. Skin relief measurements by a stylus method later confirmed microscopical findings in the present study of smoothed skin surface of morphoea plaques probably reflecting epidermal atrophy (Serup, A).

Biochemical studies

1. Analyses of collagen specific amino acids in skin

There is general agreement that accumulation of collagen in the dermis is essential in scleroderma. The amino acid hydroxylysine only appears in collagen, while hydroxyproline is also found in elastic fibres. Collagen is rich in proline, but this amino acid is a constituent of many other proteins, too. Previous studies of hydroxylysine and hydroxyproline in scleroderma skin, mainly derived from patients with generalized scleroderma, are in conflict, showing normal as well as increased and decreased concentrations (II). It is a general methodological problem that specimens studied have not been matched with respect to age and sex of the patients and with respect to body region.

In the present study, based on biopsies of morphoea plaques and regional control of normal appearing skin of the same individuals, the concentration of hydroxylysine of sclerotic skin was increased (VII). This increase was significantly larger in plaques of shorter duration and in plaques of advanced sclerosis clinically. The increase in hydroxylysine concentration was quantitatively small. Consistently, the hydroxylysine-hydroxyproline molar ratio was increased.

Concentrations of hydroxyproline and proline were similar in plaques and control regions, and no special tendencies appeared. The hydroxyproline-proline molar ratio was not changed in comparison with the normal appearing skin.

Results of this study are not in accordance with previous studies in patients with generalized scleroderma by Blumenkrantz and Asboe-Hansen (1976b; 1978a; 1978b; 1980b). Those authors found decreased concentrations of proline, hydroxyproline, and hydroxylysine in scleroderma skin of the wrist in comparison with control samples obtained from various regions. Moreover, the hydroxyproline-hydroxylysine molar ratio was lowered, and the proline-hydroxyproline molar ratio elevated.

Shuster et al. reported on increased hydroxyproline content of scleroderma skin per square mm skin area (Shuster, Raffle & Bottoms 1967; Shuster, Black & McVitie 1975; Black, Bottoms & Shuster 1970). This way of expression was later adopted by other examiners, but Rodnan, Lipinski & Luksick (1979) found that the weight of punch biopsies, being simple to measure but still invasive, provided sufficient quantitative information. However, hydroxyproline in skin under a given area and weight of punch biopsy samples both reflect the full-thickness of skin, and a direct measurement of the skin thickness appears more appropriate.

Increased concentration of hydroxylysine is also known from other conditions with active fibrosis such as dermal scar tissue and healing bone fractures, and the finding is therefore not special for scleroderma (Barnes et al. 1976; Glimscher et al. 1980).

2. Analyses of glycosaminoglycans in skin

The glycosaminoglycans of the connective tissue matrix of healthy and diseased skin have many functions, including a modulating function during formation and degradation of collagen (Comper & Laurent 1978). The common glycosaminoglycans of human skin are hyaluronic acid, dermatan sulphate, chondroitin 4/6 sulphate, and heparan sulphate.

In scleroderma skin, the total concentration of glycosaminoglycans as expressed by the concentration of uronic acid is often reported increased, but data on the fractionated glycosaminoglycans are in conflict (Dorner 1980).

According to the present study dermatan sulphate and chondroitin 4/6 sulphate concentrations were increased in the centre of morphoea plaques in contrast to a decreased concentration of hyaluronic acid (VIII). Uronic acid, hexosamine, and heparan sulphate showed no differences. The changes of morphoea plaques were consistent with known events in an inflammatory response as described in granulation tissue, in experimental damage to large vessels and in tendon regeneration (Hansen et al. 1980; Helin et al. 1971; Scott, Orfard & Hughes 1981).

Thus, the study on glycosaminoglycans in localized scleroderma skin showed, as did the study of collagen specific amino acids, changes analogous to events of self-limiting processes of reparative fibrosis.

Ultrastructural studies

1. Supporting fibres and connective tissue matrix, background
Ultrastructural alternations of collagen fibrils and other supporting fibres of the skin are considered similar in generalized and localized scleroderma (Braun-Falco & Rupec 1964; Hayes & Rodnan 1971; Fleischmajer & Prunieras 1972; Kobayasi & Asboe-Hansen 1972; Kobayasi & Asboe-Hansen 1974; Kobayasi, Asboe-Hansen & Serup 1984).

Connective tissue changes can be divided into three stages, i.e. a fibril degradation and myxoedematous stage, a cell infiltration stage, and a fibrotic stage (Kobayasi, Asboe-Hansen & Serup 1984). In the initial myxoedematous stage mucopolysaccharides are increased, and collagen fibrils show evidence of degradation. In the fibrous stage changes of collagen fibrils are distinct with increased amount, and abnormalities of arrangement, shapes, and diameter. The fibril diameter may be bimodal, but the periodicity is normal. Elastic fibres show degeneration in the initial stage. In later stages microfibril formation is seen, or fibres are lacking. Changes of elas-

tic fibres are not remarkable as those of collagen fibrils are. Cellular infiltration consists of lymphocytes, macrophages, plasma cells, and mast cells (Fleischmajer & Nedwich 1972; Fleischmajer, Perlish & Reeves 1977; Fleischmajer, Perlish & West 1977; Kobayasi, Asboe-Hansen & Serup 1984). Mast cells appear in the myxoedematous stage, persist through the stage with cellular infiltration, and disappear in strongly fibrotic areas. Fibroblasts and fibroblast-like cells appear in the stage with cellular infiltration. Seemingly these cells are originated from the pericytes of nerves and vessels as described later.

1. Vascular and perivascular alterations

Vascular alterations include gaps, vacuolization and destruction of endothelial cells with reduplication of the basal lamina, perivascular cellular infiltration, and fibroblasts and pericytes with enlarged rough endoplasmic reticula accompanied by perivascular fibrosis (Fleischmajer & Perlish 1980).

According to the present study of morphoea plaques, small vessels with pericytes presented three pathological patterns, i.e. a first pattern with endothelial cells in a stimulated condition and thickened vascular wall with cellular infiltration, a second pattern characterized by thickening of the basal lamina, and a third pattern with activated pericytes and perivascular cellular infiltration (IX). Altered endothelial cells similar to the first pattern were also seen in the second and third patterns.

The first pattern was frequent in skin which appeared normal, while the second and third patterns appeared mainly in the inflammatory and sclerotic areas. Thus, activated pericytes, in relation to manifest scleroderma, seemed a more prominent vascular alteration.

2. Neural and perineural alterations

Neural and perineural alterations in scleroderma seem not to have been studied systematically.

According to the present study, mesenchymal parts of the nerves, i.e. the epi-, peri- and endoneurium, exhibited prominent alterations with myxoedematous changes, collagen fibril degradation, cellular infiltration and abnormal fibrosis (X). Alterations of the pericytes of the perineurium were noteworthy. Pericytes contained distinct granular endoplasmic reticula, ribosomes, pinocytotic vesicles, invaginated nuclei, and cytoplasmic filaments suggesting transformation into myofibroblasts. Basal laminae were either dislocated, interrupted, or lacking. The axons and the Schwann cells showed only degenerative changes.

Alterations of mesenchymal parts of nerves and transformation of pericytes into myofibroblasts were not observed in skin, which appeared normal. Thus, those alterations appeared essential in relation to manifest scleroderma.

The myofibroblast, as described in the early 1970's, shares ultrastructural features with both fibroblasts and smooth muscle cells, including the presence of endoplasmic reticula and cytoplasmic filaments (Gabbiani et al. 1976). It has capacity for synthesis as well as contractile properties. During the 1970's the myofibroblast was established as a principal cellular component in granulation tissue of healing wounds and numerous other pathological conditions with active formation of connective tissue (Lipper, Kahn & Reddick 1980; Seemayer et al. 1980; Seemayer, Schürch & Lagacé 1981).

The exact origin of the myofibroblast is unknown. Fibroblasts, smooth muscle cells, and primitive mesenchymal stem cells are under consideration. The origin need not be uniform. The present study of cutaneous nerves in morphoea suggests that pericytes of nerves, ultrastructurally identical with those of small vessels, are precursors of myofibroblasts in the morphoea form of scleroderma.

CHAPTER 4

The Perilesional Area of Morphoea Plaques

Clinical observations

The perilesional and peripheral areas of morphoea plaques may represent special features of progression or remission.

In the present study, 14% of plaques presented a lilac ring, but this was intense in only 2.3% (I). Melanin pigmentation of the perilesional area was common occurring in around 55% of plaques. In 2.3% of plaques, visible atrophy of subcutaneous tissue underlying the perilesional area was noted. Inflammatory redness, pigmentation, and subcutaneous atrophy in some instances represented an extension of phenomena of the plaques into the surrounding skin, while in other instances changes of the perilesional area represented separate phenomena.

Quantitation and characterization by means of non-invasive methods

Because of the characteristic lilac ring, flowmetry is of special interest. Laser-Doppler blood flow measurements of the present study showed a significant increase in flow in the perilesional area, in most plaques reaching a level comparable to the increased level of the sclerotic centre (V). In lilac rings, very high flow was recorded.

Ultrasound measurements of the perilesional area showed either a normal thickness or a slight increase or reduction, depending on the clinical state (J. Serup, unpublished observations).

Biochemical studies

Analyses of hydroxylysine, hydroxyproline and proline in skin

specimens of perilesional skin showed normal concentrations or, with respect to hydroxylysine, intermediate concentrations between the sclerotic centre and regional control (VII). Despite a lilac ring in five plaques and evidence of progression in six plaques out of 18 examined, no plaque showed evidently abnormal perilesional concentration of amino acids analyzed.

Analyses of glycosaminoglycans showed increased concentration of dermatan sulphate, while chondroitin 4/6 sulphate tended to be increased (VIII). Concentrations represented intermediate values between the sclerotic centre and regional control. The hyaluronic acid concentration was increased in contrast to a decreased concentration in the sclerotic centre. Furthermore, the increase in hyaluronic acid predominantly occurred around plaques with clinical evidence of activity. A small, however, significant increase in hexosamine concentration was found together with a tendency to increased uronic acid concentration. Differences observed between sclerotic centre, perilesional area, and normal appearing skin probably reflected sequential changes in the glycosaminoglycan composition during initial fibrosis and manifest scleroderma. Increased hyaluronic acid in the perilesional area seemed the better biochemical denominator of active scleroderma.

Ultrastructural studies

Ultrastructural alterations of connective tissue of the peripheral zone of morphoea plaques are gross with numerous acid glycosaminoglycan microfibrils, disarranged collagen fibres and fibrils uneven in thickness, and degenerated elastic fibres (Kobayasi & Asboe-Hansen 1974). Alterations may represent both neoformation and degradation of collagen.

Ultrastructural alterations of vessels and nerves in the periphery or perilesional area of plaques were essentially similar to those observed in the sclerotic area including evidence of transformation of pericytes of nerves into myofibroblasts (IX; X).

CHAPTER 5

The Normal Appearing Skin of Morphoea Patients

Observations including ultrastructure

An initial light-microscopical study, including connective tissue staining methods, disclosed no alterations of the connective tissue stroma of normal appearing skin of morphoea patients, indicating scleroderma (J. Serup and N. Tinggaard-Pedersen, unpublished observations).

Ultrasound measurement of the thickness of normal appearing forearm skin of morphoea patients showed, consistently, a normal skin thickness as compared to healthy control persons (II).

The ultrastructure of the connective tissue stroma of normal appearing skin of morphoea patients showed no characteristic alterations of scleroderma affection (IX;X; T. Kobayasi and J. Serup, unpublished observations). However, ultrastructural examination revealed alterations of endothelial cells of small vessels with irregular luminal surfaces, cytoplasmic abnormalities and invaginated nuclei, i.e. alterations also observed in sclerotic plaques (IX;X). The cutaneous nerves were normal with no definit alterations of the perineurium including the pericytes and their basal lamina (IX;X). Differentiation into myofibroblasts was not observed.

In conclusion, the normal appearing skin of morphoea patients is not an entirely normal skin but presents ultrastructural alterations of the endothelium of small vessels also known from plaques of manifest scleroderma. However, in the normal appearing skin, these alterations were not associated with the intense stromal fibrosis characteristic for scleroderma.

The non-sclerotic pigmented spot

The sclerotic plaque presenting white colour, induration, and thickening is the essentiel lesion of morphoea defining the disease.

Christianson et al. (1956) in a retrospective study noted café au lait spots in 3% of patients with localized scleroderma. Pierini, Abulafia & Mosto (1970) reported an atrophoderma associating morphoea, which they named "l'atropho-sclérodermie".

A clear distinction clinically and by light-microscopy between individual lesions of non-sclerotic pigmented spots of morphoea patients, and atrophoderma Pasini-Pierini does not seem possible.

In the present study, 11 out of 21 patients with localized morphoea plaque had non-sclerotic pigmented spots presenting as such and never exhibiting the characteristic clinical signs of scleroderma (I). As lesions of atrophoderma Pasini-Pierini these spots were mainly located to the trunk.

A few spots in the present study also presented atrophy of the underlying subcutaneous tissue (I).

Ultrasound measurement of the skin thickness of non-sclerotic pigmented spots with no evidence of previous scleroderma showed reduced thickness (III; IV).

Skin elasticity measurement of non-sclerotic pigmented spots showed a reduced tensile distensibility and a normal hysteresis, i.e. the same rheology as pigmented sequels of morphoea plaques, which also had decreased skin thickness (IV).

The frequent finding of primarily pigmented spots sharing features with pigmented sequels of morphoea plaques in patients with morphoea having characteristic sclerotic plaques in other body regions may point to a temporary and regulatory defect in connective tissue formation in the skin with increased formation and accumulation in some areas and reduced formation in others and in late lesions.

Systemic manifestatons including collagen metabolism and central cornea thickness

Localized scleroderma is not assumed to involve internal organs or to influence survival.

A necropsy study including 27 instances showed no abnormalities of visceral organs related to localized scleroderma (Piper & Helwig 1955). A radiographic and manometric study of esophagus involvement in scleroderma including 14 cases of morphoea showed motor dysfunction and decreased peristalsic contractions of the upper third of the esophagus in one and two patients respectively, but the lower two thirds was affected only in generalized scleroderma (Weihrauch & Korting 1982). In the authors material, radiological examination indicated esophageal motor dysfunction in two patients (11 examined) with generalized morphoea. X-ray of the chest and lung function test in patients with generalized morphoea showed no case of pulmonary fibrosis (11 and 10 examined). Among 8 patients with localized morphoea plaques, no case of esophageal or pulmonary affection was suggested (8 radiography, 4 lung function test). In the authors material, no case of decreased renal function was noted. Thus, it cannot be excludede that the esophagus may occasionally show minor involvement in morphoea.

Arthralgia at the debut of localized scleroderma was noted in 44% of patients in the study of Christianson et al. (1956) and in 10.3% in the present study (I). Raynaud's phenomenon was present in 3.4% of patients in the study of Christianson et al. (1956) and in 10.3% in the present study, however, only in patients with generalized morphoea or extensive linear affection of an extremity (I).

Antinuclear antibodies and anti-DNA antibodies are positive or increased in some patients with localized scleroderma including morphoea indicating activation of the immune system and a rela-

tion to the group of rheumatic diseases (Barthelmes 1975; Jablonska et al. 1979; Takehara et al. 1983; Serup & Staun-Olsen, A.).

Thus, there are indications that morphoea may not simply be a local process confined to the plaques of the skin.

Alterations of connective tissue of normal appearing skin and other organs were hitherho not evaluated in morphoea. As mentioned, ultrastructural alterations of vessels of normal appearing skin are common (IX).

In patients with generalized scleroderma, we recently described increased central cornea thickness with a positive correlation to the duration of disease for the first 8 years after debut (Serup, Serup & Hagdrup 1984). Measurements were performed by the slit-lamp method. The cornea is composed of thin epithelial and endothelial linings, and a connective tissue stroma, comprising 9/10 of its thickness, with collagen fibres organized in a system of lamellae, packed with connective tissue ground substance. This special architecture renders the cornea transparent. In relation to scleroderma, it is noteworthy that the cornea is supplied with nerves but not with vessels. Cornea thickness measurements in patients with morphoea also revealed an increased central cornea thickness (XI). This increase was also correlated to duration of disease.

The excretion of urinary metabolites of collagen (hydroxyproline, hydroxylysine, proline) is known to be increased in generalized scleroderma (Blumenkrantz & Asboe-Hansen 1976a; Blumenkrantz & Asboe-Hansen 1980a). In the present study, we showed that patients with morphoea also have increased metabolites of collagen in their urine (XII). Since the plaques of morphoea only comprise a negligible part of the whole collagen mass of the body, this shows that morphoea patients have a generalized alteration of the collagen metabolism.

Localized morphoea plaque and generalized morphoea

Clinically, two entities of morphoea may be defined. In *localized morphoea plaque,* one or a few circumscribed plaques occur, limited to a few body regions. In *generalized morphoea,* several plaques, typically larger in size, occur in several body regions. A third entity of morphoea, with *guttate lesions* occurring in regions, may also be defined, but in this type, characteristic plaques of the first two entities are not found.

The present study showed that localized morphoea plaque, as compared to generalized morphoea, is characterized by

- Younger individuals are affected.
- a lesser tendency to symmetry and linearity of the skin lesions.
- plaques more frequently presenting advanced degree of scleroderma clinically and, accordingly, a larger increase in thickness as to ultrasound measurement.
- increased concentration of hydroxylysine in the plaques.
- increased urinary excretion of collagen metabolites.

Furthermore, patients with localized plaques tended to have a larger increase in cornea thickness, and the plaques exhibited a larger increase in blood flow and a more advanced reduction in sweat gland function.

In summary, despite a smaller number of plaques and a more limited regional distribution of the sclerotic skin lesions of localized morphoea plaque in comparison with generalized morphoea, the former type of morphoea represents a more advanced disease, both with respect to the dermal sclerosis and with respect to the general disturbance of collagen metabolism.

CHAPTER 9

Considerations on the pathophysiology of morphoea

The essential trait of scleroderma defining the disease is accumulation and disorganisation of the collagen fibrils of the dermis.

During human life and ageing, the collagen is replaced many times in a seemingly minutely controlled way (Robert & Robert 1973). Traumatic lesions of the skin are repaired by a self-limiting process of neoformation of connective tissue.

The present study on collagen specific amino acids and glycosaminoglycans of morphoea plaques demonstrated alterations already known from embryonal growth, healing of bone fractures, vascular damage, dermal wounds and tendon lesions (VII; VIII). Thus, the biochemical results indicate that the process of collagen formation in morphoea is widely analogous to physiological and pathophysiological processes of growth, regeneration and repair.

The accumulation of collagen in morphoea plaques associated with increased skin thickness may indicate hyperfunction of connective tissue cells while the decreased skin thickness of non-sclerotic pigmented spots and sequels of morphoea may indicate hypofunction. This points to a temporal and sequental defect in the synthetic capacity of connective tissue cells in scleroderma, in morphoea with both hyperfunction and hypofunction as a possibility. Ultrastructural studies demonstrated transformation of, at least, pericytes of nerves into myofibroblasts, i.e. a cell type associated with active formation of connective tissue (IX;X). Cornea thickness measurements and analyses of collagen metabolites in the urine indicated a more general increase in the formation of connective tissue constituents. A general and slight activation of connective tissue cells, particularly pericytes of nerves and vessels, not detectable by electron microscopy may be an essential underlying feature in morphoea. In circumscribed areas of skin as represented by the

sclerotic plaques the activation of particularly pericytes of nerves may temporarily get out of control, resulting in intense collagen formation and clinically manifest scleroderma. The sclerotic stage seems to activate a process of inhibition of connective tissue cells leading to remission and thinning of the skin. In some lesions, i.e. non-sclerotic pigmented spots, this activation may already take place in the very initial stage.

The regulation of differentiation and synthetic capacity of connective tissue cells under physiological and pathophysiological conditions is not known. The intense infiltration with inflammatory cells, including lymphocytes in and around morphoea plaques, may play a role since these cells may produce substances which stimulate as well as inhibit fibroblasts (Fleischmajer, Perlish & Duncan 1983; Lee et al. 1984; Korn 1980; Wahl, Wahl & McCarthy 1978; Tamura & Ishikawa 1983).

The considerations emerged from the present study on morphoea are in accordance with both the "abnormality of collagen metabolism" and the "immune" theories. We found no special evidence in favour of the "vascular", i.e. endothelial, theory.

Applications of non-invasive methods for quantitation of cutaneous and corneal manifestations in generalized scleroderma (progressive systemic sclerosis)

Ultrasound measurement of skin thickness by the equipment developed in the present study has already been applied to patients with acrosclerosis (Serup 1984b). Problems with the dermis-subcutaneous tissue interface being structurally less sharp on the hands were overcome by measuring skin surface-phalangeal bone distance. In a group of patients under treatment with inhibitors of collagen synthesis, the skin-phalanx distance over the middle and proximal phalanges were increased in 82% and 59% respectively, while the skin thickness of the extensor and flexor aspects of the forearm were increased in 32% and 23%. The ultrasound method is easy to use and suited for routine measurements. The skin-phalanx distance over the middle phalanx was correlated to the circumference of the finger as measured by a ring-size (Serup 1985). However, the scleroderma involvement of fingers seems to result in a comparitively larger thickening on extensor aspects as is also the case on forearms; measurement of skin-phalanx distance, therefore, seems a more sensitive method (Serup 1984b).

Skin elasticity measurement by the method introduced has at present only been performed in a few instances of generalized scleroderma. The method is technically applicable to the fingers. In practical use, some experience with the method is needed. Before the method can be used routinely to monitor generalized scleroderma, systematic studies are needed in patients with acrosclerosis representing different stages and healthy individuals to evaluate sexual, age, inter-individual, and day-to-day variations. Functional aspects of skin elasticity as evaluated in this study represents a potential source of variation.

Skin blood flow measurements by the laser-Doppler method are easy to perform. The very complex situation on hands and fingers

afflicted with acrosclerosis, with cutaneous sclerosis tending to increase the flow, arterial obstructions tending to reduce the flow, and Raynaud's phenomenon with vasospasm and reactive hyperaemia invalidates blood flow measurements for monitoring cutaneous affliction with scleroderma. Moreover, the cutaneous blood flow is an actively regulated parameter showing considerable variations by time (Tenland 1982).

The function of sweat glands after pilocarpine iontophoresis was reduced in every plaque studied (VI). The method of Gipson & Cooke (1959) appears too difficult and time-consuming for routine usage. The imprint technique is convenient and also allows assessment of skin relief, but there is a subjective aspect related to the observer when sweat "pores" are counted under a stereomicroscope. A study on patients with acrosclerosis on subjective appreciation of dryness of the hands and objective measurement of water evaporation by an easy-to-use open-chamber method (Nilsson 1977) is in progress. Further studies are needed with a comparison of different methods for measuring sweat gland function in patients with acrosclerosis before the relevance of measuring this parameter can be decided. It has to be taken into consideration that physiological sweat gland function will depend on a number of external conditions such as humidity, temperature, clothing, and recent physical stress.

Stereomicroscopy of skin relief of morphoea plaques showed smoothing of the skin surface to be characteristic (VI). However, a quantitative expression of this smoothing is desirable. The author recently showed that the roughness of scleroderma skin of morphoea plaques and normal appearing skin can be differentiated using a stylus method (Serup, A). Smoothing did not correlate with increase in thickness of the plaques or with duration of morphoea. The method appears of interest for diagnosis and differential diagnosis of scleroderma. A future study of patients with acrosclerosis and other conditions with swollen fingers, including primary Raynaud's disease with skin relief measurement by an objective

method like the stylus method and as assessed under a stereomicroscope, is of interest.

Involvement of subcutaneous structures, i.e. tendons and joints resulting in contractures, and fibrosis of peripheral nerves resulting in peripheral neuropathy may be assessed by special finger goniometres and screened by determination of two-point discrimination (Serup 1983; Serup 1984g). Radiography of the hands in acrosclerosis patients often shows joint and bony involvements as well as calcinosis of the subcutaneous tissue (Brun, Serup & Hagdrup 1983).

The cornea thickness as measured optically by a slit lamp mounted with a special device was preciously shown to be increased in patients with generalized scleroderma (Serup, Serup & Hagdrup 1984). The increase in cornea thickness correlated with duration of scleroderma. The optical method is highly reproducible in the hands of an experienced ophthalmologist, but results obtained by different ophthalmologists may vary (Ehlers & Sperling 1977; Olsen, Nielsen & Ehlers 1980). The probe of the ultrasound skin thickness measurer developed in this study is with its small size and soft gelatine plug for coupling ideal for application on the cornea surface. The cornea is with its very smooth outer and inner surfaces anatomically ideal for such measurement. A further development of the ultrasound skin thickness measurer, including computerizing of the signals and mathematical functions (integral and average), is in progress with support from the Danish Rheumatism Association. The exact velocity of ultrasound in corneal tissue needs to be determined. The specificity of increased cornea thickness in relation to other connective tissue diseaes and primary Raynaud's disease is not known and require study.

It is the hope that non-invasive methods for quantitation of scleroderma and other skin diseases are used more extensively and further developed for objective description of manifestations and the course of diseases, comprising rational premises for assessments including evaluation of effect of therapy.

The purpose of this study was to evaluate localized scleroderma (morphoea) clinically and with respect to physiology, biochemistry and ultrastructure of the plaques with particular respect to changes of connective tissue (Chapter 1). The study was also planned to evaluate whether plaques are affected exclusively or if there is a more general involvement of skin and connective tissues in morphoea. Moreover, the purpose was to develop and test non-invasive methods for quantitation of scleroderma affection of skin relevant for the monitoring of generalized scleroderma (progessive systematic sclerosis) and technically applicable to patients suffering from this disease.

Based on clinical examination of a total of 214 circumscribed scleroderma lesions, including non-sclerotic pigmented spots, as registered in 58 patients, a detailed description of the clinical presentation of localized scleroderma is given (Chapter 2). Clinically two entities of morphoea may be defined, i.e. localized morphoea plaque and generalized morphoea. Disturbances of pigmentation being frequent, and linearity of skin lesions following special patterns may point to a predisposing defect in the migration of neural crest cells during embryonal life.

In *the sclerotic centre of morphoea plaques,* the skin was increased in thickness as was shown by ultrasound measurement with a 15 MHz A-scanner developed for this study (Chapter 3). The ultrasound method prowed accurate. The increase in thickness of skin afflicted with scleroderma was comparatively larger in the more thin extremity skin. A method was developed for in vivo measurement of skin elasticity based on automatically repeated applications of suction to a standardized skin area (0.79 cm^2) and registration of resultant elevation of the skin by the principle of electrical capacitance calibrated to distance. The method was accurate. Registrations of the tensile distensibility, the resiliency, and the hys-

teresis of skin was possible. Sclerotic morphoea plaques were characterized by decreased tensile distensibility and decreased hysteresis, but during remission the hysteresis temporarily turned to increased values. In the studies, effects of oedema and previous mechanical stress on skin rheology were also evaluated. Measurement of skin colour by remittance spectroscopy proved not to be of interest for quantitation of scleroderma. Laser-Doppler flowmetry showed increased blood flow in the white-coloured centre of fibrotic plaques. This increase was not attributed to altered optical properties of scleroderma skin. The sweat gland function of sclerotic plaques as measured gravimetrically after pilocarpine iontophoresis and as assessed by stereomicroscopy of sweat "pores" in silicone rubber imprints was reduced. Glands also showed evidence of "fatigue". Studies on sweat gland function were in accordance with histological evidence of atrophy. Stereomicroscopical assessment of skin surface relief showed loss of the normal polygonal crease-pattern reflecting epidermal atrophy.

Analysis of the collagen specific amino acids hydroxyproline and hydroxylysine, and of proline, showed increased concentration of hydroxylysine in the centre of sclerotic plaques, while the other amino acids were normal (Chapter 3). This increase was correlated to the duration of morphoea as well as the degree of sclerosis of the plaques. Analyses of glycosaminoglycans showed increased concentrations of dermatan sulphate and chondroitin 4/6 sulphate while the hyaluronic acid concentration was decreased. Uronic acid and hexosamine were not changed. Biochemical changes were concluded to be in accordance with known changes of self-limiting processes of reparative fibrosis such as wound healing, healing of bone fracture, and tendon lesion.

Electron microscopy of vessels showed alterations of endothelial cells and activation of pericytes, associated with cellular infiltration (Chapter 3). Alterations of endothelial cells were also found in normal appearing skin. Electron microscopy of nerves showed activation of pericytes as well; however, pericytes of nerves also pre-

sented cytoplasmic filaments and evidence of transformation into myofibroblasts, i.e. a cell type normally associated with intense neoformation of connective tissue. Thus, pericytes of nerves and possibly vessels may have an active role in the fibrotic process of morphoea plaques.

The peripheral and perilesional areas of morphoea plaques (Chapter 4) may exhibit special features such as a lilac ring. Laser-Doppler flowmetry confirmed that some plaques have a high perilesional flow, but most plaques presented a flow intermediate between the sclerotic centre and the normal appearing skin. Hydroxylysine, and dermatan and chondroitin 4/6 sulphate concentrations of the perilesional area were intermediate between the sclerotic centre and the normal appearing skin. However, the hyaluronic acid concentration was increased in contrast to decreased concentration in the centre of plaques. Hexosamines were increased, while uronic acid was normal. Biochemical findings in the perilesional area seemed to reflect temporal changes during the development of scleroderma. The ultrastructure of vessels and nerves of the peripheral and perilesional areas was comparable to the centre of plaques.

The normal appearing skin (Chapter 5) was not increased in thickness as compared to skin thickness measurements in healthy controls. Ultrastructural alterations of vascular endothelial cells were fully developed in the normal appearing skin, but pericytes of vessels were normal. Nerves were normal, and there was no evidence of transformation of pericytes of nerves into myofibroblasts. Thus, the normal appearing skin of morphoea patients is not entirely healthy, but provides alterations of vascular endothelial cells also known from the sclerotic centre of morphoea plaques.

Non-sclerotic pigmented spots, not showing evidence of sclerosis (Chapter 6), were frequent in morphoea patients (Chapter 6). These spots presented a decreased skin thickness and a rheology comparable to sequels of fibrotic plaques. Pigmented spots of morphoea are indicative of a pathophysiological association be-

tween increased and decreased formation of connective tissue of skin.

Analysis of urinary excretion of collagen metabolites in morphoea showed increased excretion (Chapter 7). This indicates a generalized abnormality of connective tissues. Measurement of the central thickness of the cornea, representing a specially organized and avascular connective tissue stroma, showed increased thickness with a positive correlation to the duration of morphoea. Thus, a general disturbance of connective tissue is present in morphoea.

The two entities of morphoea, i.e. localized morphoea plaque and generalized morphoea, differ in many respects (Chapter 8). Despite a limited regional distribution, the former represents a more advanced disease both with respect to the degree of sclerosis of individual lesions and with respect to general disturbance of connective tissues.

The pathophysiology of scleroderma including morphoea is under debate. This study indicates that activation of pericytes of nerves and possibly vessels and their transformation into myofibroblasts is significant (Chapter 9). The study provides no special evidence in favour of an active role of vascular endothelial cells in the pathogenesis of morphoea.

Non-invasive methods for quantitation of cutaneous and corneal manifestations of scleroderma as developed or tested in this study are technically applicable to patients with generalized scleroderma, and some of the methods may already be used for follow-up of individual cases (Chapter 10). Further adjustments and supplementary studies are proposed, aiming at routine use of non-invasive methods to quantify manifestations of generalized scleroderma.

Afhandlingen har til formål dels at belyse hudsygdommen lokaliseret sklerodermi (morphæa), dels at udvikle og gennemprøve noninvasive metoder til kvantitering af sklerodermisk hudaffektion. Det videre sigte er, med lokaliseret sklerodermi som model, at belyse den systemiske form af sklerodermi og at udvikle kvantiteringsmetoder egnede til klinisk anvendelse. Projektets kliniske opgørelse omfatter en detaljeret registrering af hudforandringer hos 58 patienter med morphæa. Fortykkelsen af sklerodermisk hud er målt med et højfrekvent ultralydudstyr udviklet til studiet. Fortykkelsen udgør indtil 310% af normal hud. Måling af brunpigmenterede hudelementer, der også findes ved morphæa, viser, at disse har mindsket tykkelse. Elasticiteten in vivo af sklerodermisk og rask hud er målt med en ny metode udviklet til studiet. Forskellige rheologiske mønstre i sklerodermiaffektioners ophelingsfase beskrives. Måling af blodgennemstrømningen i sklerodermisk hud med laser-Doppler teknik viser – trods hvid hudfarve – øget gennemstrømning. Måling af svedkirtelfunktion med to metoder (gravimetrisk og stereomikroskopi af hudaftryk) efter pilocarpin stimulation viser nedsat funktion i angrebet hud. De biokemiske undersøgelser omfatter analyse af kollagenspecifikke aminosyrer i hud og urin og analyse af fraktionerede glykosaminoglykaner i hud. Der findes i sklerodermisk hud øget koncentration af aminosyren hydroxylysin og af dermatan sulfat og chondroitin 4/6 sulfat, mens koncentrationen af hyaluronsyre er nedsat. De biokemiske fund er overensstemmende med kendte ændringer ved reparativ fibrose. Elektronmikroskopiske undersøgelser af dels kar, dels nerver viser karakteristiske forandringer af karendothelet og af karrenes og nervernes pericyter med, i patogenetisk sammenhæng bemærkelsesværdigt, transformation af pericyter til myofibroblaster omkring nerver. Myofibroblaster kendes fra aktive fibrotiske tilstande. Endothelforandringerne fandtes også i normal udseende hud, og

disse forandringer er derfor ikke tæt knyttet til manifest sklerose. Fund af øget udskillelse af kollagenmetaboliter i urin og fund af øget tykkelse af øjets hornhinde, der består af avaskulært bindevæv, viser, at morphæa ikke er en simpel bindevævsabnormitet svarende til de sklerotiske plaques. Morphæa-patofysiology diskuteres med særlig vægt på transformationen af pericyter til myofibroblaster og aktiveringen og styringen af denne. Videre undersøgelser beskrives og foreslås med henblik på applikation af projektets resultater på den systemiske form af sklerodermi og kvantitering af acrosklerotisk håndaffektion.

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