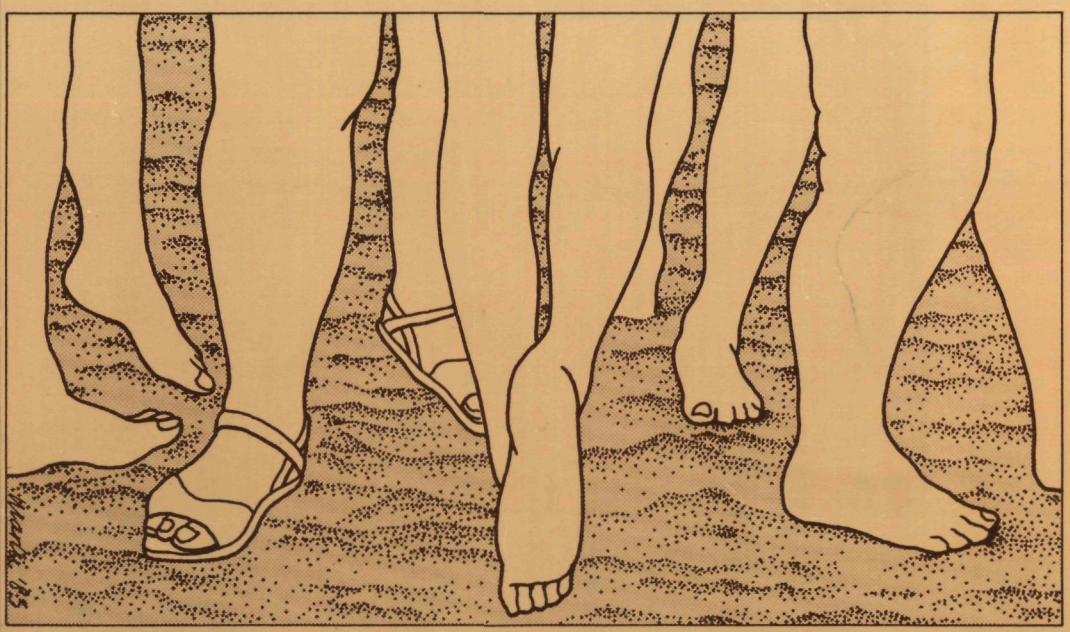
clinical investigation of **skin elasticity**



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clinical investigation of **skin elasticity**

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clinical investigation of
skin elasticity

an in vivo study of patients with varicose veins and certain connective tissue disorders

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During the last two decades the research in the Vascular Laboratory of the Department of Dermatology of the University of Nijmegen in cooperation with the Laboratory of Medical Physic and Biophysics, was concentrated on peripheral circulation in man (Brakkee and Vendrik, 1966; Kuiper, 1966). Considerable progress has been made with respect to the quantification of various parameters concerning the peripheral circulation and especially with respect to the calf muscle pump mechanism. Yet little is known to what extent the specific components of the leg such as venous wall, the muscles, the fascie sheet of the muscle compartment and the skin contribute to this calf muscle pump. Some authors believe that the skin under normal circumstances acts as a supplement to the calf muscle pump (Leu, 1972; Van der Molen, 1980). In their opinion the skin of the lower leg can be considered as an elastic stocking, counterbalancing rises in venous pressure.

In order to investigate the supposed contribution of the skin to the calf muscle pump, skin elasticity measurements were initiated. For this purpose an *in vivo* method has been developed at the Laboratory of Medical Physics and Biophysics based on uniaxial strain of the skin (Wijn, 1980). With this method the mechanical properties of the skin for small deformations of the skin can be characterized by a set of parameters (chapter 2). Normal values of these parameters as well as various factors which influence the parameter values are presented in chapter 3. Also Wijn's interpretation of the parameters in terms of elastin fibre properties will be discussed in this chapter.

Patients with primary varicose veins are presumed to have a generalized increase in venous wall distensibility. This idea is based on plethysmographic experiments by Wood (Wood and Wheeler, 1966) and Zsotér (Zsotér and Cronin, 1966) who found an increased volume distensibility of the forearm in patients with varicose veins of the leg. The volume distensibility of a leg, as measured by congesting the venous

system using an inflated cuff, depends in part on the distensibility of the venous vessels. From the measured volume distensibility of the limb a venous capacity can be calculated (chapter 2). This venous capacity reflects venous wall distensibility, which is determined by the quality of the vascular wall connective tissue and the pressure exerted on the vessels by the surrounding tissues (Van den Berg and Barbey, 1976). The increase in venous wall distensibility in patients with primary varicose veins is probably due to an altered vascular wall connective tissue. Since skin elasticity reflects the properties of the dermal connective tissue (Gibson and Kenedi, 1967), the increase in skin extensibility found by Van der Molen (1966) in patients with primary varicose veins points at a concomitant change in the dermal connective tissues in these patients. This recalls the old concept of a hereditary generalized connective tissue 'weakness' in patients with primary varicose veins postulated by Vogel (1905) and Curtius (1928). To test this concept of a so-called 'status varicosus' (Curtius, 1928), skin elasticity parameters as a measure for the skin connective tissue have been compared with the venous capacity as a measure for the quality of the vascular wall connective tissue (chapter 4).

Since it is claimed that a skin elasticity deficit contributes to the deterioration of the pump function in chronic venous insufficiency, also skin elasticity measurements were performed in patients with the chronic venous insufficiency syndrome (chapter 5). The correlation between the skin elasticity parameters and a pump function parameter, determined plethysmographically (Brakkee and Kuiper, 1975), was investigated in order to find a relationship between a possible skin elasticity change and the degree of pump function impairement. To investigate if the skin contributes to the calf muscle pump anyway, the pressure exerted by the skin on the subcutaneous tissues has been calculated from the elasticity data in normal subjects (chapter 5).

From the skin elasticity measurements in patients with chronic venous insufficiency, we learned that oedema influences

the mechanical properties of the skin. Therefore a chapter on skin elasticity in patients with oedemas has been added (chapter 6).
In the course of this investigation doubts arose about the correctness of the interpretation of the skin elasticity parameters in terms of elastin fibre properties. To improve our understanding of the biological background of the elasticity parameters, two generalized connective disorders were selected for skin elasticity measurements: one mainly affecting the elastin fibre system (pseudoxanthoma elasticum, chapter 7) and the other mainly affecting the collagen fibre system (Ehlers-Danlos syndrome, chapter 8). Again elasticity parameter values were compared with venous capacity values, in order to demonstrate a relationship between the degree of skin damage and the degree of vascular damage in these diseases.
In the last chapter (chapter 9) some results of the investigations are discussed more generally and a revised interpretation of the skin elasticity parameters is presented.

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Synopsis of the composition and function of the dermal and venous vascular wall connective tissues, in view of their mechanical properties.

1.1 Dermal connective tissue

1.1.1 Collagen and reticulin

Collagen is the major fibre constituent of the dermis. According to Weinstein and Boucek (1960) the percentage collagen of the fatfree dry weight (FFDW) of the human corium is approximately 77%. The corium can be distinguished microscopically in an upper part, with rather fine fibres (pars reticularis cutis), constituting 10% of the dermis (Smith et.al., 1982), and a lower part with coarse collagen fibre bundles (pars reticularis cutis), constituting the remaining 90% of the dermis. Total skin thickness varies from 0.95 mm for the glabellar region to 2.6 mm for the skin of the back (Pinkus, 1964). Mean skin thickness amounts about 1.3 to 1.5 mm. The dermis accounts for 95% of the total skin thickness. This figure is much lower for certain skin regions in which the epidermis has an increased thickness, such as in the palmar and plantar surfaces of hand and foot. Epidermal thickness of palmar and plantar skin can rise to 0.6 mm (Kyrle, 1925).
The upper part of the dermis contains many fine argyrophilic reticulin fibres with a diameter of 0.2 to 1µm. There is a large amount of ground substance associated with this kind of fibre. Electron microscopically the reticulin fibre shows the same periodicity as the collagen fibre. Nowadays we know that reticulin is identical with type III of the five collagen types which can be distinguished (Gay et.al., 1976). Special anchoring fibrils link the collagen fibre meshwork to the basal lamina (Swanson and Helwig, 1968), in this way connecting the dermis with the epidermis. In the lower two-thirds of the dermis coarse collagen fibres form a multiple directional system of wavy fibres and fibre bundles. The

fibre networks run mostly parallel to the skin surface (Kyrle, 1925). In relaxed state the fibres in the networks have a wavy intertwined coiled appearance. Under stress the fibre bundles straighten out. The coarse collagen fibres of the lower two-thirds of the dermis are mainly responsible for the strength of the skin (Meigel et.al., 1977). They consist of type I collagen. Four classes of collagens and a subdivision of these classes into several types are distinguished at present (Burgeson, 1982). For this review only the first class is of interest. This class consists of the major structural collagens viz. type I, II and III. The collagen type composition of the various tissues changes with ageing (Eyre, 1980). Type I collagen can be found throughout the dermis, it constitutes about 85% of the dermal collagen. Type III collagen is especially present in the upper dermis, constituting the remaining 15% of the dermal collagen (Bauer and Uitto, 1979). Collagen type II (cartilage), IV (basement membrane) and V (pericellular matrices) are not involved in the mechanical properties of the skin.

In the terminology of Grant and Prockop (1972) the structures which can be seen with the naked eye are called 'fibres'. The collagen fibre is constructed in such a way that it gains an impressive tensile strength. The fibre is made up of smaller components called 'fibrils' and the latter again of smaller so-called microfibrils. Fibrils are per definition the smallest structures visible on light microscopy (thickness 10-15 μm). The microfibrils, which are about 60 nm (600 Å) thick, are only visible with the electron microscope. The microfibrils are composed of ultra thin filaments approximately 3 to 5 nm thick. An ultra thin filament probably represents an aggregation of 3 to 5 collagen molecules, because the diameter of an ultra thin filament is too large for the dimensions of one tropocollagen molecule (diameter 1.4 nm; length 290 nm). A tropocollagen molecule consists of three polypeptide chains. Two basic chain types exist: α_1 and α_2. Collagen type I has two α_1 chains and one α_2 chain. Collagen type III consists of three

α_1 chains. Nowadays a more extensive subdivision of α-chains is used (Bornstein, 1980). The three polypeptide chains of the tropocollagen molecule are twisted in a triple helix twist to the left. Tropocollagen itself makes a righthanded twist. Apart from that, the filaments are twisted around one another too. Lateral and longitudinal covalent bonds (cross-links) inside and between the molecules contribute to the strength of the filaments. A fibre of a considerable strength is constructed in this way.
The synthesis of the precursor molecule of collagen, procollagen, occurs in the fibroblast. Also smooth muscle cells are capable of producing collagen, especially in vascular connective tissue. Among the various amino acids needed for the production of procollagen especially proline and hydroxyproline are of importance. The latter being a relatively specific marker for collagen. The intracellular enzymes prolylhydroxylase and lysylhydroxylase are required for the hydroxylation of proline and lysine. Hydroxylysine is a second amino acid characteristic of collagen. It plays a critical role in the formation of cross-links (Uitto and Lichtenstein, 1976). After passing through the cell membrane the enzyme procollagen peptidase splits the N-terminal end from the procollagen molecule, resulting in the formation of tropocollagen. Subsequently spontaneous aggregation of tropocollagen molecules into ultra thin filaments takes place by means of interactions between the charged side groups of the adjacent molecules. An extracellular enzyme, lysyloxidase, is necessary for the extracellular cross-linking of the molecules. The specific lateral alignment of the tropocollagen molecules gives the collagen microfibril the typical banded appearance, seen by electron microscopy. Extensive side to side interactions of the microfibrils give rise to the formation of fibrils (Trelstad et.al., 1982). In contrast to the extensive knowledge about collagen on the molecular level, hardly anything is known on how fibrils are united in fibres and how fibres are held together in fibre bundles (Black et.al., 1980).
The complicated collagen synthesis can go wrong at many

steps. For instance the above mentioned intracellular enzymes do not function appropriate without cofactors such as Fe^{2+}, α-ketoglutarate and vitamin C. Deficiency of vitamin C leads to the defective connective tissue of scurvy. Chemicals such as β-aminoproprionitrile block lysyloxidase, causing lathyrism in animals. Also many drugs can interfere with collagen metabolism, for instance cross-linking is impaired by penicillamine. The most obvious example of a disturbance in the synthesis of collagen leading to abnormal mechanical properties of the skin is seen in Ehlers-Danlos syndrome (chapter 7). In this heterogeneous syndrome several key enzymes of the collagen metabolism can be absent.
The main function of collagen in the dermis is its function as skeleton of the dermis, preventing the skin from injury by stretching, tearing and shearing. Other functions of the dermal collagen for instance in epidermal differentiation, in wound healing and as a matrix for antibody fixation are beyond the scope of this synopsis. For further information on collagen, the reader is refered to Harkness (1961), Grant and Prockop (1972) and Bornstein (1980).

1.1.2 Elastin

Approximately 2 to 4% of the fatfree dry weight (FFDW) of the dermis consists of elastin (Weinstein and Boucek, 1960; Hall, 1971; Uitto et.al., 1982). Elastin fibres are smaller than collagen fibres. The diameter of an elastin fibre varies from 0.5 to 3 μm (Schmidt, 1968). In the papillary dermis small elastin fibres ascent from the dermal elastin fibre meshwork perpendicularly towards the skin surface and end by attaching to the basal lamina (Tsuji, 1980). In the lower part of the cutis the elastin fibres are more coarse and show some fibre bundle formation. The major part of the elastin fibre meshwork in the lower dermis runs in the same direction as the collagen fibre meshwork, parallel to the skin surface. Unlike collagen fibres which are unbranched, the elastin fibres show many end-to-side junctions in their

network. Elastin fibres are looped spirally around the collagen fibres and are entwined with the collagen fibres. Elastin fibres show connections with collagen fibres (Dawber and Shuster, 1971; Pierard and Lapiere, 1979). In the lowermost part of the dermis elastin forms broad thin sheets with numerous branches (Tsuji, 1982).
The density of the elastin fibre meshwork is body region dependent, and even race dependent (Kyrle, 1925; Montagna and Parakkal, 1974). Abundant elastin fibres spin around hair follicles and around secretory coils of the sweat glands. Also around the microvasculature of the dermis an extensive elastic fibre network is found.
Electron microscopically the elastin fibre has two components: an amorphic core of elastin and a cortex of protein-like non-elastin microfibrils of about 11 nm in diameter. The mature elastin fibre consists of 8% microfibrils and 92% elastin (Ross, 1973). The protein elastin has the typical rubber-like properties. Microfibrils do not seem to play a role in the mechanical properties of the elastin fibre.
The production of elastin in the dermis takes place chiefly in the fibroblast and in the vascular wall especially in the smooth muscle cell (Rucker and Tinker, 1977). Elastin processing involves fewer enzymatic steps than collagen synthesis. After intracellular synthesis of tropoelastin, the native soluble elastin subunits organize around microfibrils. During the formation of the mature elastin fibre, cross-linking occurs depending on the enzyme lysyloxidase.
The structure of elastin is not yet completely elucidated. The insolubility of elastin is the major problem in the search for its structure. Presumably two types of elastin exist (Sandberg et.al., 1982). Various hypothetical molecular models have been proposed for the structure of elastin. At present approximately 70 percent of the sequence is known (Sandberg et.al., 1981). The predominance of non-polar amino acids is unique to elastin. Elastin has two special amino acids: desmosine and isodesmosine. They have a function in cross-linking. The insolubility of the elastin fibres seems to be related to the formation of the desmosine cross-links.

Elastin plays a role in several disease states. For instance in atherosclerosis often an accumulation of lipids near the elastin fibre is found. When lipid binds to elastin, there can be changes in the elastic properties of the vascular wall (Rucker and Tinker, 1977). In pseudoxanthoma elasticum the elastin fibres are markedly changed (chapter 7). The meaning of the calcium deposits on the elastin fibres in pseudoxanthoma elasticum and in atherosclerosis is unknown. Also in other diseases the role of elastin is increasingly appreciated (Sandberg et.al., 1981; Uitto et.al., 1982). The function of elastin in the mechanical properties of the skin will be discussed in section 1.1.5. For further information on structure and biochemistry of elastin the reader is refered to Ross (1973), Rucker and Tinker (1977), Sandberg et.al. (1981) and Sandberg et.al. (1982).

1.1.3 Ground substance

The amorphous matrix in which the fibre networks and cells of the dermis are embedded is called groud substance. It is a gel containing many components. There is some organisation in the gel with regard to glycosaminoglycans (GAG's) and water. Part of this organisation is the concept of the so-called 'pre-lymphatic fluid pathway'. This is a low resistance pathway for fluid and proteins, flowing from capillaries along connective tissue fibres to the initial lymphatics as postulated by Földi (1971).
The major part of the ground substance is formed by the glycosaminoglycans, which amount about 0.5 to 1% of the FFDW of the whole skin (Lindner and Schütte, 1976). Other components of the ground substance include blood products such as urea, glucose, salts, metabolic products of the connective tissue cells and water. The greatest part of the GAG fraction exists of hyaluronic acid and dermatan sulphate (= chondroitin sulphate B). Smaller amounts of chondroitin-6-sulphate (= chondroitin sulphate C) and heparan sulphate are present in the ground substance of the skin (Mier, 1972).

The basic molecular structure of GAG's is a polymeric sugar made up of disaccharide units in glycosidic linkage. These units consist of a hexosamine (either glucosamine or galactosamine) alternating with another sugar (Silbert, 1982). Heparan sulphate has a somewhat more complicated structure. Dermal GAG's are not free existing but are bound to proteins in the form of proteoglycans. The structure of the various core proteins is unknown. Interactions exist between the GAG's and collagen (Lindner and Schütte, 1976; Silbert, 1982). GAG's have an influence on collagen fibre formation (Comper and Laurent, 1978). Unlike the other GAG's hyaluronic acid is linked to little or no protein. A solution of hyaluronic acid has peculiar viscoelastic properties. It exhibits a predominantly viscous behaviour when the solution is deformed at low velocity and becomes elastic when the deformation occurs at high velocity. Increasing the concentration of hyaluronic acid makes the solution more elastic (Comper and Laurent, 1978). Depolymerisation of the polymer by hyaluronidase (bacterial or lysosomal), X-rays etc., causes a decrease in viscosity (Fabianek and Herp, 1970). Hyaluronic acid can bind a considerable amount of water. In combination with collagen still more water can be bound. Some disagreement exists on the terms 'bound' and 'free' water. The most appropriate way to deal with this problem is to define 'bound' water as the part of the total which is not available to dissolve water soluble non-electrolytes. In this way 80% of the water in the skin is 'free', while the remaining 20% will not dissolve even the smallest molecule of non-electrolyte (Yates, 1971).

Besides the above mentioned functions of the ground substance in controling water and salt distribution in the dermis, it has also an ion exchange function, a role in cell differentiation and it acts as a shock absorbing device in combination with collagen.

For reviews on GAG's the reader is refered to Mier (1972), Comper and Laurent (1978), Lindahl and Höök (1978) and Silbert (1982).

1.1.4 Ageing of the dermal connective tissue

From morphological studies it is known that collagen fibres thicken to the age of 20 years. After this age the increment lessens (Montagna and Parakkal, 1974). In senile skin the collagen fibre bundles become thinner (Braverman and Fonferko, 1982). The accumulation of so-called 'elastoid' material in sun-exposed skin of old individuals is probably denaturated collagen. The total amount of collagen in the skin decreases with age due to a decreased synthesis of collagen with ageing. Nevertheless this decrease concerns especially the soluble fraction of collagen whereas the insoluble fraction, this is the morphologically demonstrable fraction of collagen, increases (Lindner and Schütte, 1976). The number of intramolecular and intermolecular cross-links in collagen seems to increase towards senium (Lindner, 1972), although this is doubted by others (Bentley, 1979). In neonatal skin an abundance of type III collagen is found. A progressive reduction of type III collagen occurs with increasing age until at last type I collagen predominates in senile skin (Lindner and Schütte, 1976). With electron microscopy Hall (1976) could demonstrate many acute breaks and bends of the collagen fibres in the sun-exposed skin of the elderly. The fibres were covered with a mass of amorphous debris obscuring the characteristic cross-striations of the fibres. Elastin content of the skin probably decreases with age (Lindner, 1972). Other authors found increases of elastin content in the skin of older individuals (Sams and Smith, 1964; Pearce and Grimmer, 1972). With light microscopy one can find a progressive loss of the fine architecture of the elastin fibre network in the papillary dermis with advancing age. Both focal loss of elastin fibres and focal proliferation and thickening of the termal elastin fibres can be found in the papillary dermis of older persons (Braverman and Fonferko, 1982). Elastin fibres in the deeper layers of the dermis show a variable amount of degeneration with ageing (Hall, 1976). By electron microscopy marked changes are found in senile skin. The elastin fibres show a decreased

number of microfilaments, an appearance of electron dense inclusions and calcium deposits into the elastin matrix and at last desintegration of the fibre (Stadler and Orfanos, 1978; Braverman and Fonferko, 1982).
The glycosaminoglycan pattern changes from neonatal to adult skin. In neonatal skin 75% of the amount of GAG's consists of hyaluronic acid. The remaining 25% are sulphated GAG's. In adult skin the percentage hyaluronic acid is decreased to 30%, while the sulphated GAG's (mostly dermatan sulphate) increases to 70% (Lindner and Schütte, 1976). The reduction of hyaluronic acid and soluble collagen towards senium is responsible for the decreased water content of aged skin. Changes in the mechanical properties of the skin with ageing, will be discusses in chapter 3. For further information on ageing, see Lindner (1972), Hall (1976), Lindner and Schütte (1976) and Braverman and Fonferko (1982).

1.1.5 The function of the various connective tissue components, with regard to the mechanical properties of the skin.

The mechanical properties of the skin mainly depend on the dermal fibre networks, embedded in the viscous medium of the ground substance (Gibson and Kenedi, 1967). Removal of epidermis and subcutenaous fat has little influence on skin elasticity (Tregear, 1966; Vlasblom, 1967; Brown, 1973). When the epidermis is excessively thick, such as in the palms of the hands, than probably also the epidermis determines the mechanical properties of the skin (Harkness, 1971). The main fibre bulk in the dermis is collagen. The collagen fibre has a considerable strength. It requires 10 to 40 kg to break a collagen fibre with a diameter of 1 mm (Grant and Prockop, 1972). The maximal extensibility of the straight collagen fibre amounts 2 to 8% of its original length. The collagen fibres have a function in limiting skin extensibility, to prevent the skin from injury. Unlike collagen, elastin is very flexible. It has a rubberlike elasticity.

An elastin fibre can be stretched to 130% of its original length (Carton et.al., 1962). Therefore it is accepted that elastin has a role in the elastic recoil of the collagen network after deformation. Ground substance, containing hyaluronic acid has time and load frequency dependent properties. It serves as a shock absorber.
Several investigators (Gibson and Kenedi, 1967; Finlay, 1969; Brown, 1973) made microscopical observations on the dermal fibre networks under strain. When the skin is stretched, the collagen fibres which in relaxed state lay in a curled, twisted, wavy pattern, are straightened out and become aligned in the direction of stress. At the same time the elastin fibres which are looped spirally around the collagen fibre bundles and are attached to the collagen fibre bundles, become tightened. In fully stretched skin they lay between the parallel arranged collagen fibre bundles. After unloading,the elastin fibre network is presumed to act as an energy storage device in order to return the collagen fibre meshwork in its original relaxed position (Gibson and Kenedi, 1967). The degree of extensibility is not the same for each direction in which the skin is stretched. This effect is called anisotropy. For instance the skin of the calf can be stretched much farther in the direction perpendicular to the tibial axis, than in the direction parallel to the tibial axis. The effect is less pronounced in other skin areas of the body, as for instance abdomen skin. The anisotropy of the skin is already known from the experiments of Langer in 1861 (Langer, 1861). Langer mapped the cleavage lines of the skin in cadavers. These cleavage lines were later called 'Langer's lines'. The Langer's lines do not represent the lines of the greatest tension of the living human body. The greatest tension in the skin of a living human being is found along the so-called relaxed skin tension lines (Borges, 1973). It is now well established that Langer's lines run in the preferential direction of the dermal fibres (Cox, 1942; Ridge and Wright, 1966).

1.2 Venous vascular wall connective tissue

1.2.1 Composition of the venous vessel wall

The venous vessel wall consists of intima (inner layer), media and adventitia. The intima has a monolayer of endothelial cells and does not play a role in vessel wall mechanics. An elastin membrane (elastica interna) separates intima from media. The media has predominantly circular musculature. An external elastin lamina is found between media and adventitia. The adventitia contains a small quantity of longitudinal musculature, especially in the saphenous vein, less in the femoral vein. The vessel wall components: collagen, elastin and glycosaminoglycans are produced by the smooth muscle cells of the vascular wall (Burke and Ross, 1979). Media and adventitia have a variable amount of collagen, elastin, glycosaminoglycans and smooth muscle. Collagen content is about 50% of the FFDW of the internal saphenous vein wall. Elastin content amounts to approximately 13% and smooth muscle content amounts 30 to 40% of the FFDW of this vessel (Svejcar et.al., 1962; Leu, 1971). The deep veins for instance the anterior tibial vein, have about 40% smooth muscle in their vein walls (Conrad, 1971). The percentage of GAG's for example in the vena cava inferior amounts 0.4% of the FFDW (Kresse et.al., 1970). Collagen and elastin content of the vein walls decrease in distal direction while the smooth muscle content increases in the direction of the foot. The outer layer of the venous vessel wall, the adventitia, is continuous with the surrounding connective tissue. Collagen fibres branch from fascia and fat lobuli passing into the adventitia of the vessel. Small elastin fibres emerge from the adjacent connective tissue sheet and insert into the vessel wall (Schmidt, 1968).

1.2.2 Function of the venous vessel wall components in relation to their mechanical properties

The composition of the vessel wall reflects the function of the vessel. The arterial vessel has much elastin (20%, Kresse et.al., 1970) in its wall. The abundance of elastin seems to have a function in receiving and transmitting the pulse wave (Lie, 1980). The venous vessel being subjected to a much lower pressure has an adapted connective tissue: elastin and muscle content are lower than in arteries, collagen fraction becoming relatively more important (Conrad, 1971). Collagen has a function in the tensile strength of the vessel, protecting the vessel from over-extension. Elastin and smooth muscle cells cooperate in wall tension. Elastin seems to have a function in maintenance tension, while smooth muscle can also actively change the vessel diameter (Burton, 1954). The fact that smooth muscle content increases distally in the veins of the lower extremity, indicates that smooth muscle has a function in counterbalancing rises in intravenous pressure in these veins (Conrad, 1971).
The deep veins of the leg have a storage function, they act as a dynamic reservoir. Changing posture from horizontal to erect position causes an additional blood volume of about 350 ml for each lower limb (Ludbrook, 1976). In spite of such volume changes, a relatively constant supply for the cardiac pump is maintained. Some 70 percent of the blood volume is collected in the post-capillary or capacity vessels. Active changes in venous volume occur by contraction or relaxation of the smooth muscle cells in the vein wall, by flow resistance changes in the praecapillary and post-capillary region and by the calf muscle pump (including the 'skin-pump' according to Leu (1972)). Smooth muscle action is controlled by the sympathetic autonomous nervous system. Unlike the veins in the splanchnic area, the deep veins of the calf have little or no innervation (Shephard and Vanhoutte, 1975). Since the venous tone of the veins of the calf of a person in supine position at room temperature is

generally low, the capacity vessels act more or less as a passive reservoir in response to changes in intravenous pressure (Ludbrook, 1976). Under these conditions increases in limb volume as a result of internal venous pressure rise are largely due to passive wall distension, depending on venous wall quality and on the pressure exerted by the surrounding tissues on the vessel. The surrounding tissues become more important in determining vessel wall distensibility when the vessel wall is thinner, such as in capillaries and initial lymphatics. The mechanical properties of such small vessels do not depend on the connective tissues of their vessel walls, but are completely dependent on the mechanical properties of the surrounding tissues (Ryan, 1978).

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Methods.

2.1 Basic mechanical concepts for skin

The mechanical properties of a piece of skin can be described by means of a stress-strain relationship. The stress σ is the force exerted on that piece of skin divided by its cross-sectional area; the strain ε is the relative increase of its length caused by the stress.

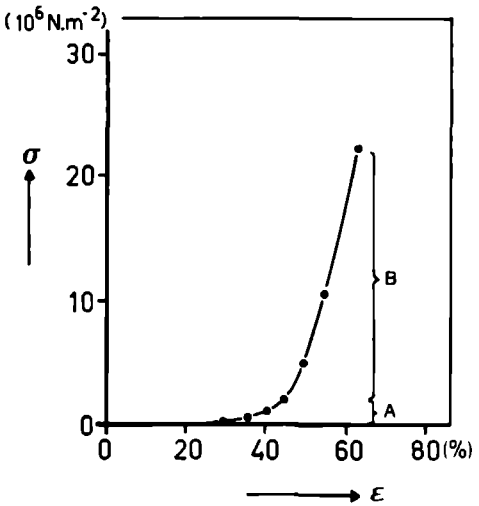

Fig.2.1 A stress-strain relationship of human skin.

Figure 2.1 shows a stress-strain relationship of human skin (according to Gibson et.al., 1976), in which two parts can be distinguished: part A in which low stress values cause a rather large extension of the skin and part B in which the skin can hardly be elongated further. In the literature part A is called the 'toe-part'. The toe-part reflects the straightening out of the collagen fibres (Brown, 1973) and the simultaneous stretching of the elastin fibres.

Part B corresponds to the stress exerted on fully stretched collagen fibres and therefore reflects collagen fibre properties. In this thesis only the 'toe-part' will be considered (maximum stress used is 0.05×10^6 $N.m^{-2}$). The 'toe-part' is said to be determined mainly by the elastin fibre meshwork (Dick, 1951; Daly, 1969; Wijn, 1980).
Coefficients of elasticity can be calculated directly from the stress-strain relationschip. This relationship is a modification of Hooke's law. In 1678 Robert Hook found that the extension of a metal wire (Δl) was proportional to various weights (F) hanging on the metal wire ($F=c.\Delta l$; figure 2.2.).

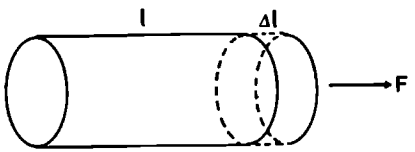

Fig.2.2 Extension of a wire.

Thomas Young made this law more precise, also considering the cross-sectional area of the wire. He showed that for the same material for each dimension of the wire, the force divided by cross-sectional area (stress σ) has the same proportionality to the relative increase in length of the wire (strain ϵ): $\sigma=E.\epsilon$. E is called the Young's modulus. The Young's modulus is only defined for linear elastic materials (e.g. steel). From figure 2.1 it is evident that a more complicated *non-linear* stress-strain relationship exists for human skin.
In addition the skin is not a purely elastic material. The strain depends on the duration of the applied force and does not return to zero when the stress is released. These two facts indicate that a liquid flow can take place in the skin. Therefore the skin is called *visco-elastic*.
In literature Young's moduli for biological materials are

calculated by taking the 'best' linear part of the stress-strain curve. The Young's modulus of elastin, as deduced from measurements on ox ligamentum nuchae, is reported to be approximately $3x10^5$ $N.m^{-2}$ and the Young's modulus of collagen, derived from experiments on fascia lata, is about 10^8 $N.m^{-2}$ (Caro et.al., 1978). Because the stress-strain relationship for human skin appears to be non-linear, the Young's modulus will not be used in this thesis. Instead of the Young's modulus the initial coefficient of elasticity is introduced. This initial coefficient of elasticity is defined as the slope of the stress-strain curve for zero stress. This is illustrated with figure 2.3, which shows a stress-strain curve, as obtained from our experiments in the 'toe-part' region.

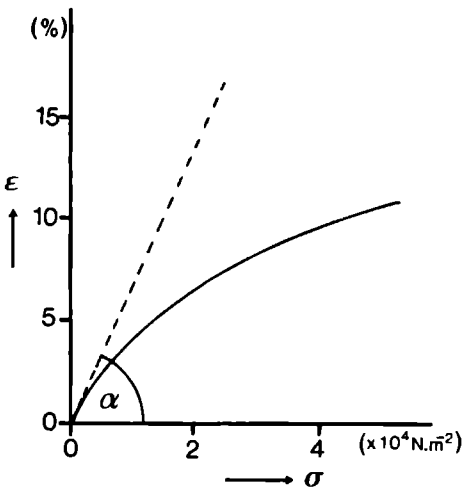

Fig.2.3 Initial coefficient of elasticity for zero stress: cotgα.

Because we have choosen in our experiments for the stress as the independent variable, the stress and the strain axis are interchanged in comparison with figure 2.1; therefore the initial coefficient of elasticity is cotgα.
A representative value for the initial coefficient of elas-

ticity for human skin in vivo, determined on the skin of the calf in the direction of the tibial axis is 1.5×10^6 $N.m^{-2}$ and in the perpendicular direction 0.1×10^6 $N.m^{-2}$ (chapter 3). These figures indicate that the extensibility of the skin, is not the same in each direction. This is called *anisotropy*.

2.2 Basic vessel wall mechanics

Two kinds of strain experiments can be performed on isolated blood vessels. Firstly, a piece of a blood vessel can be stretched longitudinally and secondly a fixed length of vessel can be distended by various known transmural pressures, while the vessel diameter change is recorded. In this way Young's moduli can be calculated for longitudinal and circumferential stress.
The stress-strain relationship for blood vessels has also a biphasic shape. The initial part seems to reflect the contribution of smooth muscle to the vessel wall and to a lesser extent the contribution of elastin to the vessel wall. The second part reflects primarily the collagen fraction of the vessel wall (Somlyo and Somlyo, 1968).
Young's modulus for smooth muscle depends on the level of contraction. In relaxed state it is similar to that of elastin and in the active state it is about 2×10^6 $N \cdot m^{-2}$. For the inferior vena cava Young's modulus amounts 0.4×10^5 to 1×10^5 $N.m^{-2}$ and for the femoral artery this is 9×10^5 to 12×10^5 $N.m^{-2}$.
The relationship between pressure and stress can be described by Laplace law. According to this law the pressure required to distend a tube against a given tension in the wall is inversely proportional to the radius of the tube. The wall tension is the product of the stress in the wall and the wall thickness.
An extensive exposé on vessel wall mechanics is beyond the scope of this introduction. More information on this topic can be found in Caro et.al. (1978).

2.3 Uniaxial strain measurements of the skin in vivo

2.3.1 Introduction

Investigations into the mechanical properties of human skin are not of a recent date. Langer (1861) made the first systematical investigations into this field. He stabbed a round shaped awl into the skin of cadavers. The resulting hole was not round but oblong, due to the tension in the skin. By applying this method systematically to the whole surface of the skin, he found the lines of the greatest tension in cadaver skin (Borges, 1973; figure 2.4).

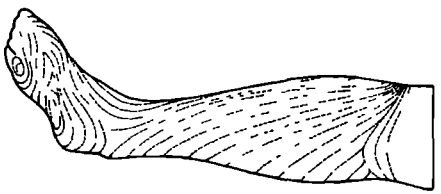

Fig.2.4 Langer's lines at the calf (after Arzt and Zieler,1934).

Langer also measured the extensibility of excised strips of skin (Langer, 1862). The first quantitative skin elasticity measurements in vivo, were performed by Schade (1912). He used an indentation method. Stricktly speaking he did not measure skin elasticity, since indentation of the skin refers more to the subcutaneous tissues than to the skin itself (Ridge and Wright, 1965). Since Schade's elastometer many methods were employed to measure skin elasticity in vivo (for survey see Wijn, 1980). Today most investigators use uniaxial strain or torsion measurements for their skin elasticity research. Uniaxial strain measurements have the advantage of the possibility to determine the degree of anisotropy in the skin. As could be expected already after Langer's experiments, a considerable anisotropy is found in human skin.

The present investigation is a continuation of the work of Wijn (1980) who studied extensively both methods and designed

a new uniaxial strain method. For a detailed description of instrumentation and signal analysis the reader is refered to this author. A short explanation of the method is given in the next sections.

2.3.2 Instrumentation

In uniaxial strain measurements, stepwise loads are applied to the skin and the resulting deformations are detected. The instrument used, is an improved version of the device presented earlier by Wijn et.al. (1976).

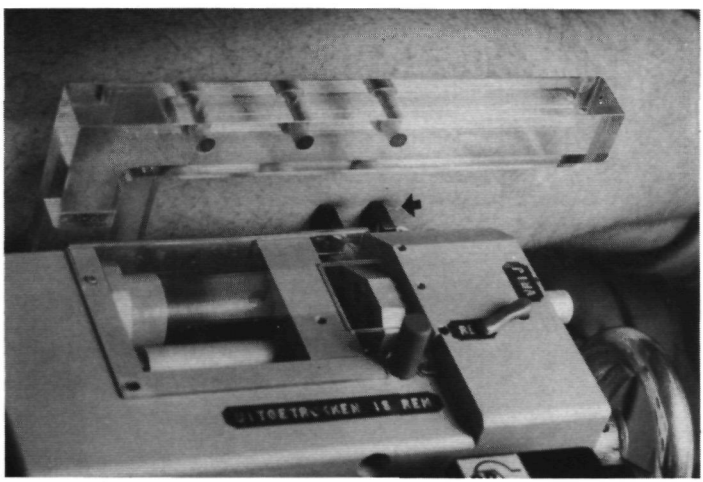

Fig.2.5 Uniaxial strain apparatus, viewed from underneath, with the two tabs (arrow) fixed at the dorsal side of the skin of the calf.

The skin is stressed by means of two parallel rectangular tabs (figures 2.5 and 2.6). The tabs are attached to the skin by double sided adhesive tape (Scotch 400). The size of the contact surface between tab and skin is 75 mm^2 (5 mm to 15 mm pro tab). In order to secure the reproducibility of the experiment, the attachment of the tabs to the skin of the calf is performed by an automatic procedure. This is done as follows: after placing the tabs against the

skin of the calf, the tabs are indented one centimeter by an indentation automaton for half a minute. After this time the tabs are pulled back automatically, leaving a residual indentation of one millimeter. One of the tabs is fixed to the frame of the apparatus and the other tab is connected to a permanent bar-magnet. The magnet can move with low friction along the axis of a cylindrical coil due to air-bearings (figure 2.6).

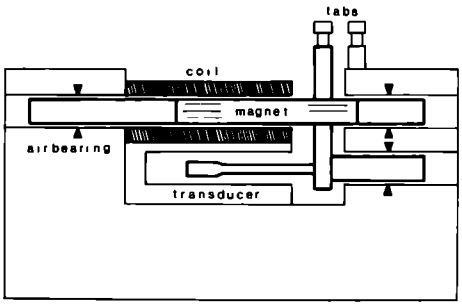

Fig.2.6 Schematic reproduction of the strain device.

Forces generated by current in the coil pull the tabs apart parallel to the surface of the skin. A preconditioning of the skin appears to be necessary to diminish the artefact of the attachment procedure and to improve the reproducibility of the experiment. For this purpose we use six short loadings (load duration is two seconds, interval time is also two seconds). The first regular load program starts one minute after the preconditioning of the skin. Six stepwise loads with increasing amplitudes (0.1, 0.2, 0.4, 0.6, 0.8, 1.0 N) are generated by a programmable source. The load duration is 10 seconds and the interval time is 20 seconds. The displacement of the movable part is detected as a function of time by an inductive displacement transducer (Philips PR 9314/10). The programmed forces and the deformation of the skin are recorded on paper (Rikadenki three channel recorder) for visual control during the experiment

and are sampled on line by a minicomputer (Digital MNC 11/02) for further analysis. The sample rate is 10 Herz. The apparatus is mounted on a specially designed couch (fig. 2.7). It can be shifted in any direction by a coordinate manipulator.

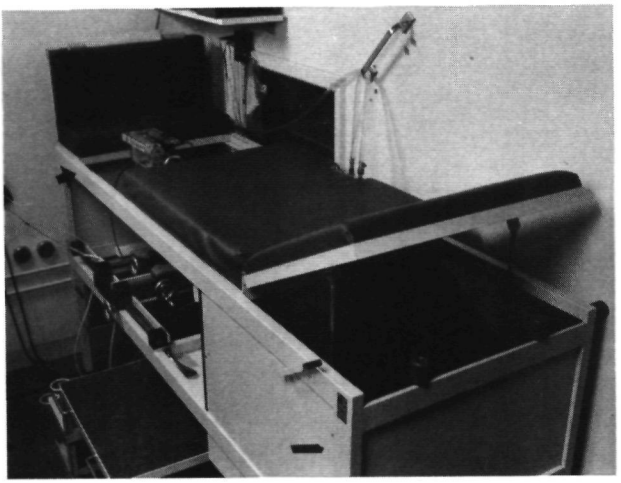

Fig.2.7 Strain apparatus and couch.

Measurements on the calf can be performed in a horizontal plane, in every direction, without changing the position of the patient on the couch. In one experiment two measurements are performed: one in the direction of the tibial axis and the other in the perpendicular direction. In normal circumstances one experiment requires about twenty minutes. Room temperature, outdoor temperature and relative humidity are noted to evaluate the possible effects of these factors on the results.

2.3.3 Signal analysis

2.3.3.1 Introduction

All the procedures described in this section are realized by a number of specific computer programs.

Signal analysis starts by correcting the measured displacements for the small deformations in the adhesive tape and in the apparatus. Furthermore the sizes of the piece of skin actually involved in the experiment, are deduced from the distance between the tabs and their width (Wijn, 1980). With these values and using a constant value for skin thickness of 1.3 mm, force and deformation are converted into stress: $\sigma(N/m^2)$ and strain: $\varepsilon(\%)$ respectivily (see section 2.1.).

2.3.3.2 Viscoelastic model

The schematic response of the skin on a load program of six loads with increasing amplitudes is presented in figure 2.8.

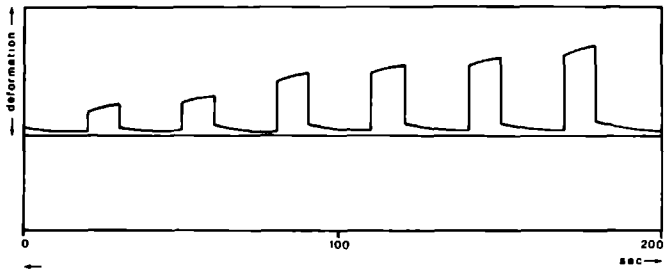

Fig.2.8 Schematic response of the skin on a loadprogram.

Figure 2.9 shows the response of the skin on one of the six loading cycles schematically. Three regions are distinguished: an instantaneous deformation (ε_a), a delayed deformation (ε_b) and a permanent deformation (ε_c). These three regions are considered as the results of three different mechanical processes in the skin: a purely elastic process, a delayed elastic process and a purely viscous process.

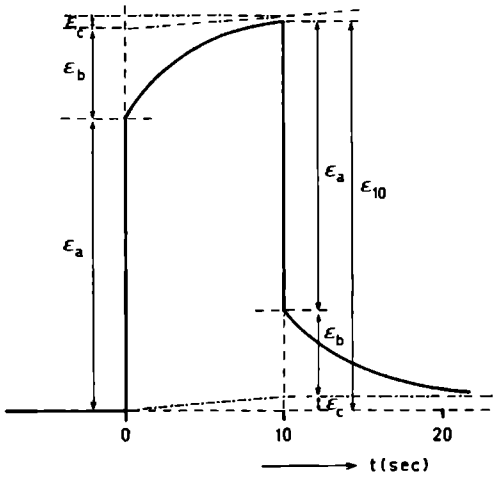

Fig.2.9 Schematic response of the skin on one loading cycle of 10 sec.

The response of the skin corresponds with the response of a mechanical model consisting of:

- a serial spring (Young's modulus E_s),
- a spring and a dashpot in parallel (Young's modulus E_p and viscosity η_p),
- a serial dashpot (viscosity η_s).

This is the so-called Burger's model (figure 2.10).

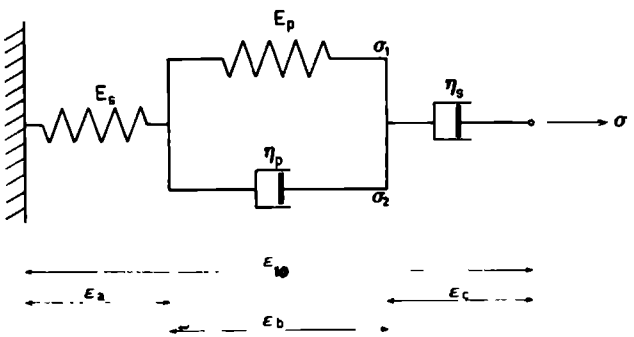

Fig.2.10 Burger's model

2.3.3.3 Initial coefficients of elasticity and coefficients of alinearity

For each mechanical process of section 2.3.3.2, strain values can be plotted against the stress values, resulting in stress-strain relationships. Since the deformations of the skin during loading and after loading are not similar, stress-strain relationships are calculated for the three different mechanical processes both during loading and after loading. Parameters derived from a process during loading will be indicated with the subscript 'u' for 'up' and parameters derived from a process after loading will be indicated with the subscript 'd' for 'down'.
Moreover in each experiment two measurements are performed, one in the direction of the tibial axis and the other in the perpendicular direction. Parameters derived from the measurement in the direction of the tibial axis, will be indicated with the subscript '//' and parameters in the perpendicular direction to the tibial axis with the subscript '$\perp$'. An example of the stress-strain relationships of the instantaneous elastic process down, determined along the tibial axis and perpendicular to the tibial axis is presented in figure 2.11.

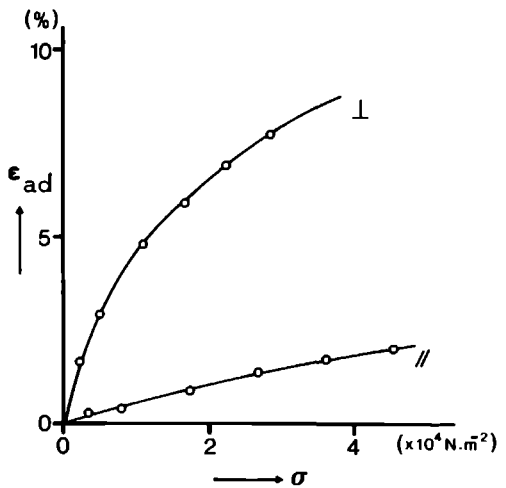

Fig.2.11 Stress-strain curves of the purely elastic process after loading, measured along (//) and across ($\perp$) the tibial axis.

All stress-strain relationships appear to be non-linear. For each process the stress-strain relationship can be described with

$$\frac{d\sigma}{d\epsilon} = E' = E_o + k.\sigma \quad \text{(Wijn et.al., 1981)}$$

In this equation E' is called the tangent modulus of elasticity, E_o is the initial coefficient of elasticity for zero stress and k is a coefficient of alinearity. The coefficient of alinearity is a measure for the degree of curvature of the stress-strain curve (figure 2.11). Henceforth the initial coefficients of elasticity will be presented without the subscript zero (E in stead of E_o).

2.3.3.4 Anisotropy and time constants

As shown in figure 2.11, the skin is more stiff in the direction along the tibial axis than in the perpendicular direction. Since the coefficients of alinearity determined along and across the tibial axis appear to be approximately equal, the anisotropy of the skin is only reflected in the initial coefficients of elasticity. Therefore the anisotropy is defined as:

$$A = \frac{E_{//}}{E_{\perp}}$$

The step response of a spring (E_p) and dashpot (η_p) in parallel (fig.2.10) results in an exponential curve, from which the time constant (τ) is equal to η_p/E_p (Wijn, 1980). The time constants of the delayed elastic process do not show anisotropy.

2.3.3.5 The selected set of parameters

For each of the three mechanical processes an initial coefficient of elasticity and a coefficient of alinearity can be calculated both during loading and after loading, and also both in the direction of the tibial axis and perpendicular

to the tibial axis. Altogether 34 parameters can be calculated, the anisotropy parameters and the time constants included.
The parameters of the purely elastic process will be indicated with the subscript 's' and the parameters of the delayed elastic process will be indicated with the subscript 'p'. Since the time constants are only calculated in the delayed elastic process, no subscript 'p' will be added to these parameters.
The parameters of the purely viscous process will not be used in this investigation, because these parameters cannot be determined with high accuracy.
If two parameters are highly correlated than the parameter which can be determined with the highest accuracy is selected. The 'up' and 'down' versions of several parameters are highly correlated: for instance $E_{su//}$ and $E_{sd//}$, $k_{su\perp}$ and $k_{sd\perp}$ (Wijn, 1980). However in the delayed elastic process, the initial coefficients of elasticity $E_{pu//}$ and $E_{pd//}$, and the time constants $\tau_{u\perp}$ and $\tau_{d\perp}$, are not correlated. Therefore both 'up' and 'down' versions of these parameters are included in the selected set of parameters.
In view of the high correlation between several parameters and the problematical determination of other parameters, finally the following set of ten parameters has been selected:

- purely elastic process : $E_{sd//}$, $E_{sd\perp}$, $k_{sd\perp}$ and A_{sd},
- delayed elastic process: $E_{pu//}$, $E_{pd//}$, $k_{pd\perp}$, A_{pd}, $\tau_{u\perp}$ and $\tau_{d\perp}$.

2.3.4 Reproducibility

Wijn (1980) found an overall reproducibility of the parameters determined along the tibial exis within 10%. The reproducibility of the parameters determined perpendicular to the tibial axis was a little worse. Insignificant errors are introduced when the measurements are performed not

exactly in the direction of the tibial axis or not exactly in the perpendicular direction (Manschot et.al., 1982).
A comparison between the parameter data of both legs in normal subjects, results in a reproducibility within 10% of the parameters of the purely elastic process and of the time constants. The reproducibility of the other parameters remains within 20%.

2.4 Determination of the venous capacity and calf muscle pump function by strain-gauge plethysmography

2.4.1 Introduction

Strain-gauge plethysmography was introduced by Whitney in 1953. A practical design for routine investigation was developed by Brakkee and Vendrik (1966). Kuiper (1966) demonstrated the value of plethysmographic investigations for patients with chronic venous insufficiency. The method is of importance for the demonstration of the degree of calf muscle pump impairement in these patients.
With a strain-gauge plethysmograph changes in leg volume can be detected. Volume increases can be induced by inflation of a pneumatic cuff around the thigh to pressures between systolic and local venous pressure which cause a congestion of the venous system. Plotting the congestion pressures against the corresponding increases in limb volume results in a so-called p_v-V relation. From this relationship a venous capacity can be calculated (2.4.2). The venous capacity is among other things determined by the (mechanical) properties of the venous vessel wall.
A quantification of the calf muscle pump function is possible by calculating the venous pressure fall in the foot due to activity of the calf muscles (2.4.3).

2.4.2 Determination of the venous capacity of the calf.

The subject is laying on a couch in a supine position (figure 2.12).

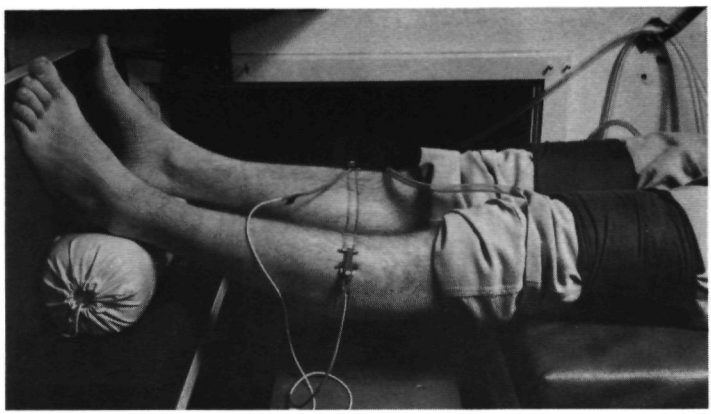

Fig.2.12 Subject on the couch with strain-gauges on the calf.

A pneumatic cuff (width 13 cm) is placed around each thigh. Mercury in rubber strain-gauges are put around the greatest circumference of the calf. Inflation of the cuffs to pressures p_c successively of 40, 30, 20 and 10 mm Hg, causes a congestion of the venous system. The congestion results in an increase in limb volume and reaches a stable phase when the enhanced venous pressure p_v is in equilibrium with the cuff pressure p_c. The venous pressure p_v just before deflation is not equal to the cuff pressure p_c, due to the elastic properties of the tissues between cuff and venous vessel wall. For a cuff with a width of 13 cm, $p_v=0.7x\ p_c$ (Kuiper, 1966). Plotting the relative increases of limb volume against the corresponding p_v-values, results in a p_v-V relation (figure 2.13).

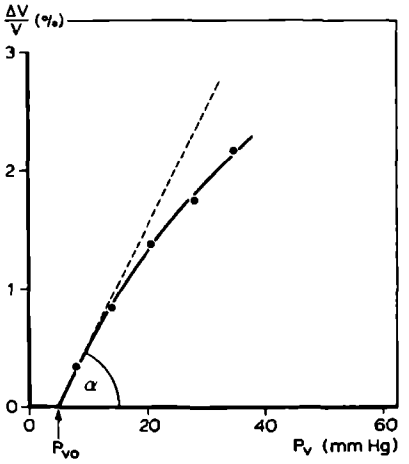

Fig.2.13 p_v-V relation of the left calf (male 45 yr).

The venous resting pressure p_{vo}, is found at the point of intersection of the p_v-V relation with the pressure axis. A value for the venous capacity C_o at p_{vo}, is found from the slope of the tangent in p_{vo} (tgα in figure 2.13). The venous capacity is expressed in %/mm Hg.

2.4.3 Determination of a calf muscle pump function parameter

As a measure for the calf muscle pump function the venous pressure fall in the foot due to tiptoe movements can be used ('walking venous pressure fall': WVp). This pressure fall is expressed as a percentage of the pressure in a foot vein of a motionless subject in the erect position ('standing venous pressure': SVP). The venous pressure fall in the foot can be derived from the corresponding volume decrease measured plethysmographically with strain-gauges around the forefoot. For the conversion of volume decrease into pressure decrease the p_v-V relationship of the foot, measured in the same way as described for the calf in section 2.4.2, is used. The SVP, which is the hydrostatic pressure in the foot, follows from the distance between the right heart and

the foot (Kuiper and Van de Staak, 1970). Knowing the pressure fall in the foot during tiptoe movements and knowing the SVP, the muscle-pump function WVp is found in a non-invasive way.

2.5 Statistical analysis

All parameters described in the previous paragraphs are calculated with their accuracy range (based on computer program BMDØ7R, Dixon, 1974). Parameter values with an accuracy worse than 40% are excluded, except for the time-constants which are excluded when their accuracy is worse than 25%. The parameters of the purely elastic process usually show an accuracy better than 10%.
Normal ranges of the parameters are determined from the inner limits and outer limits of the percentiles P_5 and P_{95}, with a distribution free technique (Rümke and Bezemer, 1972). For parameters which appear to be age dependent, a linear regression is performed with age in which the exact ages of the subjects are taken into account. For each data point the difference in parameter value with respect to the regression line is calculated (the residual). The normal limits of P_5 and P_{95} are now determined from these residuals. Parameters outside the outer limits are considered as abnormal. Values between the inner limits and the outer limits are in the warning area. Values between the inner limits are considerated to be normal.
Spearman correlation coefficients are calculated between the parameter values and age, outdoor temperature, relative humidity and venous capacity.
Patient groups are compared with the normal group by means of the Mann-Withney U test (Guilford and Fruchter, 1973).
If no p-value is presented, the following symbols will be used to indicate the level of significance:

**	: highly significant	$p \leq 0.01$
*	: significant	$0.01 < p \leq 0.05$
(*)	: dubious	$0.05 < p \leq 0.1$
-	: not significant	$p > 0.1$

The parameter values of the patient groups will also be presented as relative results in percentages of the mean values of the normal group, if the p-value is < 0.1.
Because of the age dependency of some parameters, both normal subjects and patients have been divided into four age groups: under 27.5 years of age; between 27.5 and 42.5 years; between 42.5 and 57.5 years and older than 57.5 years of age respectively. Statistical analysis is performed for each age group. The p-values of the Mann-Whithney U test for the different age groups of one disease state are combined (Rümke and Eeden, 1961). Parameter values of males and females of the patient groups, are compared with the parameter values of the same sex of the normal group. Some patient groups are not only compared with the normal group, but also with other patient groups by means of the Mann-Whitney U test.
Mean values and standard deviation (SD) of the parameter values of the patient groups will be presented to get an impression of the divergence of the parameter values in the patient groups.
In certain patient groups, patients are classified according to the severity of the disease and parameter values are compared with the Kruskal-Wallis test (Guilford and Fruchter, 1973).

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Chapter 3

Normal values of the skin elasticity parameters and of the venous capacity.

3.1. Skin elasticity measurements in normal subjects

3.1.1 The normal subjects

Normal values were determined in 92 persons. None of the persons of the normal group showed an abnormal skin of the calf clinically. The group included the 48 normal subjects measured by Wijn (1980), recruited by requests for volunteers in the local newspaper. The other 44 subjects were medical students and patients with minor skin diseases such as acne, superficial mycosis or wrats. Combination of both groups was allowed because no significant differences were found between the values of the used parameters of both groups (Mann-Whitney U test).
Experiments were performed on the skin of the calf of both legs of each person, both in the direction parallel and perpendicular to the main (tibial) axis of the leg. The results of the most accurate experiment of only one of the legs were used for determining the normal values.

3.1.2 Normal values related to sex and age.

The parameter values did not show a normal distribution, therefore a distribution free technique was choosen for statistical analysis (see 2.5).
Mean values and standard deviations of the parameters are presented in table 3.1, to give an indication of the divergence of the various parameters. For the age dependent parameters, mean values and standard deviations (SD) of the parameters of the four age groups are presented.

Table 3.1 Mean values and standard deviation of the parameter values.

MALES				FEMALES			
Parameter	mean	SD	n	Parameter	mean	SD	n
$E_{sd//}$	1.7	0.85	48	$E_{sd//}$	1.7	0.98	41
" (1)	1.9	0.95	14	" (1)	2.0	0.88	11
" (2)	1.8	0.91	16	" (2)	1.8	0.78	11
" (3)	1.6	0.47	9	" (3)	1.3	0.63	10
" (4)	1.0	0.61	9	" (4)	1.6	1.5	9
$E_{sd\lfloor}$	0.15	0.08	50	$E_{sd\lfloor}$	0.12	0.08	42
" (1)	0.19	0.08	16	" (1)	0.16	0.09	12
" (2)	0.14	0.07	16	" (2)	0.13	0.06	11
" (3)	0.13	0.05	9	" (3)	0.10	0.04	9
" (4)	0.11	0.09	9	" (4)	0.10	0.10	10
A_{sd}	13	6.4	48	A_{sd}	17	10	40
$k_{sd\lfloor}$	17	6.6	50	$k_{sd\lfloor}$	20	6.4	42
" (1)	15	6.1	16	" (1)	15	4.9	12
" (2)	17	3.3	16	" (2)	19	5.6	11
" (3)	22	10	9	" (3)	21	5.0	9
" (4)	19	6.2	9	" (4)	25	5.5	10
$E_{pu//}$	3.7	2.6	29	$E_{pu//}$	4.5	3.0	23
$E_{pd//}$	5.5	4.2	28	$E_{pd//}$	6.9	4.3	31
" (1)	7.2	4.9	10	" (1)	9.2	4.5	9
" (2)	4.8	4.2	8	" (2)	8.4	3.0	7
" (3)	5.5	3.7	6	" (3)	4.6	3.2	10
" (4)	2.5	1.8	4	" (4)	5.0	5.5	5
A_{pd}	14	10	20	A_{pd}	18	12	22
$k_{pd\lfloor}$	423	193	40	$k_{pd\lfloor}$	393	115	39
$\tau_{u\lfloor}$	3.6	0.77	45	$\tau_{u\lfloor}$	3.3	0.43	36
$\tau_{d\lfloor}$	6.0	0.44	49	$\tau_{d\lfloor}$	6.0	0.35	41
" (1)	5.8	0.46	14	" (1)	5.9	0.24	11
" (2)	5.9	0.24	17	" (2)	6.0	0.47	10
" (3)	6.1	0.43	9	" (3)	6.2	0.28	11
" (4)	6.4	0.35	9	" (4)	6.1	0.35	9

Legend to table 3.1: E-values: $x10^6$ $N.m^{-2}$, τ-values: in seconds.
Age related parameters are subdivided into the following age groups:
(1): under 27,5 years of age;
(2): between 27,5 and 42,5 years;
(3): between 42,5 and 57,5 years;
(4): above 57,5 years.

Figures 3.1 to 3.10 show the ten selected parameters of the group of 92 normal subjects in relation to age. The middle straight thick line in each figure stands for the linear regression line when a relation between the parameter and age could be demonstrated or stands for the mean value, indicated as a horizontal line, when no influence of age on the parameter could be detected.
The dotted lines are the inner limits and the straight thin lines are the outermost limits of the percentiles P_5 and P_{95}. The parameter values of the males are indicated with small circles and the parameter values of the females are indicated with closed dots.
Figure 3.1 to 3.4 show the results for the purely elastic process after deformation.

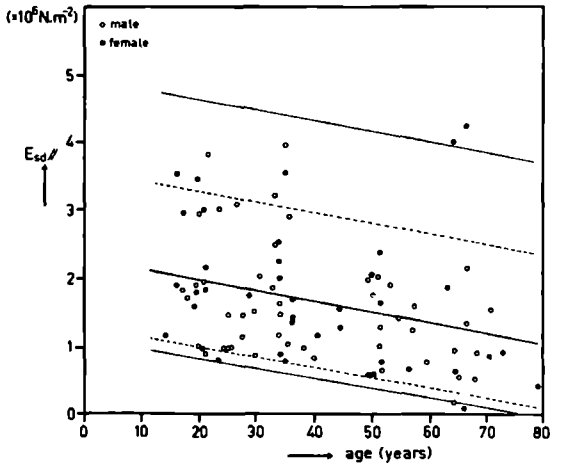

Fig.3.1 Initial coefficient of elasticity: $E_{sd//}$

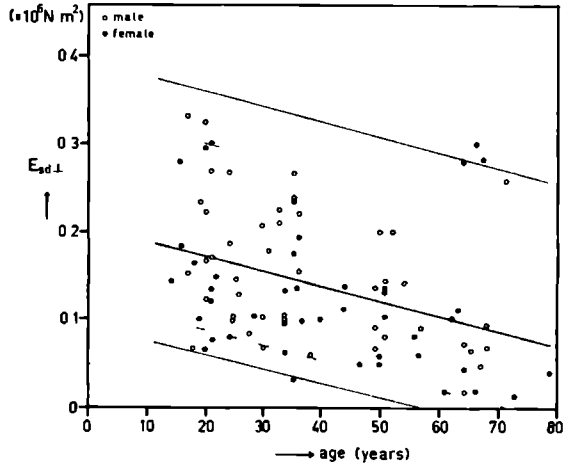

Fig.3.2 Initial coefficient of elasticity: $E_{sd\perp}$

The initial coefficients of elasticity of the purely elastic process: $E_{sd//}$ and $E_{sd\perp}$ decrease with age. The alinearity parameter $k_{sd\perp}$ increases with age. The anisotropy parameter, A_{sd} appears to be age independent.

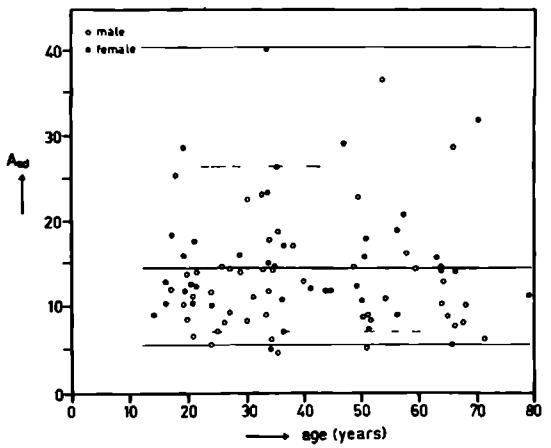

Fig.3.3 Anisotropy parameter: A_{sd}

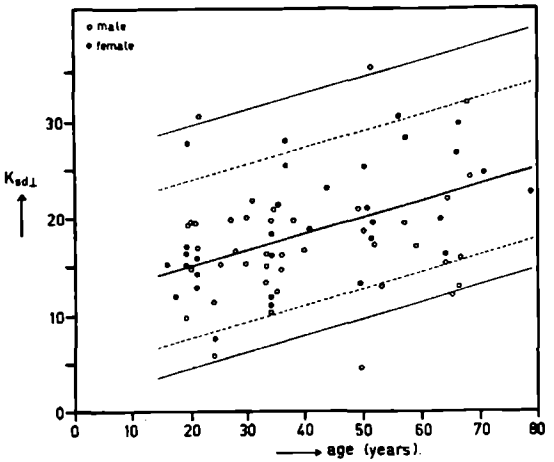

Fig.3.4 The alinearity coefficient: $k_{sd\perp}$

Figures 3.5 to 3.10 show the selected parameters of the delayed elastic process.

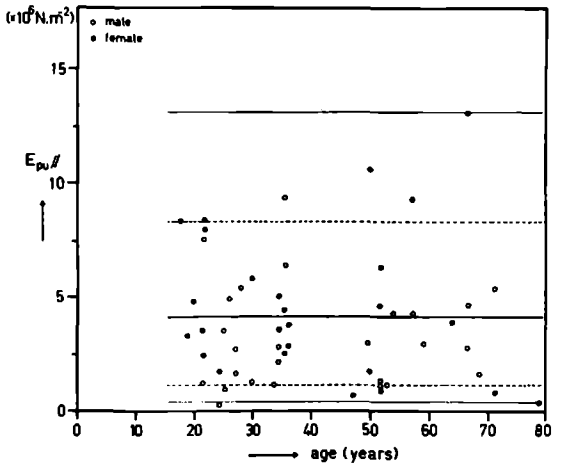

Fig.3.5 Initial coefficient of elasticity: $E_{pu//}$

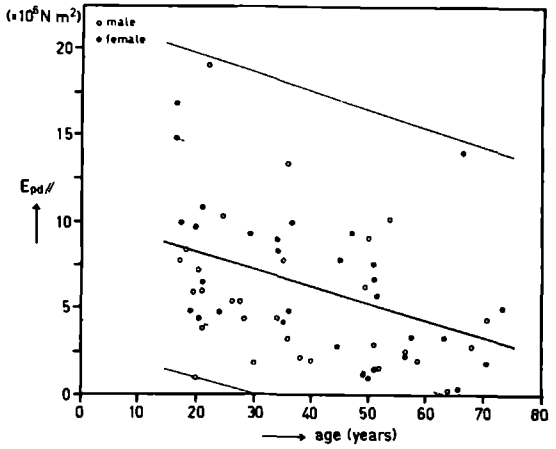

Fig.3.6 Initial coefficient of elasticity: $E_{pd//}$

In contrast to $E_{pu//}$, $E_{pd//}$ decreases with age in a similar way as $E_{sd//}$ and $E_{sd\perp}$. $E_{sd\perp}$ and $E_{pd//}$ appear to be highly correlated.

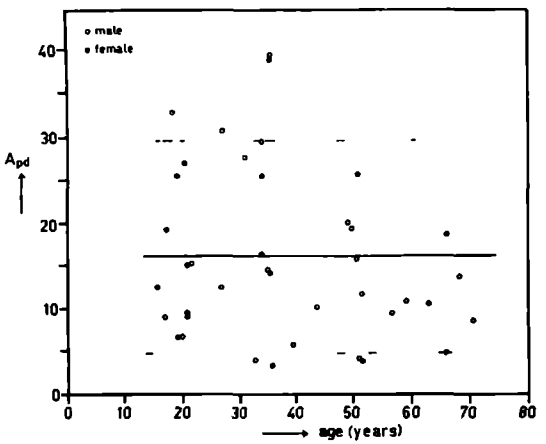

Fig.3.7 Anisotropy parameter: A_{pd}

The number of correct measurements of A_{pd} was too small to calculate outer limits.

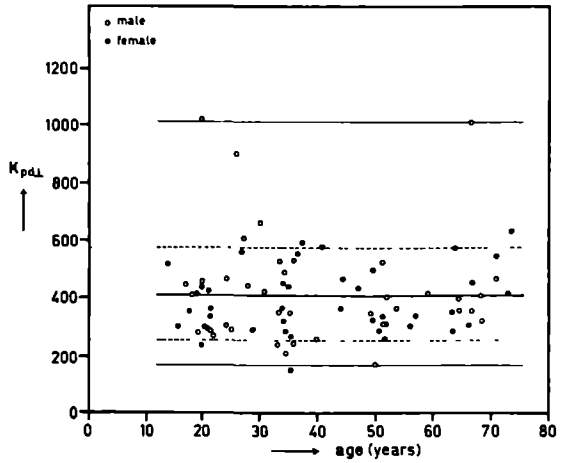

Fig.3.8 The alinearity coefficient: $k_{pd\perp}$

The alinearity parameter $k_{sd\perp}$ differs from the alinearity parameter $k_{pd\perp}$ in its age dependency.

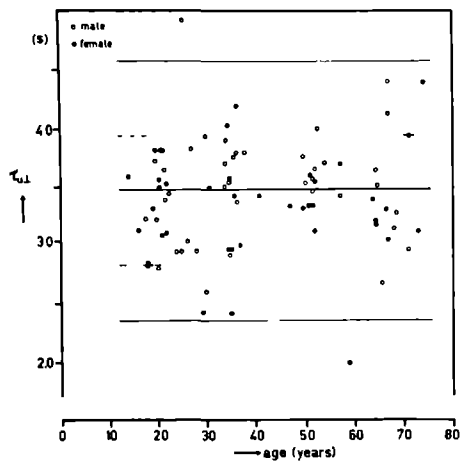

Fig.3.9 The time constant: $\tau_{u\perp}$

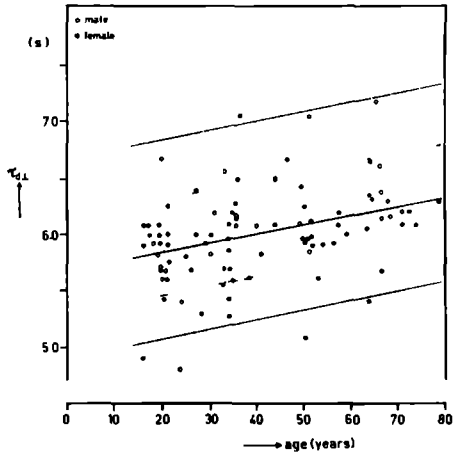

Fig.3.10 The time constant: $\tau_{d\perp}$

Both a comparison of the age dependency of $E_{pu//}$ with $E_{pd//}$ and $\tau_{u\perp}$ with $\tau_{d\perp}$, indicate that different processes take place in the skin during loading (stretching) and after loading (relaxation).

3.1.3 Discussion on factors influencing the parameter values

3.1.3.1 The influence of sex and age

In this investigation no significant differences between the parameter values of males and females were found. A slight difference existed between the sexes with regard to the A_{sd} parameter. This parameter shows lower values for males (table 3.1). In the literature differences between the skin elasticity of males and females in the 'toe-part' of the stress-strain curve (see 2.1) are reported. Grahame (1970) found, with a suction method, higher values for the moduli of elasticity in females than in males. Leveque et.al. (1980), using a torsion device, found a less extensible skin in females in comparison with males. On the other hand

Pierard and Lapiere (1977) found a more extensible skin in females than in males, with a traction method.
The influence of age on our parameters is demonstrated in figures 3.1 to 3.10. All the initial coefficients of elasticity appear to be age dependent, except $E_{pu//}$. The found lower initial coefficients of elasticity stand for a loss of skin elasticity with ageing. This means that old skin is more extensible than young skin. The only age dependent alinearity parameter is $k_{sd\perp}$.
The reports in literature on the influence of age upon the 'toe-part' of the stress-strain curve, only concern coefficients of elasticity. Dick (1951) found an increased skin extensibility in ageing skin in vitro. Jansen and Rottier (1957), using a uniaxial strain method in vitro, could not demonstrate changes with ageing. Daly and Odland (1979) showed in vitro a pronounced skin elasticity loss in senile skin. Graham (1970), using a suction cup method in vivo, found a progressive rise of the modulus of elasticity with ageing in contrast to our results. However in view of the high moduli of elasticity, which he found, his results might be related to the more stiff part of the stress-strain curve and are therefore incomparable with our results.

3.1.3.2 The influence of skin thickness

Skin thickness influences the elasticity parameter values, since stress is defined as force divided by cross-sectional area.
There are a few methods available for skin thickness measurements in vivo, of which only an X-ray method and an ultrasound echo method are sufficiently accurate. However in our opinion the X-ray method was not acceptable for this investigation. An ultrasound method was not available when we performed our experiments. Therefore a constant value for skin thickness of 1.3 mm was used (Pinkus, 1964).Recently Tan et.al. (1982) described the variability of skin thickness in vivo and the decrease of skin thickness with ageing

in normal individuals, using an ultrasound method. The influence of the interindividual variability of skin thickness of about 10% (Tan et.al., 1982) on the initial coefficients of elasticity cannot be neglected. Since skin thickness is directly proportional to the value of the initial coefficients of elasticity, an increase in skin thickness of 10%, gives a increase of 10% of these initial coefficients. Skin thickness decreases from the age of 20 to the age of 70 with 10 to 15% (Tan et.al., 1982). The initial coefficients of elasticity decrease in this period with about 50%. Therefore the decrease of the initial coefficients with ageing cannot be explained on the basis of a thinner skin with growing age.
To get an idea of the influence of an increased skin thickness on the parameter values, 12 patients with neurodermitis disseminata (disseminated atopic dermatitis) in a stable phase were measured. In all these cases the skin of the calf was involved. In such patients the papillary dermis may be 20 times its normal thickness (Ackermann, 1978), which means about a two to three fold increase of the total skin thickness.
In these patients both initial coefficients of the instantaneous elastic process, $E_{sd//}$ and $E_{sd\perp}$, showed an average increase by a factor of two. This corresponds to the above mentioned skin thickness increase. Patients with atopic dermatitis without involvement of the skin of the calf (n=5), did not have increased initial coefficients of elasticity. Because of the expected and demonstrated significance of the skin thickness for the initial coefficients of elasticity, our measurements were performed when possible, exclusively on clinically non-involved skin.

3.1.3.3 The influence of the stratum corneum and the relative humidity

According to Park and Baddiel (1972) the modulus of elasticity of the stratum corneum can reach a value of 10^9 $N.m^{-2}$.

This figure depends on the relative humidity. The cohesion of the stratum corneum cells might play a role in the mechanical properties of the skin.
In table 3.2 an example of a measurement in a patient with ichthyosis vulgaris is given. Ichthyosis vulgaris is a disorder of keratinization. In this disease the horny layer is slightly or moderately increased in thickness and has an abnormal composition. Elasticity parameters were determined before and one month after the use of an ureum containing ointment (Calmurid^{R}), which softens the stratum corneum in such patients. Relative humidity was the same during both experiments: 60%. Both initial coefficients of elasticity of the instantaneous elastic process showed a decrease by factor of about 2.5 after the use of the ointment.

	Before		After	
Parameter	abs.res.	rel.res.	abs.res.	rel.res.
$E_{sd//}$	4.8	253**	1.8	95
$E_{sd\perp}$	0.23	121	0.07	37
A_{sd}	21	162*	24	185*
$k_{sd\perp}$	19	127	16	107
$E_{pu//}$	-		3.4	92
$E_{pd//}$	6.0	83	6.1	85
A_{pd}	9.5	68	20	143
$k_{pd\perp}$	304	72	256	61*
$\tau_{u\perp}$	2.9	81*	3.3	92
$\tau_{d\perp}$	5.8	100	6.0	103

Table 3.2 Skin elasticity measurements on the skin of the calf of a man (20 yr) with a substantial ichthyosis vulgaris of the calf. Before and one month after the use of an ureum containing ointment.
abs.res.: absolute results, E: $x10^{6}$ $N.m^{-2}$, τ: seconds
rel.res.: relative results with respect to normals (%)
* : significance (see 2.5).

It is tempting to assume that the decreases of the initial coefficients of elasticity after the use of the ointment, are the result of a changed stratum corneum. However once more the higher initial coefficients of elasticity determined on the skin before therapy might also be partly the result of an increased skin thickness, due to an increased stratum corneum in the ichthyotic skin. In normal skin the thickness of the horny layer is about 15µm (Montagna and Parakkel, 1974), which is 1% to 2% of the total skin thickness (chapter 1). In ichthyosis vulgaris the stratum corneum can be increased to about five times its normal thickness (Pinkus and Mehregan, 1981). Therefore skin thickness alone cannot account for the increase of $E_{sd//}$ by a factor of 2.5 (see 'before' table 3.2). So the influence of the stratum corneum itself has to be reckoned with in case of an abnormal and increased stratum corneum.
The modulus of elasticity of isolated stratum corneum widely depends on the water content of the stratum corneum, the latter is influenced by the relative humidity (Park and Baddiel, 1972). According to Leveque et.al. (1980) the state of hydration of the stratum corneum cannot be neglected in skin elasticity measurements in vivo. Since usually the stratum corneum contributes only 1% to the total skin thickness, it is improbable that the hydration of the stratum corneum has a great influence on our skin elasticity parameters. This is supported by the fact that no correlation has been found between our skin elasticity parameters and the relative humidity (measured outdoors).

3.1.3.4 The influence of temperature

Since the mechanical properties of the skin are of a *visco*-elastic nature, some temperature dependency has to be expected. However Wijn (1980) could not detect any influence of room temperature or skin temperature on the parameter values. Also in the present investigation the skin elasticity parameters appear not to be correlated with outdoor

temperature. No season dependency of the parameters could be demonstrated.

3.1.3.5 The influence of posture

Subjects were measured in supine position. To investigate the influence of muscle pull on the mechanical properties of the skin, patients were measured with the leg in elevated position and experiments were performed with the foot in dorsal flexion and subsequently in exorotation. Nor elevation of the leg for 15 cm, nor dorsal flexion or exorotation of the foot did significantly influence the results. Yet Finlay (1971) performing torsion experiments, did found a stiffening of the skin by tensing the underlaying muscles. In our patients we observed repeatedly a slight increase of skin extensibility, when these persons were laying on the couch in supine position for a longer period of time. This could be an effect due to muscle relaxation.

3.1.4 Interpretation of the skin elasticity parameters

When the epidermis is sufficiently small, as in normal skin, the mechanical properties of the skin are mainly determined by the dermal fibre networks embedded in a viscous medium (Kenedi et.al., 1975).
In the initial part of the stress-strain curve only an untwining of collagen fibres, an orientation of collagen fibre bundles in the direction of stress and a simultaneous stretching of the elastin fibres occurs. With the small loads we used, no stress is exerted on fully stretched collagen fibres. Wijn (1980) gives an interpretation of the set of parameters chiefly in terms of elastin fibre properties. In his view the initial coefficients of elasticity are related to the amount of effectively involved elastin fibres in the straining process of the skin along ($E_{sd//}$, $E_{pu//}$, $E_{pd//}$) and across ($E_{sd\underline{|}}$) the tibial axis. The alinearity para-

meters are interpreted as a specific property of the elastin fibre itself ($k_{sd\perp}$) or as a structural parameter of the meshwork ($k_{pd\perp}$). The anisotropy parameters (A_{sd}, A_{pd}) are a measure of the angular distribution of the elastin fibres. The time constants of the delayed elastic process ($\tau_{u\perp}$, $\tau_{d\perp}$) are related to the velocity of the shifting of the bulk of the collagen fibre meshwork through the viscous interstitium.

The interpretation of Wijn (1980) of the elasticity parameters in terms of elastin fibre properties, although supported by some reasonable arguments, needs to be regarded with prudence. One of the reasons is that the initial coefficients of elasticity found in the skin of normal subjects are too high to be based solely on elastin. The values for the initial coefficients of elasticity in normal subjects are about 0.1 to $2x10^6$ $N.m^{-2}$ (table 3.1). The Youngs modulus for elastin is about $3x10^5$ $N.m^{-2}$ (see 2.1). Since the skin consists of only 2% elastin (see 1.1.2), the modulus of elasticity for skin would be $6x10^3$ if only elastin was involved in the mechanical properties of the skin for small deformations.

The alinearity parameters can also have another meaning than indicated by Wijn (1980). Our modified interpretation will be presented in chapter 9, as a result of the investigations into the mechanical properties of the skin of patients with a collagen defect and an elastin defect of the skin (chapters 7 and 8).

3.1.5 Discussion on the variability of the results of the uniaxial strain measurement

A large variability of the skin elasticity parameter values exists between the normal individuals. This variability cannot be explained solely on basis of skin thickness. The divergence of the initial coefficients of elasticity is much greater (table 3.1) than the divergence of the skin thickness of the leg (Tan et.al., 1982).

Another factor which might influence the variability of the parameter values, is a variability in fibre position in the skin of normal individuals. Differences in parameter values can be induced if interindividual differences exist for the preferential direction of the fibres in the skin, since the measurements were always performed in the direction of the tibial axis or in the perpendicular direction. Light microscopical and scanning electron microscopical investigations of the dermal architecture show a marked individual variability (Smith et.al., 1982), which supports the view of a dermal fibre distribution variability. The large divergence of the anisotropy parameters also points at this variability. The Langer lines as depicted in figure 2.4 are a simplification. They will not run in the same direction for each person.
Season dependency of some parameters can also cause some variability. We could not demonstrate such dependency, comparing the parameter values of different subjects in different seasons. These experiments must be repeated with persons which are measured several times in different seasons to get an appropriate impression of the season dependency. Factors as hormonal influences might cause a variability of the parameter values, although no differences between the parameter values of males and females were found in our normal group (except for A_{sd}).

3.2 Determination of the venous capacity in normal subjects

3.2.1 Normal values related to sex and age.

Normal values for the venous capacity were determined in 33 subjects. These subjects included patients with minor skin diseases (superficial mycosis, wrats, acne vulgaris etc.) and volunteer students. None of these persons was suffering from vascular disease.
The method of statistical analysis was the same as described in section 2.5. Figure 3.11 shows the venous capacity for the 33 testees. Outer limits could not be calculated due to

the rather low number of investigated persons.

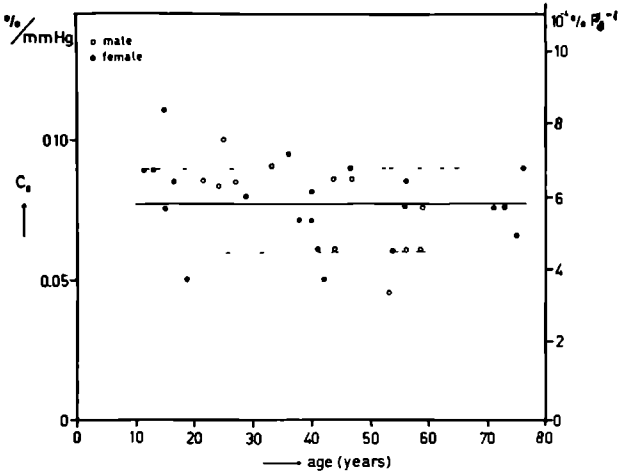

Fig.3.11 Venous capacity of the calf for 33 healthy subjects.

Neither age dependency nor sex dependency could be demonstrated in our material. Mean value for the venous capacity amounted 0.077 %/mm Hg (SD: 0.015 %/mm Hg). Mean value for the venous resting pressure appeared to be 6.3 mm Hg (SD: 2.7 mm Hg).

3.2.2 Discussion on venous capacity

The venous capacity parameter C_o as used in this study depends on a number of factors which are related to the mechanical properties and the morphological features of the various tissue constituents of the leg. The dependency on the venous wall properties has been proven in, in vivo studies using venoactive drugs (Brakkee and Kuiper, 1974). The mechanical properties of the vessel wall are determined by its connective tissue components. Since the mechanical properties of the skin are determined by the dermal connec-

tive tissue components, some correlation between venous capacity and the skin elasticity data had to be expected. However such a correlation was not found in the normal group. No age dependency could be demonstrated for the venous capacity of the normal group. Van den Berg and Barbey (1976) found a higher venous capacity with increasing age. The rather low number of testees in our investigation might be the reason that the venous capacity did not increase with age in our material.

Van den Berg and Barbey (1976) ascribed the increase in venous capacity to a loss of elastin fibres in the venous vessel wall with increasing age. Since the initial coefficients of elasticity decrease with age, presumably due among other things to a loss of functional elastin fibres in the skin (Wijn, 1980), a correlation between initial coefficients of elasticity and venous capacity could have been expected. However in the 13 subjects of our venous capacity group, in which on the same day also skin elasticity measurements were performed, no such correlation could be demonstrated.

In spite of the fact that room temperature was kept constant, still a rather large variability in C_o values existed. This could be due to factors as posture and muscle relaxation, which are difficult to keep constant. The position of the foot influences the venous capacity value, possibly due to calf muscle tensing (Barendsen and Van den Berg, 1976; Tripolitis et.al., 1980).

The sex did not influence the venous capacity in our material. In contrast to our results Van den Berg and Barbey (1976) found a lower venous capacity for females compared with males.

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Chapter 4

Skin elasticity measurements in patients with primary varicose veins.

4.1 Introduction

The incidence of varicose veins is striking. Borschberg (1967) estimated that in the western countries more than ten per cent of the population above the age of twenty is suffering from varicose veins. When even the slightest varix is counted, the percentage can rise to more than eighty for persons above the age of sixty (Da Silva et.al., 1974: Basle study II, Widmer et.al., 1978).
Already Hippocrates (± 300 B.C.) mentioned enlarged tortuous veins on the legs and associated these with leg ulcers (Anning, 1976). Many theories on the origin of varicose veins have been published since the time of Hippocrates (table 4.1; for reviews see: King, 1950; Rowden Foote, 1960; Schneider and Fischer, 1969; Alexander, 1972 and Anning, 1976). These theories have appeared to be unsatisfactory in one or other respects. One of the earliest theories proposed a generalized connective tissue 'weakness', the so-called 'status varicosus', as the cause of varicose vein formation (Curtius, 1928). This status varicosus included among other things: varices, haemorrhoids, herniae, striae and flat feet. Later on indeed connective tissue abnormalities were found. Aberrations were demonstrated especially in the connective tissues of the vein walls of patients with varicose veins (table 4.1). These connective tissue changes can be demonstrated both biochemically and microscopically (Svejcar et. al., 1963; Leu, 1971).
Microscopical examination of varicose veins shows a smooth muscle hypertrophy in the early phase of varicose vein formation. In later stages a disappearance of muscle sheet and elastic tissue can be found. These tissues are progressivily replaced by collagen, leading to fibrosis and subsequentely sclerosis of the vessel wall (Gans and Steigleder, 1957; Leu, 1971)

Table 4.1 Factors in the pathogenesis of varicose vein formation.

I Abnormality of the connective tissues:

- (generalized) increase in venous wall distensibility (Wood and Wheeler, 1966; Zsoter and Cronin, 1966; Eiriksson and Dahn, 1968; Prerovsky et.al., 1969; Mellmann, 1981)
- decrease of collagen, increase of elastin and hexosamine content of the venous wall (Svejcar et.al., 1963; Prerovsky, 1981)
- decrease of collagen and elastin content of the venous wall (Andreotti and Cammelli, 1979)
- decrease of insoluble collagen and increase of hyaluronic acid of the venous wall (Niebes et.al., 1977)
- abnormal collagen and elastin fibres in the venous vessel wall (Zwillenberg et.al., 1971)
- abnormal elasticity of isolated varicose segments (Thulesius, 1974)
- skin elasticity deficit (Van der Molen, 1966)
- generalized connective tissue 'weakness'(Curtius, 1928)

II Valvular incompetence:

- Congenital absence of valves (Curtis and Helms, 1947; Lodin, 1961)
- destruction of perforator valves (Dodd, 1976)
- valvular incompetence (Ludbrook, 1962, 1963; Wisemann, 1976)

III Mechanical stow:

- pressure on iliac veins by overfilled colon (Cleave, 1960; Dodd, 1964)
- obesitas as an aggravating factor (Ludbrook, 1964; Haeger, 1968)
- non adaption to erect posture of man (Rowden Foote, 1960; Bailey, quoted by Burkitt, 1972)

IV Smooth muscle defect:

- abnormal and less smooth muscle in the vein wall (Wagner and Herbut, 1949)
- injurious agents change smooth muscle cells (Staubesand, 1978)

V Dietary factors:

- constipation from low residue diet (Burkitt, 1972; Latto et.al., 1973; Melet, 1981)
- marginal vitamin E deficiency (Melet, 1981)
- abundant alcohol consumption (Tanyol and Menduke, 1961)

VI Other factors:

- hormonal influences (King, 1950; Arnoldi, 1957; Kappert, 1969; Leu, 1971)
- arterio-venous shunts (Piulachs and Vidal Barraquer, 1956)
- vasomotor disturbances (Rappert, 1961)
- lysosomal enzymes (Niebes and Laszt, 1971)
- false hemodynamic loading (Lechner, 1982)

Collagen and elastin contents of primary varicose veins were found to be lowered biochemically (Svejcar et.al., 1963; Andreotti and Cammelli, 1979). The hexosamine content (glycosaminoglycan fraction) and the smooth muscle content of the varicose veins were found to be increased (Svejcar et.al., 1963; Niebes et.al., 1977; Prerovsky, 1981). Smooth muscle function seems to be impared, showing a decreased contractibility in varicose veins (Laszt, 1971).
Plethysmographic findings in patients with varicose veins also suggest a functional disturbance of the venous vessel wall. The apparently non-involved forearm veins in patients with varicosities of the legs, showed an increased volume distensibility (Wood and Wheeler, 1966; Zsoter and Cronin, 1966; Prerovsky et.al., 1969).
This finding seems in agreement with the forementioned generalized venous wall 'weakness' in patients with varicose veins.
Besides the connective tissue lesion in the venous vessel walls (table 4.1), also the skin connective tissue might be involved in patients with varicose veins. This is suggested by Van der Molen (1966), based on his skin fold thickness measurements in patients with varicose veins. A skin elasticity loss in patients with varicose veins might be the dermal expression of a generalized connective tissue change in these patients.

To investigate a supposed skin elasticity deficit in patients with varicose veins, we used the recently developed uni-axial strain apparatus presented in chapter 2.

4.2 Material

Patients were recruited from the phlebological divison of the department of dermatology of the university. The clinical classification of the varicose veins was based on Basle study II (Da Silva et.al., 1974). Two groups of patients with primary varicose veins were selected: one group with truncal varicosities (TV) and the other group with reticular varicose veins (RVV).

Truncal varicosities were defined as tortuous veins belonging to the long or short saphenous veins or to the major branches of these vessels. Reticular varicose veins are disseminated small superficial tortuous veins not belonging to the major trunks.
The TV group was divided into two subgroups: one subgroup consisting of 16 males (mean age 39,4 years) and the other subgroup consisting of 19 females (mean age 39,9 years).
The RVV group consisted of 2 males and 15 females (mean age 40,1 years). It was not possible to divide the RVV group in a male and a female subgroup, due to the low number of males in the group. Men do not seem to seek medical attention as quickly as women for their reticular varicose veins. The RVV group consisted not solely of patients with isolated reticular varicose veins. Several patients of this group had reticular varicose veins in combination with so-called hyphenwebs ('Besenreiser' varices). Patients with primary varicose veins and an overt chronic venous insufficiency syndrome were excluded.

4.3 Measurements

4.3.1 Truncal varicosities

Tables 4.2 and 4.3 show the results of the skin elasticity measurements in the patients with truncal varicosities (TV). Also relative results in percentages of the normal values are presented if $p<0.1$, in the last but one column of the tables.

Parameter	mean	SD	n	percentage of normal	p-value
$E_{sd//}$	1.3	0.63	16	72	0.08
$E_{sd\perp}$	0.12	0.07	16		-
A_{sd}	16	15	16		-
$k_{sd\perp}$	19	7.5	16		-
$E_{pu//}$	4.2	3.2	10		-
$E_{pd//}$	3.2	1.9	10	67	0.06
A_{pd}	14	15	16		-
$k_{pd\perp}$	433	171	16		-
$\tau_{u\perp}$	3.8	0.86	9		-
$\tau_{d\perp}$	6.0	0.37	15		-

Table 4.2 Skin elasticity in 16 males with truncal varicosities.
E-values: $x10^6$ $N.m^{-2}$, τ-values: seconds.

No significantly abnormal parameter values were found in the TV group, except for $E_{pd//}$ in the female TV group. No statistical differences existed between the male TV subgroup and the female TV subgroup.

Parameter	mean	SD	n	percentage of normal	p-value
$E_{sd//}$	1.3	0.69	19		-
$E_{sd\perp}$	0.10	0.05	19		-
A_{sd}	16	8.2	19		-
$k_{sd\perp}$	16	5.0	19		-
$E_{pu//}$	3.8	2.2	10		-
$E_{pd//}$	2.8	1.3	11	33	<0.01
A_{pd}	8.6	3.8	6	48	0.07
$k_{pd\perp}$	382	136	17		-
$\tau_{u\perp}$	3.5	0.43	7		-
$\tau_{d\perp}$	6.1	0.52	17		-

Table 4.3 Skin elasticity in 19 females with truncal varicosities.
E-values: $x10^6$ $N.m^{-2}$, τ-values: seconds.

The TV group was classified into grades of truncal varicosity according to Kappert (1972), in order to investigate the correlation between the grade of severity of the TV and the elasticity parameter values. Grade I TV is a truncal varicosity without cross insufficiency (incompetence of the sapheno-femoral valve or the parva-popliteal valve); grade II is a TV with cross insufficiency; grade III with perforator insufficiency and in grade IV also trophical changes of the skin are present. No correlation has been demonstrated between the grade of varicosity and the elasticity parameter values.

Also the volume distensibility of the calf was measured plethysmographically. A significant higher venous capacity was found for the patients with TV in comparison with the normal group. Mean C_o value for the male TV subgroup was 0.12 %/mm Hg and for the female TV subgroup 0.10 %/mm Hg. The correlation was investigated between the skin elasticity data of the calf skin and the venous capacity of the same calf, in 7 males and 7 females (table 4.4). Only in the male TV subgroup some parameters correlated with the venous capacity.

	MALES (n=7)	FEMALES (n=7)
Parameter	r	r
$E_{sd//}$	-0.44	-0.23
$E_{sd\perp}$	-0.87**	0.50
A_{sd}	0.55	-0.49
$k_{sd\perp}$	0.11	-0.50
$E_{pu//}$	-1.0 **	0.20
$E_{pd//}$	-0.84*	-0.1
A_{pd}	0.23	-1.0
$k_{pd\perp}$	-0.22	-0.05
$\tau_{u\perp}$	1.0 **	-0.46
$\tau_{d\perp}$	-0.81*	0.28

Table 4.4 Correlation between skin elasticity parameter values and venous capacity in 14 patients with truncal varicosities.
r: correlationcoefficient, *: significance (see 2.5)

4.3.2 Reticular varicose veins

Table 4.5 shows the results of the skin elasticity measurements of the calf in the patients with reticular varicose veins (RVV), compared with the normal group. A comparison between men and women could not be made for the RVV group

due to the low number of men in the group.

Parameter	mean	SD	n	percentage of normal	p-value
$E_{sd//}$	0.84	0.42	17	47	<0.01
$E_{sd\perp}$	0.06	0.03	17	46	<0.01
A_{sd}	16	12	17		-
$k_{sd\perp}$	15	4.7	17	79	0.02
$E_{pu//}$	1.8	0.76	12	40	<0.01
$E_{pd//}$	2.9	2.5	14	35	0.01
A_{pd}	12	11	6		-
$k_{pd\perp}$	397	121	11		-
$\tau_{u\perp}$	3.3	0.57	10		-
$\tau_{d\perp}$	6.0	0.42	13		-

Table 4.5 Skin elasticity in 17 patients with reticular varicose veins.
E-values: $x10^6$ $N.m^{-2}$, τ-values: seconds.

All the initial coefficients of elasticity in the RVV group are significantly lowered, compared with the normal group. A comparison between the parameter date of the woman of the RVV group with the parameter data of the female TV subgroup, showed significantly lowered $E_{sd//}$, $E_{sd\perp}$ and $E_{pu//}$ values for the RVV group.
Plethysmographic experiments were performed on the same leg on which the elasticity measurements were performed in 11 patients. In contrast to the TV group, no deviation of the venous capacity was found in the RVV group. No correlation could be demonstrated between the venous capacity and the skin elasticity data of the RVV group, except for A_{sd} ($r=-0.67$).

4.4 Discussion

Most of the theories on the pathogenesis of varicose veins ascribe the development of the tortuous veins to some or other connective tissue factor. Collagen and elastin deviations have been demonstrated in varicose veins (table 4.1). Unfortunately not always a distinction is made between truncal varicosities (TV) and reticular varicose veins (RVV) in these investigations, whereas these two types of varicose veins probably have a different etiology (Lechner, 1982). The different prevalance of truncal varicosities and reticular varicose veins also points to a different etiology for these two conditions. The differences we found between the TV group and the RVV group both for the initial coefficients of elasticity and for the venous capacity, might support this view. There are some indications that TV are the result of haemodynamic disturbances with secondary vessel wall damage and RVV are the result of a hereditary generalized vessel wall lesion (Lechner, 1982).
If a generalized vessel wall lesion in primary varicose veins is a part of a generalized connective tissue defect as suggested by Curtius (1928), then it is interesting to investigate skin elasticity as a measure for the quality of the dermal connective tissue.
Although there is a tendency for lower initial coefficients of elasticity in the TV group, no significant abnormalities of the skin elasticity parameters of the apparently normal skin of the calf were detected, except for a lower $E_{pd//}$ value in the female TV subgroup. The significance of the latter finding is not clear yet.
In the RVV group significantly lower values for the initial coefficients of elasticity were found (about 50% of the normal values; table 4.5). These values were also lower compared with the TV group. Patients with RVV apparently have a more lax skin than normal. The abnormal skin elasticity in these patients points to a connective tissue defect of the skin. The idea of a generalized connective tissue defect is not supported by our plethysmographic experiments in

patients with RVV. One would expect an increased venous capacity, due to an increased venous wall distensibility as the result of a defective vascular wall connective tissue in these patients. However the venous capacity of the RVV group was normal and also no correlation existed between the venous capacity and the skin elasticity parameters. This might be due to the fact that we are comparing different parts of the connective tissues of skin and vascular wall by comparing skin elasticity data (mainly reflecting elastin fibre properties) with venous capacity data (reflecting smooth muscle, elastin and collagen properties of the vessel wall). The venous capacity determined plethysmographically, is known to be increased in patients with primary varicose veins (Wood and Wheeler, 1966; Zsotèr and Cronin, 1966). This is supposed to be the result of an increased venous wall distensibility due to a hereditary abnormal 'weak' connective tissue of the vessel wall. The pressure-volume curve of the limbs of patients with primary varicose veins is markedly different from the normal curve (Prerovsky, 1981). For small pressures the volume increase is greater in varicose patients than in normals. For high pressures the volume increase is diminished in comparison with normal subjects. Stress-strain relationships of isolated segments of the greater or shorter saphenous veins show a similar pattern: namely increased strain values for small loads (Laszt, 1971). It is tempting to assume that both the deviations in the pressure-volume curve and in the stress-strain relationship are due to the same pathological process in the connective tissue of the vein wall. A similar change in the stress-strain curve of the skin could not be found for the TV group. Nevertheless a correlation existed between several initial coefficients of elasticity of the male TV subgroup and the venous capacity (table 4.4). Such a correlation could mean a common factor both damaging venous wall connective tissue and dermal connective tissue. However in contradiction with this assumption is the fact that no correlation could be found between the grade of severity of the truncal varicosities and the elasticity

parameters.
In view of the forementioned results it is unlikely that the skin is of pathogenetic importance in the development of TV.
The strain experiments described in this chapter were performed with small loads. The parameters derived from the stress-strain curves obtained with such experiments mainly reflect elastin fibre properties (see 3.1.4). For testing the collagen part of the skin, larger loads are required. Therefore the forementioned results do not exclude a collagen defect in the skin of sufferers from varicose veins.

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Skin elasticity measurements in patients with the chronic venous insufficiency syndrome.

5.1 Introduction

In the vascular laboratory of the department of dermatology of the University of Nijmegen, peripheral circulation research has been performed by strain gauge plethysmography for some twenty years (Brakkee and Vendrik, 1966; Kuiper, 1966). This has resulted in a quantification of the calf muscle pump function by objective plethysmographical criterions (Kuiper and Brakkee, 1971).
Hardly anything is known about the contribution of the various components of the leg: venous wall itself, muscles, fascie and skin to the musculo-venous pump.
Some authors believe that the skin acts as a supplement to the calf muscle pump (Schneider und Fischer, 1969; Leu, 1972; Van der Molen, 1980). The skin of the lower leg is supposed to act as an elastic stocking (Földi et.al., 1974).
Failure of this skin-pump mechanism due to a hypothetical hereditary skin elasticity deficit, should attribute to the failure of the muscle calf pump mechanism in the chronic venous insufficiency syndrome, resulting in venous stasis (Van der Molen and Kuiper, 1962; Leu, 1972). According to Van der Molen (1966) indeed a skin elasticity loss exists in patients with the chronic venous insufficiency syndrome.
To investigate the supposed loss of skin elasticity in patients with the chronic venous insufficiency syndrome, we performed skin elasticity measurements in two groups of patients with chronic venous insufficiency (5.2). A plethysmographically determined parameter for the muscle calf pump function (WVp; see 2.4.3), was compared with the skin elasticity parameters, in order to see if the degree of pump function impairement was related to the degree of skin elasticity loss.
To evaluate the contribution of the skin to the calf muscle

pump, we estimated the pressure exerted by the skin on the subcutaneous tissues, by means of Laplace's law.

5.2 Material

Patients were recruited from the phlebological department of the university hospital. To make a distinction between idiopathic chronic venous insufficiency and chronic venous insufficiency as the late sequelae of deep vein thrombosis, a subdivision of the patient group was made. One subgroup consisted of 14 patients with post-thrombotic chronic venous insufficiency (CVI-PT) and the other group consisted of 16 patients with chronic venous insufficiency not due to deep vein thrombosis (CVI-NT). The CVI-PT group (mean age 40,9 years) consisted of 6 males and 8 females and the CVI-NT group (mean age 49,1 years) consisted of 6 males and 10 females. The number of patients in the various age groups was too small to make a comparison between men and women. Because compression of the skin might influence the results of the skin elasticity measurements, by expelling fluid from the (sub)-cutaneous tissues, it was attempted to measure only patients without elastic stockings. In the CVI-PT group, 5 patients who wore elastic stockings were included, because the symptoms of the CVI in these patients were too severe to stop them from wearing elastic stockings for some time. None of the patients of the CVI-NT group wore elastic stocking at the time of the skin elasticity measurements.

5.3 Measurements and results

Both legs of each patient were measured. In tables 5.1 and 5.2 only the results of the measurements which were performed on the skin of one of the legs of each person, viz. the affected leg, are presented. In both CVI groups the initial coefficients of elasticity measured in the direc-

tion of the tibial axis are lowered, compared with the normal group. All the anisotropy parameters are significantly lowered in both groups. The alinearity parameters are slightly lower. Comparison of the skin elasticity data of the CVI-PT group with the data of the CVI-NT group, showed no significant differences between the skin of the affected legs of both CVI groups.
Tables 5.1 and 5.2 show the results of the skin elasticity measurements in the CVI-PT group and the CVI-NT group, compared with the normal group.

Parameter	mean	SD	n	percentage of normal	p-value
$E_{sd//}$	0.70	0.45	14	39	<0.01
$E_{sd\underline{\vert}}$	0.13	0.09	14		-
A_{sd}	6.2	3.4	14	41	<0.01
$k_{sd\underline{\vert}}$	15	6.4	14	83	0.06
$E_{pu//}$	2.3	1.5	6		-
$E_{pd//}$	2.1	1.6	5	32	0.05
A_{pd}	3.4	2.2	3	21	0.01
$k_{pd\underline{\vert}}$	364	124	11		-
$\tau_{u\underline{\vert}}$	3.8	0.32	5	110	0.06
$\tau_{d\underline{\vert}}$	6.1	0.46	10		-

Table 5.1 Skin elasticity in 14 subjects with post-thrombotic CVI
E-values: $x10^6$ $N.m^{-2}$, τ-values: seconds.

Parameter	mean	SD	n	percentage of normal	p-value
$E_{sd//}$	0.85	0.60	16	59	0.02
$E_{sd\perp}$	0.10	0.07	16		-
A_{sd}	9.8	7.0	16	68	0.01
$k_{sd\perp}$	17	4.3	16	79	0.04
$E_{pu//}$	3.1	3.3	8		-
$E_{pd//}$	3.5	4.5	13	69	0.05
A_{pd}	11	10	11	76	0.04
$k_{pd\perp}$	374	150	16		-
$\tau_{u\perp}$	3.2	0.50	7		-
$\tau_{d\perp}$	5.9	0.39	15		-

Table 5.2 Skin elasticity in 16 subjects with non-post-thrombotic CVI.
E-values: $x10^6$ $N.m^{-2}$, τ-values: seconds.

The parameters derived from the skin elasticity measurements of one leg, were compared with the grade of CVI of the same leg. As a measure for the degree of CVI was used the plethysmographically determined pumpfunction criterion: the 'walking venous pressure fall' WVp (Brakkee and Kuiper, 1975; see 2.4.3). If no WVp determination was done, the clinical classification of Da Silva et.al.(1974) was used to estimate the grade of CVI (table 5.3).

CVI-grade		WVp	clinical symptoms
I	(slight)	50%<WVp≤65%	corona phlebectatica
II	(moderate)	30%<WVp≤50%	+ oedema + pigmentation
III	(severe)	WVp≤30%	+ ulcer history

Table 5.3 CVI criterions used in this investigation

A significant correlation (r=-54) was found between the grade of CVI and the $E_{sd//}$ parameter. Highly significant correlations (r=-84) were found between the grade of CVI and all the anisotropy parameters. This means that patients with severe CVI had low anisotropy values. Especially in patients with severe CVI and much oedema of the leg very low anisotropy values were found. Because also in patients with oedema due to other causes than CVI, lower anisotropy values could be demonstrated (chapter 6), it became apparent that the lower anisotropy values found in the patients with CVI could be due to the dermal oedema.
To investigate if the lower initial coefficients of elasticity and lower anisotropy parameters in the patients with CVI (tables 5.1 and 5.2) were due to a congenital skin elasticity deficit or were the result of the dermal oedema, we compared the affected legs of the CVI patients with their apparently healthy legs. Most of the non-affected legs of the CVI-PT group showed normal initial coefficients of elasticity and normal anisotropy parameters. In patients with an idiopathic CVI, a comparison between the elasticity data of both legs is more difficult, since frequently both legs show signs of CVI. Nevertheless the legs with slight symptoms of CVI in the CVI-NT group showed almost normal skin elasticity parameter values.
A significant difference between the limb volume distensibility of the CVI-PT group and the CVI-NT group existed. In the CVI-PT group the mean C_o value was normal. The mean C_o value for the CVI-NT group was increased (0.094 %/mm Hg). The venous capacity (C_o) did not correlate with any of the elasticity parameters of the CVI-PT group. In the CVI-NT group only a highly significant correlation (r=-0.70) between C_o and $k_{sd\perp}$ was found.

5.4 Discussion

In both CVI groups lower initial coefficients of elasticity, determined on the skin in the direction of the tibial axis

($E_{sd//}$, $E_{pd//}$) of the legs with CVI were found. The initial coefficients of elasticity determined in the perpendicular direction to the tibial axis ($E_{sd\perp}$), were normal (tables 5.1 and 5.2). This resulted in lower anisotropy values (A_{sd} = $E_{sd//}$: $E_{sd\perp}$). Abnormal values of the skin elasticity parameters were not found in the non-affected legs of the patients of the CVI-PT group and slightly abnormal values were found for the skin elasticity parameters of the non-affected legs of the patients of the CVI-NT group. It is likely that the noted deviations of skin elasticity in CVI skin are the result of the CVI and are not due to a hypothetical hereditary skin elasticity deficit, because in the latter case both legs should show similar abnormal skin elasticity values.

It is known that long lasting dermal oedemas cause fragmentation of elastin fibres in the stratum reticulare (Braun-Falco, 1964). Since the initial coefficients of elasticity are mainly related to the amount of elastin fibres involved in the strain procedure (3.1.4), it seems reasonable to ascribe the found lower initial coefficients of elasticity in CVI skin to the dermal oedema in such skin. However if this assumption is correct, one would expect that both $E_{sd//}$ and $E_{sd\perp}$ values are lowered in the skin of patients with CVI. Yet the $E_{sd\perp}$ value of the affected leg of the patients with CVI was normal, causing the lower anisotropy values.

An explanation of the lower anisotropy values in CVI skin could be the following. Oedema which involves the whole leg, causes an increase in circumference of the leg, stretching the skin considerably in the direction perpendicular to the tibial axis. The skin in the direction of the tibial axis is hardly stretched. Because of the tightening of the skin by the oedema, a stiffer skin is measured especially in the direction perpendicular to the tibial axis. Therefore $E_{sd\perp}$ increases more than $E_{sd//}$ and a lower anisotropy is found (A_{sd} = $E_{sd//}$: $E_{sd\perp}$). However another factor influences the E-values.

Longstanding dermal oedemas in CVI cause a considerable

elastin loss, resulting in lower E-parameter values, because these parameters are mainly related to the dermal elastin fibres.
In the perpendicular direction elastin loss, causing lower $E_{sd\perp}$ values and tightening of the skin by the oedema, causing higher $E_{sd\perp}$ values compensate each other, resulting in almost normal $E_{sd\perp}$ values in the CVI groups (tables 5.1 and 5.2).
In the direction along the tibial axis however, the tightening of the skin by the oedema is not so great because of the shape of the leg, therefore the elastin loss dominates, causing lower $E_{sd//}$ values in the CVI groups (tables 5.1 and 5.2).
When the oedema of the leg is treated by compressive bandages the $E_{sd\perp}$ will decrease and to a lesser extent the $E_{sd//}$ value, the anisotropy will become normal (chapter 6; table 6.2).
In the decongested leg the $E_{sd\perp}$ value will be decreased to the same level as the $E_{sd//}$ value due to the elastin loss, for instance to about 60% of the normal values. In the non-decongested limb the $E_{sd\perp}$ value is not found to be decreased to 60%, but is normal (table 5.2). In this case the increase in $E_{sd\perp}$ value due to the oedema is 0.04×10^6 $N.m^{-2}$. From the formula in 2.3.3.3 it can be calculated that an $E_{sd\perp}$ value of 0.04×10^6 $N.m^{-2}$, corresponds with a strain of 6% in the direction perpendicularly to the tibial axis.
Such a value for the circumference increase of the leg in oedema seems to be reasonable in view of clinical data.
A highly significant correlation ($r=-0.84$) was found between the grade of CVI and the anisotropy parameter. This indicates that a quantitative relationship exists between the amount of oedema in the leg and the anisotropy value. The higher the grade of CVI, the more oedema in the leg and the lower anisotropy value.
Another way to investigate whether a loss of skin elasticity contributes to the development of the chronic venous insufficiency syndrome, is to determine the correlation between skin elasticity parameters and pump function data in

patients with CVI. No correlation existed between a measure for the pump function: WVp (walking venous pressure fall) and the initial coefficients of elasticity. This supports the view that the skin does not contribute to the development of CVI.

If skin connective tissue is not directly involved in the development of the chronic venous insufficiency syndrome, but is supposed to reflect the state of the vascular wall connective tissue, than the highly significant correlation between venous capacity and $k_{sd\perp}$ value of the CVI-NT group is interesting. The alinearity parameter $k_{sd\perp}$, is probably a structural parameter of the collagenous dermal fibre meshwork (chapter 9). This might point at a collagen defect in the CVI-NT group.

Some authors believe that the skin acts as an elastic stocking. In chronic venous insufficiency this elastic stocking should fail, due to a skin elasticity loss (5.1). However since skin elasticity does not seem to be involved in the pathogenesis of chronic venous insufficiency, one wonders if the skin acts as an elastic stocking under normal circumstances anyway.

By means of skin elasticity measurements in normal subjects, an estimation can be given of the pressure exerted by the skin on the subcutaneous tissues. This pressure gives an impression of the contribution of the skin to the muscle calf pump mechanism.

An estimation of the 'skin-pressure' on the subcutaneous tissues can be obtained using Laplace law: $p = \frac{A}{r} \times \sigma$, by considering the limb as a cylinder with a radius r, covered by skin with a thickness A, and a radial tension (stress) σ. The radial tension in the skin can be derived from the initial coefficients of elasticity in the perpendicular direction to the tibial axis ($E_{sd\perp}$).

It is known from plethysmographic experiments, that the volume change of the calf in vivo, during muscular exercise of the calf muscles amounts to a few percent (Kuiper, 1966). Such a volume change causes a circumferential change of the calf of half that value, say of about 1%, which means

1% strain in the direction perpendicular to the tibial axis. With a normal value for $E_{sd\perp} = 0.13 \times 10^6$ N.m^{-2}, the stress (σ) for a 1% strain is 0.13×10^4 N.m^{-2}. For a calf with a radius of e.g. 6 cm, the pressure p exerted by the skin under this strain is 28.2 N.m^{-2} (=0.21 mm Hg). Such a pressure (at calf level) has no significance for the calf muscle pump mechanism, realising that Arnoldi et al. (1966) have determined in the great saphenous vein at calf level, a mean pressure difference of 58 mm Hg between contraction and relaxation of the muscle pump. Moreover Arnoldi et.al. (1966) showed with phlebography that no change in cross sectional area of the great saphenous vein occurs during muscle contraction of the calf. If the skin is not able to compress the saphenous vein, than it will certainly not act as an elastic stocking. For all these reasons it is rather improbable that the skin contributes to the musculovenous pump.

The 'skin-tendon-pump' of Van der Molen (1980) should act in the knee region and in the lower third of the leg as a supplement to the calf muscle pump. According to this author, repeated dorsal flexion of the foot, causing rhythmic expansion and relaxation of the skin of the lower leg, should squeeze blood through the venae communicantes in the direction of the deep system.

On the basis of the estimated circumference increase of the lower leg (1 to 1.5 cm) by Van der Molen (1980), we calculated 'skin-pressures' of 1 to 2 mm Hg by determing initial coefficients of elasticity in that skin region. Therefore also the existance of a 'skin-pump' in the lower leg must be doubted. As a result of knee movements which might stress the skin beyond the 'toe-part' of the stress-strain curve, a compression of the great saphenous vein between bone and skin might take place.

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Chapter 6

Skin elasticity measurements in patients with oedema

6.1 General introduction

As argued in the forgoing chapter, the oedema of chronic venous insufficiency may influence the initial coefficients of elasticity and the anisotropy parameters of the skin of the calf. Therefore we became interested in the influence of various kinds of oedema on the mechanical properties of the skin.
Firstly, we measured the initial coefficients of elasticity of the instantaneous elastic process and the anisotropy parameters in oedemas of different origin, to see if the same parameter changes occur in all kinds of oedema as in the oedema of chronic venous insufficiency (table 6.1). Secondly a section on lymphoedema was added (section 6.3), since Ryan (1978) suggested that skin elastic tissue plays a role in the development of lymphoedema. Finally, a section on a pseudo oedematous state: lipoedema was included, to investigate whether a distinction could be made between the excess of fluid in the skin of lipoedema and the oedema due to chronic venous insufficiency by means of skin elasticity measurements (section 6.4).

6.2 Determination of initial coefficients of elasticity and anisotropy parameters in patients with oedemas of different origin

6.2.1 Material

17 Patients of various ages with pronounced clinical (mostly pretibial) oedemas were recruited at the dermatological department of the university hospital.
Among these patients were subjects with oedema due to chronic venous insufficiency, cardiac oedema, nephrogenic oedema, dependency oedema, oedema due to a post-traumatic

sympathethic dystrophy (causalgia minor), lymphoedema, hypodermitis and lipoedema. Also some patients with an acute exacerbation of their atopic dermatitis were measured, because in patients with a disseminated atopic dermatitis the whole skin seems to be oedematous in the acute phase. Some patients with oedema due to chronic venous insufficiency and some patients with cardiac oedema, were measured several times, before and after dehydration of the leg, to investigate the influence of the amount of oedema on the parameter values. An example of these measurements is given in table 6.2.
Finally the effect of locally induced oedema on the parameter values was investigated in healthy medical volunteer students. An example of such an experiment is presented in table 6.3.

6.2.2 Results

Table 6.1 shows the results of the skin elasticity measurements for the instantaneous elastic process for 17 subjects with heavy oedema.
The most remarkable result from table 6.1 is that all patients with oedema have much lower anisotropy values (mean normal value for $A_{sd} = 15$), regardless the cause of the oedema. Also the three patients with a neurodermatitis disseminata (generalized atopic dermatitis) in the acute phase, showing a swollen face clinically, had a decreased anisotropy value for the skin of the calf, on which the oedema was clinically less evident.
Patients treated with compressive bandages have lower initial coefficients of elasticity. Especially the $E_{sd\perp}$ values of these patients are lowered in comparison with similar patients without compressive bandages. Patients with huge oedema of the leg, without compressive bandages show an enormous increase in $E_{sd\perp}$ values (cases 2, 4 and 11 to 17 in table 6.1).

no	$E_{sd//}$	%	$E_{sd\perp}$	%	A_{sd}	remark
1	0.28**	14	0.08**	48	3.7**	causalgia minor; c.b.
2	1.5	78	0.37*	231	4.2**	causalgia minor
3	0.71	70	0.15	136	4.7**	dependency oedema; c.b.
4	0.55	42	0.19	111	2.9**	C.V.I. + lipoedema
5	0.08**	6	0.03*	36	2.5**	C.V.I. + lipoedema c.b.
6	0.14*	11	0.02	23	7.0(*)	C.V.I. + hypodermitis; c.b.
7	0.36**	20	0.08	54	4.8**	C.V.I.; c.b.
8	1.4	73	0.25	132	5.7*	acute phase atopic dermatitis
9	1.8	92	0.38*	200	4.7**	acute phase atopic dermatitis
10	1.0*	53	0.11	58	9.3	acute phase atopic dermatitis
11	1.1	85	0.20*	218	5.6*	congenital lymphoedema
12	0.87	55	0.77**	770	1.1**	elephantiasis; C.V.I. + lipoedema
13	1.1	69	0.76**	760	1.4**	hypodermitis + sec.lymphoedema
14	1.5	112	0.22	244	6.7(*)	C.V.I. + sec.lymphoedema
15	2.1	132	0.32*	317	6.6(*)	C.V.I. + hypodermitis
16	1.9	121	0.84**	840	2.3**	cardial + nephrogenic+lymphoedema
17	4.5**	249	1.7**	1199	2.7**	huge longstanding sec.lymphoedema

Table 6.1 Skin elasticity in 17 subjects with various kinds of oedemas.
% : relative results to normal
E-values: $x10^6$ $N.m^{-2}$
c.b. : compressive bandage used on the leg
C.V.I. : chronic venous insufficiency syndrome
* : significance (see section 2.5)

Therapy with compressive bandages causes a decrease of the initial coefficients of elasticity and an increase of the anisotropy parameter. An example of such a treatment is presented in table 6.2.

date	$E_{sd//}$	%	$E_{sd\perp}$	%	A_{sd}	remarks
24-08-81	0.69	69	0.12	109	5.8*	no therapy
28-08-81	0.42	42	0.06	55	7.4	4 days c.b.
05-10-81	0.51	51	0.06	55	9.0	e.s. (± 50 mm Hg)
25-01-82	0.28	28	0.03	27	9.4	e.s.

Table 6.2 Skin elasticity in a man (68 yr), with severe oedema of the leg due to CVI, during decongestive therapy.
% : relative results
E-values: $x10^6$ $N.m^{-2}$
c.b. : compressive bandages
e.s. : elastic stocking

Also elasticity measurements were performed on the skin of the calf of normal volunteers with a locally induced oedema (table 6.3). The oedema was induced by an intracutaneous injection of 0.01 ml histamine (0.01 mg/ml) in the skin of the calf. In the case of locally induced oedema, the anisotropy values did not decrease in comparison with the situation before injection. The initial coefficients of elasticity increased after the urticarial swelling developed.

$E_{sd//}$	%	$E_{sd\perp}$	%	A_{sd}	remarks
1.4	74	0.16	84	8.6	before injection
1.8	95	0.21	111	8.5	25 minutes after injection
2.0	105	0.19	100	10.5	90 minutes after injection

Table 6.3 Skin elasticity in a man (26 yr), with locally induced oedema of the skin of the calf, induced by an intracutaneous injection of 0.01 ml histamine (0.01 mg/ml).
% : relative results
E-values: $x10^6$ $N.m^{-2}$

6.2.3 Discussion on oedemas of different origin

The oldest reports from the literature on skin elasticity measurements in patients with oedema are from Schade (1912) and Schwartz (1916). They saw a residual deformation after

removal of an indenting load and considered this as an elasticity loss. These authors found that the mechanical properties of the skin became normal again after the disappearance of the oedema. Other investigators, using different methods, found corresponding changes of skin elasticity in oedema (Bönninger, 1905; Sodeman and Burch, 1938; Dick, 1951). The maximal extensibility of the skin seems to depend on its water content (Rollhäuser, 1950). Increased water content of the skin should increase the maximal extensibility (low tensile strength). Most of this forementioned work was done in vitro.

In the present investigation a great divergence of initial coefficients of elasticity was found between the various patients with oedema of the leg (table 6.1). No relation between the degree of abnormality of the E-values and the type of oedema could be detected. Dehydration of the oedematous legs by compressive bandages caused a decrease of the initial coefficients of elasticity (table 6.2). This has been demonstrated in all types of oedemas. The degree of deviation of the initial coefficients of elasticity in oedema seems to be related to the duration of the oedema and not to the type of oedema.

In non-inflammatory shortlasting oedema, the dermal fibres become tightened and orientated by the oedema, causing a stiffening of the skin, which results in higher initial coefficients of elasticity (table 6.3). When the whole leg is oedematous, especially $E_{sd\perp}$ increases (see also 5.4). In such shortlasting non-inflammatory oedemas the elastin fibre meshwork appears not to be affected (Braun-Falco, 1964).

In longlasting oedemas, inflammatory or non-inflammatory, the elastin fibres in the stratum reticulare of the dermis become fragmented (Dick, 1951; Braun-Falco, 1964). The damage to the elastin fibre meshwork is reflected in the lower initial coefficients of elasticity determined on the skin of dehydrated legs (table 6.2).

The collagen part of the skin behaves different in oedema. In shortlasting oedemas, the dermal collagen fibre mesh-

work appears swollen and the lymphvessels are enlarged histologically. In later stages the dermal collagen content grows and only a tissue poor in cells and rich in collagen is left (Gans and Steigleder, 1957). It must be expected that this will change the mechanical properties of the skin. Although the initial coefficients of elasticity for small deformations, do not reflect collagen fibre properties especially, it is likely that in cases of severe sclerosis of the dermis these parameters will be affected by the increase of dermal collagen (e.g. case 17 in table 6.1).
In all sorts of oedema the anisotropy parameter decreases (table 6.1). The anisotropy decrease does not depend on the type of oedema, but does depend on the amount of oedema. Reducing the oedema by means of compressive bandages causes an increase of the anisotropy value (table 6.2). The anisotropy value is inversely proportional to the degree of CVI, which means the more CVI the more oedema, the lower the anisotropy (see 5.3 and 5.4). The anisotropy decrease occurs only when the whole lower leg is involved, causing a larger increase in $E_{sd\perp}$ as compared to $E_{sd//}$. In locally induced oedema such an anisotropy decrease does not take place (table 6.3).
The alinearity parameters (not indicated in the tables) were normal in most of the patients with oedema. In some cases of longstanding lymphoedema, which causes a vast increase in dermal collagen, higher $k_{sd\perp}$ values were found. For instance the $k_{sd\perp}$ value for case 17 in table 6.1, amounted 155% of the normal value.
No change in time-constants was observed in the patients with oedema. The interpretation of this finding is not clear yet. Skin elasticity measurements can be used to detect the development of generalized oedema in the skin of for instance atopic patients. For example on the skin of the calf of a patient with cheiropompholynx (dishidrotic eczema) of the hands, a significantly lower anisotropy was found, probably as an expression of a disseminated oedema. Unfortunately anisotropy parameter values can only provide quantitative information about oedema rather roughly.

Probably more detailed information can be obtained from the further analysis of the parameters of the purely viscous process, which are not discussed in this thesis.

6.3 Skin elasticity measurements in patients with lymphoedema

6.3.1 Introduction

Skin elasticity might have pathogenetic significance for the propulsion of interstitial fluid into the initial lymphatics (Ryan, 1978; Olszewski, 1981). In lymphoedema the propulsing mechanism seems to be impaired. For this reason skin elasticity measurements were performed in patients with lymphoedema.
In longstanding lymphoedemas the collagen content of the dermis is increased, in contrast to shortlasting cases. Because this has an influence on the mechanical properties of the skin, a division of the lymphoedema group in two subgroups was made. One group consisted of women with shortlasting lymphoedema praecox (section 6.3.2.1) and the other group consisted of patients with longlasting lymphoedema (section 6.3.2.2). In the latter group patients with longlasting congenital lymphoedema and secondary lymphoedema were classed.

6.3.2 Material and methods

6.3.2.1 Measurements in patients with shortlasting lymphoedema praecox

Lymphoedema praecox affects predominantly women. The condition starts spontaneously with a puffiness about foot or ankle, in the second or third decade of life. The swelling progresses up the leg in months or years. The oedema is more pronounced during menses and in warm weather. Mostly one leg is affected but the other leg is also prone to become lymphoedematous. After years the oedema is no longer

'pitting' due to the collagen increase (Juergens et.al., 1980).

Skin elasticity measurements were performed on 10 women (mean age 29.3 yr) with lymphoedema praecox. The duration of the oedema varied from several months to four years. Only patients with a soft, pliable oedema (pitting oedema) were measured. None of the patients wore elastic stockings at the time of the measurements. Table 6.4 shows the results of the skin elasticity measurements of the legs affected by lymphoedema praecox, in comparison with the normal group.

Parameter	mean	SD	n	percentage of normal	p-value
$E_{sd//}$	0.67	0.31	10	37	<0.01
$E_{sd\perp}$	0.11	0.05	10		-
A_{sd}	7.7	4.2	10	45	<0.01
$k_{sd\perp}$	16	4.0	10		-
$E_{pu//}$	1.2	0.77	5	27	<0.01
$E_{pd//}$	2.2	1.2	6	26	<0.01
A_{pd}	8.1	4.5	3		-
$k_{pd\perp}$	386	147	9		-
$\tau_{u\perp}$	3.6	0.32	4		-
$\tau_{d\perp}$	6.2	0.40	4		-

Table 6.4 Skin elasticity in 10 women with shortlasting lymphoedema praecox.
E-values: $x10^6$ $N.m^{-2}$, τ-values: seconds

Both legs of each patient were measured. The results of the apparently non-involved legs of the patients (not presented in table 6.4) also showed lower initial coefficients of elasticity. Both $E_{sd//}$ and $E_{sd\perp}$ parameters of the skin of the non-involved leg were lowered to about 55% of the normal value. The $E_{pd//}$ value of the non-involved leg was lowered to 35% of the normal value. The anisotropy para-

meter was normal in the non-involved leg.
No deviations of the alinearity parameters could be found in the affected leg, nor in the non-affected leg.
None of the plethysmographically determined parameters was abnormal. No correlation could be found between elasticity parameter values and venous capacity.

6.3.2.2 Measurements in patients with longlasting chronic lymphoedema.

Skin elasticity measurements were performed in 15 patients (5 men and 10 women), with chronic non-pitting lymphoedema, lasting for more than ten years. The group was divided in a male subgroup with a mean age of 29.2 years and a female subgroup with a mean age of 55.8 years.
Five patients of the group (3 men and 2 women) had a congenital lymphoedema, which became manifest shortly after birth. Congenital lymphoedema is due to an aplasia or hypoplasia of the lymphvessels. The other 10 patients (2 men and 8 women) had a secondary lymphoedema, due to different causes: lymphmetastasis, secondary to CVI, secondary to inguinal lymphnode dissection etc.
Three males with congenital lymphoedema and one female with secondary lymphoedema were wearing elastic stockings before the skin elasticity measurements were performed.
The parameter values of the 10 women of the chronic lymphoedema group, were compared with the women of the normal group. Due to the low number of men in the chronic lymphoedema group, no statistical evaluation was possible for the male subgroup.
Although no p-values are presented for the male subgroup in table 6.5, yet some relative results are given to indicate the increase of those parameters, which appear to be abnormal also in the female subgroup.

	MALES (n=5)			FEMALES (n=10)			
Parameter	mean	SD	percent. of norm.	mean	SD	percent. of norm.	p-value
$E_{sd//}$	2.3	0.39	128	1.8	0.80	138	0.06
$E_{sd\perp}$	0.45	0.69	321	0.35	0.26	350	<0.01
A_{sd}	19	8.8	146	8.0	6.2	47	<0.01
$k_{sd\perp}$	25	6.8	147	17	6.4		-
$E_{pu//}$	11	-		4.3	2.4		-
$E_{pd//}$	3.0	-		5.8	3.6		-
A_{pd}	5.7	-		11	12		-
$k_{pd\perp}$	484	155		480	356		-
$\tau_{u\perp}$	3.6	0.46		3.6	0.35		-
$\tau_{d\perp}$	6.1	0.42		6.2	0.36		-

Table 6.5 Skin elasticity in 15 patients with longstanding chronic lymphoedema.
E-values: $x10^6$ $N.m^{-2}$, τ-values: seconds

The initial coefficients of elasticity $E_{sd//}$ and $E_{sd\perp}$ are increased in both subgroups. The discrepancy in A_{sd} values between males and females is probably due to the fact that three males were wearing elastic stockings thereby reducing the amount of oedema, which resulted in normal A_{sd} values; in the female subgroup only one woman wore elastic stockings.
The parameters of the delayed elastic process did not show abnormal values. The time constants were normal.
The venous capacity of the affected leg of the patients with longstanding chronic lymphoedema was normal. No correlation could be found between the venous capacity and the skin elasticity data.

6.3.3 Discussion on lymphoedema

There are two obvious differences between the results of

both lymphoedema groups. In lymphoedema praecox the elasticity of the skin is reduced and in the longstanding lymphoedema group the skin is stiffer than normal. Furthermore the apparently non-involved legs in lymphoedema praecox are affected too, whereas the non-affected legs in the chronic lymphoedema group are normal.
The lower initial coefficients of elasticity of both legs of the patients with lymphoedema praecox could be the result of the dermal oedema causing elasting waste (see 5.4). It is known that in many patients with lymphoedema praecox, both legs are prone to become lymphoedematous. The lower initial coefficients of elasticity of the apparently non-involved legs might be the result of a nascent lymphoedema.
Another explanation of the skin elasticity loss in both legs of the patients with lymphoedema praecox could be that the lower initial coefficients of elasticity are an expression of a congenital elastin deficit in the skin of these patients. Mortimer et.al. (1983) suppose that elastin fibres play an important role in the normal functioning of the lymphatics. Skin elasticity failure might lead to an impaired lymphpumping, because skin elastic tissue seems to play a role in transmitting arterial pulse waves on the nearby lymphatics, which results in a propulsion of the interstitial fluid (Ryan, 1978). A skin elasticity deficit could along with hormonal factors and lymph vessel abnormalities contribute to the development of lymphoedema praecox.
The increase of the initial coefficients of elasticity of the affected legs of patients with longstanding chronic lymphoedema can be explained as follows. The accumulation of plasma proteins in chronic lymphoedema, stimulates the invasion of cells from the mononuclear phagocytic system for phagocytosis and breakdown of the extra-vascular proteins. The altered proteins can probably maintain a state of chronic inflammation. In some unknown way this results in progressive fibrotic changes and loss of elastin tissue in the dermis (Piller and Clodius, 1980). The initial coefficients of elasticity in chronic lymphoedema probably

increase because of this progressive fibrosis of the skin (table 6.5). The higher $k_{sd\perp}$ value of the male subgroup of the lymphoedema group, is probably also the result of the dermal collagen increase.
The decrease of the anisotropy parameter values found in oedema, appears to be present also in the two investigated types of lymphoedema (table 6.4 and 6.5). The anisotropy parameters give no information on the type of oedema.
The parameters of the delayed elastic process did not add more information on skin elasticity in lymphoedema.

6.4 Skin elasticity measurements in patients with lipoedema

6.4.1 Introduction

Lipoedema does not get much attention in medical literature. In womens magazines the ugly appearance of lipoedema on the thighs and buttocks is usually called 'cellulite'. Allen and Hines (1940) described the condition for the first time in medical literature under the name of 'lipoedema'. In german literature the condition is known as 'Sulzbein oder Fettbein', 'Säulenbein' or 'Rot-Dick-Schenkel'. Finally Piulachs (1956) named the disorder adipocyanosis. Moncorps et.al. (1940) draw the attention to a syndrome, consisting of local fat deposits around ankles, wrists and hips ('Matronenspeck'), erythrocyanosis crurem puellarum and follicular hyperkeratosis, which occurs in young women. These women showed menstrual cycle disturbances and were prone to develop striae distensae. Patients with this constitution were called 'typus rusticanus', because these girls showed the rosy rural appearance of farmer's daughters. Today the 'typus rusticanus' is considered to be a subdivision of lipoedema (Schmitz, 1980).
The condition begins at puberty. The erythrocyanotic component of the disease state fades away after the age of about 25 (Wernsdörfer, 1955). In our experience the Moncorps rustic type is certainly not confined to the younger

age groups. Also middle aged women can still show the combination of lipoedema with erythrocyanosis crurem puellarum and cinnaber spots.
Unlike lymphoedema, lipoedema is always symmetrical and does not affect the feet usually. The typical fat collars are present around ankle and wrist joints. Hereditary transmission is usual.
At the phlebological outdoor department the patients with lipoedema of the rusticanus type are overrepresented in comparison with other lipoedema patients, because the patients of the rusticanus type have the most complaints. These patients complain of a dull spontaneous pain more prominent at the end of the day. These symptoms resemble those of venous stasis, but the swelling and aching does not recede at rest (Stallworth et.al., 1974). Pressure on the lipoedema is painful; the oedema is non-pitting.
By direct venous pressure determination in the metatarsal vein of the foot, Kuiper (1966) found a moderate functional disturbance of the muscle calf pump, in patients of the Moncorps rustic type. Although his patients did not show varicose veins, he found in most patients signs of the so-called 'corona phlebectatica marginalis pedis', being an indication for the existence of a moderate chronic venous insufficiency syndrome. Kuiper (1982) considers the group of patients with lipoedema of the Moncorps rustic type as a subdivision of the idiopathic musculo-fascial pump insufficiency group of Arnoldi (1968). Yet phlebological pathology is denied by several authors (Stallworth et.al., 1974 ; Schmitz, 1980). Phlebograms showed no valvular incompetence and lymphangiograms revealed no lymphvessel abnormalities. Histological examination of the skin in lipoedema showed no abnormality, except for an unusual amount of free fluid fat in the biopsies (Stallworth et. al., 1974).
Taken into account the soft pliable skin, the often found genu valgi, the moderate impaired pumpfunction, the predisposition for striae distensae and the menstrual cycle disturbances, lipoedema of the Moncorps rustic type might

be a real connective tissue disorder under hormonal influence.
During the investigations of skin elasticity in patients with primary varicose veins, several patients with accompanying lipoedema, were found to have lower initial coefficients of elasticity, indicative of a dermal connective tissue change. This prompted us to investigate skin elasticity and venous capacity in patients with lipoedema of the Moncorps rustic type, expecting that connective tissue abnormalities could be detected.
Furthermore skin elasticity in lipoedema was investigated to see if lipoedema, lymphoedema and oedema due to CVI could be distinguished on basis of skin elasticity measurements.

6.4.2 Material and results

Patients were recruited from the phlebological outdoor department of the university hospital. All patients fullfilled the following criteria: fat collars around the ankles (sometimes also around the wrists), 'stove pipe' legs, follicular hyperkeratosis and erythrocyanosis crurem puellarum with typical cinnaber spots. The group consisted only of women (mean age: 43.7 yr).
The lipoedema group has been compared with the normal group (table 6.6) and with the non-postthrombotic chronic venous insufficiency group. The lipoedema group differed from the normal group in E_{sd}, $E_{pu//}$ and $E_{pd//}$ parameters and from the CVI-NT group in the anisotropy parameters (being lower in the CVI-NT group).
The plethysmographically determined parameter for the pump-function (WVp; see also 2.4.3) was decreased in the lipoedema group. Mean WVp value amounted 48% in the lipoedema group (normal WVp value is 65 to 70%; see also table 5.3). However no correlation could be found between the pump function data and the skin elasticity parameter values of the lipoedema group.

A slightly lower mean value (0.069 %/mm Hg: 90% of the normal value) for the venous capacity was found in the lipoedema group. No correlation could be found between venous capacity values and the skin elasticity data of the lipoedema group.

Parameter	mean	SD	n	percentage of normal	p-value
$E_{sd//}$	0.69	0.43	15	53	<0.01
$E_{sd\perp}$	0.07	0.04	15	70	0.07
A_{sd}	12	6.0	15		-
$k_{sd\perp}$	20	5.3	15		-
$E_{pu//}$	1.8	1.2	10	40	<0.01
$E_{pd//}$	3.1	2.8	12	67	0.05
A_{pd}	21	11	5		-
$k_{pd\perp}$	371	66	13		-
$\tau_{u\perp}$	3.6	0.36	8		-
$\tau_{d\perp}$	6.0	0.35	13		-

Table 6.6 Skin elasticity in 15 patients with lipoedema of the Moncorps rustic type.
E-values: $x10^6$ $N.m^{-2}$, τ-values: seconds.

6.4.3 Discussion on lipoedema

The soft pliable skin of lipoedema was described by Allen and Hines (1940) as a typical feature of this pathology. Such skin is not found in erythrocyanosis crurem puellarum without lipoedema. No abnormal skin elasticity could be detected in several patients with erythrocyanosis crurem puellarum without lipoedema (not indicated in the table). Therefore the lower initial coefficients of elasticity obtained, seem to be due to the lipoedema component of the typus rusticanus. The observed skin elasticity changes might be the result of the dermal excess of free fluid fat

in lipoedema. The idea that the skin elasticity loss in lipoedema is the dermal expression of a generalized connective tissue lesion, was not supported by the venous capacity data. No increase in venous capacity could be found, as an expression of a vascular wall connective tissue defect. The observation made by Kuiper (1966) of a moderate impairement of the calf muscle pump in patients with lipoedema of the Moncorps type was confirmed. This pump function impairement might be the result of a musculo-fascial insufficiency as Kuiper (1982) suggests, for example due to a 'weaker' connective tissue of the fascial compartment. Skin elasticity might reflect the state of the connective tissue of the fascial sheet. Yet no correlation could be found between skin elasticity data and pump function data (WVp data). This is probably due to the fact that skin elasticity parameters mainly reflect the elastin part of the skin and a fascial insufficiency is probably of collageneous nature. To investigate whether the lower initial coefficients of elasticity are not simply the result of dermal oedema due to an idiopathic CVI in patients with lipoedema of the Moncorps type, we compared the lipoedema group with the CVI-NT group of chapter 5. It appeared that a statistical difference existed between both groups only with respect to the anisotropy parameters. Taking into account the moderate impairement of the pump function and the corona phlebectatica found in the lipoedema group, the only difference between the lipoedema group and the CVI-NT group might be a larger amount of oedema in the CVI-NT group, causing lower anisotropy values in the CVI-NT group compared with the lipoedema group.

However since in lipoedema both legs show similar deviations of the elasticity parameters and the $E_{sd|}$ value in lipoedema is decreased in contrast to a normal or slightly increased $E_{sd|}$ value in CVI (chapter 5), we suggest that the skin elasticity loss in lipoedema of the rusticanus type is not solely due to elastic tissue degeneration as the result of venous stasis oedema, but is also the result of an intrinsic connective tissue defect in the skin of these patients.

6.5 Closing remarks on oedema

From all the results discussed in this chapter it is very evident that skin elasticity is impaired by oedema. The initial coefficients of elasticity are not suited for the determination of the type of oedema or the quantity of the oedema (table 6.1).
The anisotropy is lowered in all the types of oedema studied. The anisotropy parameter gives quantitative information on the oedema (chapter 5; table 6.2). By means of the A_{sd} parameter the development of oedema or the decline of oedema of the leg in a patient can be followed. Unfortunately no distinction between lymphoedema, lipoedema and oedema due to CVI can be made with the anisotropy parameter. Of the other parameters only the alinearity coefficient gives an indication of the type of oedema. This parameter seems to be related to the collagen fibre meshwork (chapter 9). In longstanding lymphoedema in which the dermal collagen content increases, both $k_{sd\perp}$ and $k_{pd\perp}$ tend to increase (table 6.5); in stasis oedema due to CVI $K_{sd\perp}$ is decreased (tables 5.1 and 5.2).
The reported skin elasticity measurements in oedema are performed with small loads. For large loads the maximal extensibility of the skin (tensile strength) varies with the water content of the collagen fibres. The tensile strength appears to be maximal by a water content of about 10 to 20% (Harkness, 1961). It is possible that skin elasticity measurements with large loads will provide a tool to distinguish more exactly lipoedema from lymphoedema and stasis oedema, on the basis of their mechanical properties.

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Chapter 7

Skin elasticity measurements in patients with pseudoxanthoma elasticum.

7.1 Introduction

During the investigations of the skin elasticity in patients with primary varicose veins and chronic venous insufficiency, the need was felt for a better understanding of the finding of the lowered initial coefficients of elasticity in these patients. Wijn (1980) interpreted the initial coefficients of elasticity mainly in terms of elastin fibre properties. To test this interpretation, we performed skin elasticity measurements in a genodermatosis mainly affecting the elastin fibres viz. pseudoxanthoma elasticum (PXE: chapter 7) and in a genodermatosis mainly affecting the collagen fibre system, viz. Ehlers-Danlos syndrome (E-D syndrome: chapter 8).
Since both genodermatoses show a generalized connective tissue defect, also affecting the vascular system, we measured in these patients the venous distensibility. In this way we hoped to get more information about the influence of defective connective tissue of the vascular wall on the venous capacity. Finally the correlation between the elasticity data and the venous capacity was determined, to investigate if the degree of the presumed skin elasticity loss in these genodermatoses, can be used as a parameter for the severity of the vascular lesions.
This chapter deals with skin elasticity measurements in pseudoxanthoma elasticum. PXE is a rare disease. The prevalence of the disease in England has been estimated between: 1:160,000 and 1:1000,000 (Danielsen, 1979). PXE is a severe disease, because of the vascular, especially arterial complications. Since elastin is abnormal in PXE, the arteries with their high elastin content are involved. Other tissues more or less rich in elastin like the skin and Bruch's membrane of the eye, are also affected. Ruptures of Bruch's

membrane, resulting in vision problems, are an early phenomenon in PXE (Stolte et.al., 1977). These ruptures appear on fundoscopy as 'angioid streaks' of the retina. The disturbed elastin fibre system of the skin is clinically evident in the form of papules in the neck and in the folds. Calcium deposits on the elastin fibres give the papules the typical yellow appearance of the so-called 'pseudoxanthomas'. Not only the pseudoxanthomas contain abnormal elastin fibres, but also clinically apparently normal skin of the PXE patients (Ross et.al., 1978; Pasquali et.al., 1981). Collagen seems to be more or less normal in PXE (Ross et.al., 1978). The amount of acid mucopolysaccharides is increased (Pinkus and Mehregan, 1981); this increase is related to parts of immature reticulin fibres (Danielsen, 1979).
Four genetic types of PXE are distinguished today (Pope, 1975). The dominant type I group shows the classical flexural cutaneous changes. Vascular complications in this group are common; the chief ophtalmic complication is a severe choroidoretinitis. The dominant type II group has an atypical macular rash, almost no vascular complications and mild retinal abnormalities. The recessive type I group resembles the dominant type I group, although vascular and retinal degeneration are milder; haematemeses are common. Recessive type II is very rare, this type shows a generalized cutaneous laxity, without systemic complications.
It can be expected that the four genetic types are related to different molecular defects in elastin synthesis, in conformity with the situation in Ehlers-Danlos syndrome, in which the types appear to be related to specific molecular defects (table 8.1). Therefore the results of the skin elasticity measurements will also be presented for the individual types of PXE.

7.2 Material and results

In thirteen PXE patients (4 males and 9 females) skin elasticity measurements and plethysmographic experiments were

performed. The mean age of the male patients was 28.0 years and of the female patients 34.4 years. In all patients the diagnosis was confirmed histologically by a biopsy of the involved skin.
The skin elasticity measurements were performed on the apparently normal skin of the calf of both legs in each patient. The most accurate results of the experiments of one of the legs were used. The results are presented in table 7.1.

Parameter	mean	SD	n	percentage of normal	p-value
$E_{sd//}$	0.94	0.65	13	52	<0.01
$E_{sd\perp}$	0.08	0.05	13	62	0.02
A_{sd}	13	7.6	13		-
$k_{sd\perp}$	16	5.8	13		-
$E_{pu//}$	2.5	1.8	9		-
$E_{pd//}$	3.2	2.4	12	44	<0.01
A_{pd}	7.5	7.3	7	45	0.01
$k_{pd\perp}$	343	156	10		-
$\tau_{u\perp}$	3.7	0.87	7		-
$\tau_{d\perp}$	5.9	0.36	9		-

Table 7.1 Skin elasticity in 13 patients with PXE. E-values: $x10^6$ $N.m^{-2}$, τ-values: seconds.

Table 7.1 shows that the initial coefficients of elasticity, both in the direction of the tibial axis and in the perpendicular direction are lowered. The alinearity parameters and the time constants are normal.
The results of the same patients of table 7.1, are given classified according to the genetic type in table 7.2.
In our material no patients with the recessive type II of PXE were present.

Parameter	dom.I	dom.II	rec.I
$E_{sd//}$	63	22**	63
$E_{sd\perp}$	48*	35**	78
A_{sd}	95	50(*)	109
$k_{sd\perp}$	102	90	94
$E_{pu//}$	106	25*	61
$E_{pd//}$	111	45*	37**
A_{pd}	-	-	45**
$k_{pd\perp}$	94	104	78
$\tau_{u\perp}$	103	104	105
$\tau_{d\perp}$	105	96	99
	(n=1)	(n=4)	(n=8)

Table 7.2 Relative results in percentages of the normal values, of 13 patients with PXE, classified according to the genetic type.
E-values: $x10^6$ N.m^{-2}, τ-values: seconds,
dom.: dominant, rec.: recessive,
* : significance (see 2.5).

The dominant type II group shows significantly lower initial coefficients of elasticity. The recessive type I group only shows lower initial coefficients of elasticity in the delayed elastic process. The alinearity parameters and the time constants did not show abnormal values for the various genetic subgroups.
Plethysmographic experiments revealed a significantly greater venous capacity in PXE patients (mean C_o-value: 0.11 %/mm Hg, which is 143% of the normal value). A difference existed between the C_o-values of the dominant PXE group (mean C_o-value: 0.13) and the recessive PXE group (mean C_o-value: 0.10). No correlation between venous capacity and the elasticity data was found in the PXE patients.

The present investigation has shown a skin elasticity loss of the apparently non-affected skin of the calf of patients with PXE. The initial coefficients of elasticity were lowered both in the direction of the tibial axis and in the perpendicular direction, especially in the dominant type II of PXE. This seems to be in accordance with the skin elasticity measurements in PXE patients by Harvey et.al. (1975). Using a suction cup device, these authors found an increase of the cutaneous extensibility of the forearm in vivo, in patients with the dominant types of PXE. Less understandable is that these investigators found a decrease of the skin extensibility in the recessive type I group.
Since the elastin fibre system is abnormal in PXE (Ross et. al., 1978; Danielsen, 1979), it is plausible to ascribe the skin elasticity loss to this abnormality. The lowered initial coefficients of elasticity might point to a decreased number of functionally involved elastin fibres in PXE. Since according to Pasquali-Ronchetti et.al. (1981) the elastin content of the skin is increased in PXE, which is in agreement with the reported increase of the number of elastin fibres in PXE (Grüneberg and Theune, 1969; Pasquali-Ronchetti, 1981), the lower initial coefficients might also be due to a changed quality of the elastin fibres in PXE. This view is supported by electron microscopical findings. Electron microscopically,the elastin part of the elastin fibre, which shows the rubber-like properties, appears to be abnormal (Ross et.al., 1978). The microfibrillar component of the elastin fibre is unchanged in PXE.
The alinearity parameter, $k_{sd|}$, which is said to reflect elastin fibre quality (Wijn, 1980), is not changed in PXE. Also the PXE patients described by Wijn (1980), did not show abnormal $k_{sd|}$ values. Therefore it is rather unlikely that $k_{sd|}$ reflects the quality of the elastin fibres, taken into account the forementioned electron microscopic findings in PXE.
In PXE an increase of glycosaminoglycans is found histo-

logically (Pinkus and Mehregan, 1981). The effect of an increased amount of glycosaminoglycans on the mechanical properties of the skin, is not reflected in the time constants in PXE.

An interesting finding is the greater venous capacity of the calf in patients with PXE. Apparently not only the arterial but also the venous system is affected in PXE. The degree of skin elasticity loss does not reflect the severity of the vascular lesions, since no correlation could be found between the skin elasticity data and the venous capacity. This is not so surprising. Already Touraine (1940) noticed PXE patients with isolated angioid streaks and vascular lesions without skin lesions. Besides he found PXE patients with pseudoxanthomas of the skin without vascular lesions.

Venous capacity seems to be a rather good measure for the severity of the vascular lesions in PXE. Especially patients with a history of gut bleeding or arterial aneurysms showed increased C_o values.

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Chapter 8

Skin elasticity measurements in patients with Ehlers-Danlos syndrome.

8.1 Introduction

The first description of the so-called Ehlers-Danlos syndrome (E-D syndrome), was made by the Dutch surgeon Job van Meeckeren (1668). He described a Spaniard with an exceptional extensible skin. The number of publications dedicated to the syndrome is not proportional to the frequency of the disease; E-D syndrome has an estimated incidence of approximately 1:150,000 (Beighton, 1968).
The Ehlers-Danlos syndrome is mostly a generalized connective tissue disorder affecting many parts of the body. Sporadically localized forms are described. In E-D syndrome the skin is hyperextensible and more vulnerable, it bleeds quickly. Poor wound healing leads to abnormal 'fishmouth' scars, especially over the joints and to so-called 'molluscoid pseudotumors' (Jansen, 1954). The skin is thin and velvety-like. Another essential symptom is the joint hypermobility, due to the lax ligaments and tendons, leading to luxations and secondary deformity. Internal involvement mainly concerns the cardio-vascular system. Vascular symptoms include varicosis, arterial aneurysms and a fragility of the skin vessels leading to ecchymoses. Ocular symptoms can include ptosis, ectropion, keratoconus and chorioretinal bleeding. Sometimes also neurological symptoms are present such as hyperreflexie (Leiber and Olbrich , 1981).
E-D syndrome is the result of a defective collagen fibre system. Elastin content of the skin and elastin fibre structure are reported to be more or less normal in E-D syndrome (Jansen, 1954; Sevenich et.al., 1980). The skin, joint and vascular symptoms are the result of the abnormal collagen fibres.
Today nine variants of the disease are distinguished on the basis of clinical, genetical and biochemical data (Arneson

et.al., 1980; table 8.1). E-D types I, II, III and VIII are inherited in an autosomal dominant way; type V is sex-linked; the types VI, VII and IX are inherited as an autosomal recessive trait and type IV shows autosomal dominant and recessive forms.

E-D type	molecular defect	symptoms skin 1)	symptoms joints 2)	complications
I gravis	unknown	+++	+++	prematurity
II mitis	unknown	+	+	
III hypermobile	unknown	+	+++	
IV ecchymotic	absence of type III collagen	-	only digital	transparant skin; gut rupture; large vessel rupture
V sex-linked	lysyloxidase deficiency	+++	+	slight skin fragility
VI ocular	prolyl-hydroxylase-deficiency	++	+++	chorio-retinal bleeding, scoliosis
VII arthrochalasis multiplex congenita	procollagen-peptidase deficiency	++	+++	joint dislocations, short stature
VIII periodontal	unknown	-	+	pretibial scarring
IX fibronectin	dysfunctional fibronectin	++	+++	striae, platelet aggregation defect

Table 8.1 Variants of Ehlers-Danlos syndrome.

(Compiled from data of McKusick, 1972; Krieg et. al., 1981; Leiber and Olbrich, 1981; Nelson and King, 1981).

1): skin hyperextensibility

2): joint hypermobility

8.2 Material

The E-D syndrome group consisted of three males and six females. Mean age of the group was 28.1 years.
For classification of the type of E-D syndrome, clinical and genetical data were used in first instance. Unfortunately many of the E-D patients do not fit the discribed types closely (Arneson et.al., 1980). The best way to determine the type of E-D syndrome is to demonstrate the molecular defect. However the molecular defect is only known for the recessive types of E-D syndrome. In two cases (case 1 and case 9, table 8.3) also biochemical analysis of a skin biopsy was performed. The E-D syndrome group included four patients with E-D syndrome type I, two patients with type II, one patient with type IV and two patients with type V (table 8.3).
On the skin of the calf of both legs in each patients, skin elasticity measurements were performed. Only the results of one leg were used. At the same day also the venous capacity of the legs of the E-D patients was determined plethysmographically.

8.3 Results

Table 8.2 shows the results of the skin elasticity measurements in eight E-D patients. One young E-D patient (case 7 in table 8.3) is not included in table 8.2, because of the lack of a corresponding young age subgroup in the normal group, which is needed for an appropriate statistical analysis. Skin elasticity parameters of the E-D group were compared with the parameter values of the normal group (table 8.2, column 6), as well as with the parameter values of the pseudoxanthoma elasticum group (table 8.2, column 7). Initial coefficients of elasticity were found to be lowered enormously in the E-D group. Also the alinearity parameter $k_{sd\underline{|}}$ and the anisotropy parameters were found to be lowered. Evenmore if compared with the parameter values of the PXE

group, the E-D group shows lower $E_{sd//}$ and $k_{sd\perp}$ values.

Parameter	mean	SD	n	perc.[1]	compared to normal group p-value	compared to PXE-group p-value [2]
$E_{sd//}$	0.29	0.24	8	16	<0.01	<0.01
$E_{sd\perp}$	0.04	0.02	8	30	<0.01	-
A_{sd}	7.8	7.0	8	52	<0.01	0.04
$k_{sd\perp}$	10	4.5	8	56	<0.01	0.02
$E_{pu//}$	1.7	1.6	5	41	0.03	-
$E_{pd//}$	1.0	0.86	6	15	0.01	0.02
A_{pd}	2.7	1.8	3	17	<0.01	0.09
$k_{pd\perp}$	274	144	4		-	-
$\tau_{u\perp}$	3.9	-	1		-	-
$\tau_{d\perp}$	5.9	0.30	5		-	-

Table 8.2 Skin elasticity in 8 patients with E-D syndrome. E-values: $x10^6$ $N.m^{-2}$, τ-values: seconds

1): percentage of normal

2): comparison between E-D group and PXE group (Mann-Whitney U test); all E, A and k-values are lower in the E-D group (see also table 7.1).

Different types of E-D syndrome might show different mechanical properties of the skin, therefore also the relative results in percentages of the normal values are presented for the individual E-D patients in table 8.3.
In all the mentioned types of E-D syndrome the initial coefficients of elasticity are lowered (table 8.3). The alinearity parameter $k_{sd\perp}$, is especially lowered in the dominant types (types I and II) of E-D syndrome.
Lower anisotropy parameters were found. This was not due to oedema. Not the slightest sign of oedema was observed

on the legs of the investigated E-D patients. Plethysmographic experiments showed a slightly lower venous capacity for the E-D patients. No correlation could be demonstrated between skin elasticity data and the venous capacity.

Type E-D	V	I	I	I	IV	II	I	II	V
Parameter									
$E_{sd//}$	23**	3**	6**	10**	39**	14**	4**	18**	19**
$E_{sd\perp}$	49*	23**	24**	33**	48*	25**	29**	18*	34*
A_{sd}	47*	10**	18**	22**	80	59*	10**	98	58*
$k_{sd\perp}$	93	34*	35*	34*	84	52*	38*	62*	81
$E_{pu//}$	78	-	10*	-	88	18*	-	-	10**
$E_{pd//}$	16*	-	6**	6**	30*	-	7**	13	18*
A_{pd}	12*	-	-	-	27*	-	-	11*	-
$k_{pd\perp}$	79	86	111	50*	58*	99	99	71	88
$\tau_{u\perp}$	75*	101	118	-	96	77*	92	116	84
$\tau_{d\perp}$	95	100	107*	-	99	95	96	102	102
Age (yr)	32	15	26	20	16	37	9	64	34
sex	m	f	f	f	f	m	f	f	m

Table 8.3 Results of the skin elasticity measurements of the individual E-D patients in percentages of the normal values.
*: significance (see 2.5).

8.4 Discussion

A considerable decrease of initial coefficients of elasticity was found in E-D syndrome (table 8.2). The initial coefficients of elasticity are partially influenced by the reduction of skin thickness in E-D syndrome. The skin thickness in E-D syndrome amounts about 75% of the normal skin

thickness (Grahame and Beighton, 1969). However this is not enough to explain the lower initial coefficients of elasticity found in E-D syndrome. The lower E-values seem to be the result of the abnormal dermal collagen fibre network in E-D syndrome.

The deviations found in the stress-strain curve are comparable with the deviations of skin elasticity found in E-D syndrome by Grahame and Beighton (1969), using a suction cup device.

Both in E-D syndrome and PXE lower initial coefficients of elasticity were found. Therefore it is likely that both collagen and elastin influence the stress-strain curve. This is not so surprising since the collagen and the elastin fibre meshworks in the dermis are highly intermingled. The fact that these fibre meshworks are closely related is also expressed in the connective tissue genodermatoses. In Ehlers-Danlos syndrome for instance, also minor deviations in the elastin fibres are found (Holbrook and Byers, 1982). Therefore the lower initial coefficients of elasticity found in E-D syndrome might also be the result of abnormal elastin fibres. However by comparing initial coefficients of elasticity in E-D syndrome with the values for these parameters in PXE, significantly lower $E_{sd//}$ values were found in E-D syndrome (table 8.2). It seems unlikely that the abnormal elasticity of the skin found in E-D syndrome is solely due to elastin abnormalities.

A considerable decrease of the alinearity parameter $k_{sd\perp}$ was found in the dominant types I and II of E-D syndrome. None of the other patient groups, which were investigated in this thesis, showed such a decrease of the alinearity parameter. Since the collagen part of the skin is mainly affected in E-D syndrome, the $k_{sd\perp}$ decrease seems to be related to an abnormal collagen fibre system. With the small loads we used, no properties of the stretched collagen fibres themselves are measured, but only a straightening out of the collagen fibres and an orientation of the fibre bundles in the direction of stress (see 1.1.5). This is probably the reason why no abnormal $k_{sd\perp}$ values were found

in the patients with E-D syndrome type V. In E-D syndrome type V lysyloxidase is absent, an enzyme which is needed for adequate cross-linking of the tropocollagen molecules (chapter 1). The cross-linking defect is probably reflected in abnormal mechanical properties of individual collagen fibres. In the patient with E-D syndrome type IV also a normal $k_{sd\perp}$ value was found. In this type of E-D syndrome collagen type III is absent. The collagen fibre system of the lower two-thirds of the dermis, which consists mainly of type I collagen and which is mainly responsible for the mechanical properties of the skin (chapter 1), remains largely intact. This might explain the normal $k_{sd\perp}$ value found in this patient.

The molecular defect of the dominant types of E-D syndrome is unknown. Electron microscopical investigations showed a defective lateral aggregation of collagen fibrils in these patients (Vogel et.al., 1979; Sevenich et.al., 1980). The defect does not seem to be at the cellular level, but consists in a defective organisation of fibrils into fibres, respectively into collagen fibre bundles (Jansen, 1955; Julkunen et.al., 1970; Black et.al., 1980). Taking into account the abnormalities of the collagen fibre wickerwork, observed by electron microscopy by the forementioned authors, the observed changes in the alinearity parameter, $k_{sd\perp}$, in the dominant types of E-D syndrome, might be related to this abnormal composition of the collagen fibre meshwork. Therefore we suggest that the alinearity parameter $k_{sd\perp}$ is related to the degree of interaction between the collagen fibres of the network. This interaction presumably depends on collagen interfibre fibrils or on the proteoglycan coat of the collagen fibres. These structures might be responsible for the different slip of the fibres over one another during stress in the dominant types of E-D syndrome.

A considerable anisotropy was found in the E-D syndrome. This has not previously been reported in the literature. It was not the result of oedema in the legs. The anisotropy change may also reflect the changes in the dermal fibre

meshwork in E-D syndrome.

An increase in venous distensibility was expected in E-D syndrome, because of an abnormal connective tissue in the vein wall. However a slight decrease of the venous capacity was found. This is probably due to the slightly increased venous resting pressure in the E-D patients (mean p_{vo} value amounted 9.3 mm Hg), caused by overextension of the knee joints of these patients, when they were laying on the measuring couch. Because of the non-linearity of the P_v-V relation the volume distensibility of the leg decreases with increasing intravenous pressure.

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Chapter 9

Concluding remarks

9.1 Introduction

In this thesis the stress-strain curve is discussed only for small deformations. Wijn (1980) interpreted the elasticity parameters, derived from the first part of the stress-strain curve mainly in terms of elastin fibre properties. Other authors suggest that this part of the stress-strain curve is mainly related to the straightening out process of the collagen fibres during strain (Brown, 1973; Hall, 1976). To improve our understanding of the morphological background of the elasticity parameters, skin elasticity has been investigated in patients with certain connective tissue disorders (chapter 7 and 8). An addition to the interpretation of the elasticity parameters, based on our experiments, will be presented in this chapter.
A relationship between skin elasticity parameters and venous capacity was expected (see introduction of this thesis). The former being determined by the dermal connective tissue, the latter being influenced among other things by the connective tissues of the venous vascular wall. An explanation for the fact that such a relationship could not be demonstrated will be given in section 9.3.
Finally the clinical usefulness of the skin elasticity parameters and the possibilities of the method for further research on the dermal connective tissue is discussed in section 9.4.

9.2 Discussion on the meaning of the skin elasticity parameters

9.2.1 Initial coefficients of elasticity

It is difficult to create experimental conditions for skin elasticity research, in which selectively the collagen fibre

system or the elastin fibre system are affected; usually both systems are involved. In connective diseases of the skin frequently both fibre systems show abnormalities (Holbrook and Byers, 1982). Also drugs do not influence solely collagen or elastin. For instance corticosteroids not only influence collagen synthesis (Booth et.al., 1982) but also elastin synthesis (Burnett et.al., 1982).
Some authors used selective enzymatic digestion of one of the fibre components of the dermis, in vitro, as a method to distinguish between the contribution of the two fibre systems to the stress-strain curve. Daly (1969) showed a change in the initial part of the stress-strain curve, by treating skin specimens with elastase. However he did not degrade elastin exclusively. In his paper Daly himself states: "Elastase is not truly specific for elastin and does degrade some other proteins". According to Viidik (1982) this is due to some collagenase in crude elastase. His statement is based on the experiments of Oxlund and Andreassen (1980), who found that crude elastase can weaken collagen films considerably. For this reason the experiments of Daly do not prove that the stress-strain curve is exclusively determined by elastin fibres. It seems to be justified to repeat the experiments of Daly with pure elastase, in order to clarify the influence of elastase on the stress-strain curve.
The stress-strain curve of the skin is almost certainly influenced by both the collagen and the elastin fibre networks of the dermis, as suggested in chapter 8. The collagen contribution has to be seen as resulting from the deforming process of the collagen fibre meshwork during loading. This supposition is supported by the results of the measurements in normal subjects (chapter 3). In normal persons a relative increase in length of the piece of skin of 2 to 5 percent in the direction of the tibial axis and an increase of 10 to 20 percent in the perpendicular direction is found after loading of the skin of the calf. In view of the wavy pattern of the collagen fibre bundles in unstretched skin, such strains cannot align collagen fibres com-

pletely. Scanning electron microscopical investigations showed only a straightening out of the collagen fibres and an orientation of the fibre bundles in the direction of stress in case of such strains (Brown, 1973).
Another reason for believing that the stress-strain curve is not only related to the elastin fibre meshwork of the dermis, is the fact that the values found for the initial coefficients of elasticity in normal subjects are too high to be based solely on elastin (see 3.1.4).
The initial coefficients $E_{sd//}$ and $E_{sd\perp}$ are determined after loading, when the collagen fibre meshwork regains its original position by means of the tightened elastin fibres. It is likely that the mass of the collagen fibre bundles which must be pulled back by the elastin fibres also determines the purely elastic process after deformation (ε_a in figure 2.9). Therefore we suppose that $E_{sd//}$ and $E_{sd\perp}$ are determined by the amount (and properties) of both the elastin fibres and collagen fibres which are functionally involved in the straining procedure.
The used initial coefficients of elasticity of the delayed elastic process $E_{pu//}$ and $E_{pd//}$, must have a different meaning because of their different age dependency. The values for $E_{pu//}$ in normal individuals are higher than the initial coefficients of elasticity of the purely elastic process in normal subjects. Because of these high values, $E_{pu//}$ is more likely related to collagen than to elastin. The significantly lower values found for $E_{pu//}$ in Ehlers-Danlos syndrome support this view (table 8.2).

9.2.2 Alinearity coefficients

Several indications exist that the alinearity parameter $k_{sd\perp}$ is related to collagen and not to elastin. In Ehlers-Danlos syndrome, a typical collagen disease, lower $k_{sd\perp}$ values are found. However in pseudoxanthoma elasticum, an elastin disease, the $k_{sd\perp}$ parameter is normal. The increase of $k_{sd\perp}$ with ageing also points in the direction of colla-

gen, because the insoluble fraction of collagen increases with ageing, while the elastin content of the skin decreases with ageing (chapter 1).
As suggested in chapter 8, $k_{sd\perp}$ might depend on collagen fibre-fibre interactions, because properties of stretched collagen fibres are not measured with small loads. The nature of this fibre-fibre interaction is unknown at present (Black et.al., 1980). Fibre-fibre interaction could depend on inter fibre-fibrils or on the proteoglycan coat of the fibres, by which the fibres are lubricated so that the collagen fibre bundles can move freely along each other. In the delayed elastic process more and more collagen fibre bundles become orientated in the direction of stress. The alinearity parameter of this process, $k_{pd\perp}$, seems to be related to the relaxation of the collagen meshwork, because especially in diseases in which the amount of collagen increases such as chronic lymphoedema and systemic sclerosis (scleroderma), the $k_{pd\perp}$ parameter shows higher values.

9.2.3 A resumé of the interpretation of the skin elasticity parameters

The skin elasticity measurements in patients with Ehlers-Danlos syndrome and pseudoxanthoma elasticum have changed our view with regard to the interpretation of the parameters. Especially for the interpretation of the purely elastic process additional information became available. Because of the divergence of the parameter values of the delayed elastic process in the patient groups, little can be added to the interpretation of these parameters. Anisotropy parameters and time constants are interpreted in the same way as Wijn(1980) did.
Reviewing our revised interpretation of the various parameters, leads to the following statements with respect to:
the purely elastic process:
$E_{sd//}$ and $E_{sd\perp}$: measures for the amounts of elastin and

collagen fibres actually involved in the uniaxial strain experiment of the skin of the calf, respectively in the direction of the tibial axis and in the perpendicular direction.

A_{sd} : measure for the angular distribution of the fibres involved in the purely elastic process.

$k_{sd\perp}$: measure for the degree of collagen fibre-fibre interaction.

the delayed elastic process:

$E_{pu//}$: measure for the actually involved number of collagen fibres during the orientation of the collagen fibre bundles in the direction of stress.

$E_{pd//}$: measure for the amount of elastin fibres and orientated collagen fibres, involved in the process after deformation of the skin, trying to restore the collagen fibre meshwork in its original position.

A_{pd} : measure for the angular distribution of the fibres involved in the delayed elastic process.

$k_{pd\perp}$: measure for the relaxation of the collagen fibre meshwork after deformation of the skin, depending on fibre-fibre interactions and fibre-fluid interactions of the meshwork.

$\tau_{u\perp}$: time-constant related to the velocity of shifting of the bulk of the collagen fibres through a viscous medium of glycosaminoglycans.

$\tau_{d\perp}$: time constant related to the velocity of shifting of the bulk of the collagen fibres, after removal of the load, in the direction of the original position of the fibre meshwork, depending on the tightened elastin fibres and on the collagen fibre-fluid interactions of the meshwork.

9.3 Discussion on the relation between skin elasticity data and venous capacity

It was hypothesized that the plethysmographically determined volume distensibility of the leg, reflects venous

wall distensibility. Venous wall distensibility depends among other things on the quality and the composition of the venous vascular wall connective tissue.
Since skin elasticity reflects the properties of the dermal connective tissue, some correlation between venous wall distensibility (venous capacity) and the skin elasticity parameters was expected. However no correlation could be found between skin elasticity data and venous capacity, neither in the normal group nor in most patient groups.
The reason why no correlation could be found between the skin elasticity data and the venous capacity in most persons lies probably in the fact that the venous capacity is not only determined by the vascular wall connective tissue, but also by the amount of venous vessels in the limb and by the muscles and fascie sheet surrounding the veins, which are variable factors.
The venous capacity as measured from the initial part of the pressure-volume curve is presumably determined, at least partly, by the elastin part of the venous vessel wall. This is also suggested by Van den Berg and Barbey (1976). The observed increase in venous capacity in patients with pseudoxanthoma elasticum is in accordance with this view (chapter 7).
However no correlation could be found between the venous capacity data of the PXE patients and the skin elasticity data of the same group. The skin elasticity parameters used, do not seem to be a good measure for the quality of the vascular wall connective tissue for the individual patients.

9.4 Perspectives of skin elasticity measurements for clinical and research purposes

Skin elasticity measurements have their limitations for diagnosing skin diseases. These limitations depend largely on the great inter-individual variability of the parameter values. Factors as skin thickness (3.1.3.2), influence of posture (3.1.3.5), season dependency, hormonal influences

and unsuspected factors such as the influence of physical training on skin elasticity (Suominen et.al., 1978), need more attention in the future.
But skin elasticity measurements can provide additional information for the diagnosis, like a microscopical investigation of a skin biopsy does. For instance in some patients with systemic sclerosis (scleroderma) we could demonstrate an increased skin stiffness of the skin of the calf, before a sclerosis of the skin became clinically evident. Since the intra-individual variability of the parameter values is much smaller than the inter-individual variability, the method seems to be appropriate for the follow up and the evaluation of therapy of a disease process such as scleroderma. Classification of certain diseases can be made more accurate by skin elasticity measurements. A clinically, genetically and biochemically heterogenous syndrome like Ehlers-Danlos syndrome, can probably be classed more accurately on the basis of skin elasticity measurements.
In the future quantification of dermal oedema seems to be possible, especially with the parameters of the purely viscous process. This will provide a method for the follow up of patients with for instance atopic dermatitis.
Skin elasticity measurements can also be used for research purposes, for example for drug research. The influence of local or systemic corticosteroids on the dermal connective tissue can be investigated by means of skin elasticity measurements. For such investigations accurate skin thickness measurements will be an additional necessity.
Our elasticity parameters only reflect elastin fibre properties and collagen fibre meshwork properties. When skin elasticity measurements with large loads become available then also the properties of the collagen fibres itselves can be described adequately. With both types of experiments, using small loads and large loads, the mechanical properties of the skin can be characterized more completely.

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Addendum I : List of symbols

σ : stress, force exerted on a piece of skin divided by the cross sectional area of that piece of skin.

ε : strain, relative increase of length of a piece of skin, caused by a stress.

$\frac{d\sigma}{d\varepsilon} = E + k.\sigma$: stress-strain relation

E : initial coefficient of elasticity

k : alinearity parameter

A : anisotropy parameter = $\frac{E_{//}}{E_{\perp}}$

η : viscosity

τ : timeconstant = $\frac{\eta}{E}$

subscript: indicates a parameter:

's' : of the purely (instantaneous) elastic process (see 2.3.3.2)

'p' : of the delayed elastic process

'u' : derived from the response of the skin to a load (see figure 2.9)

'd' : derived from the deformation curve of the skin after loading

'//' : derived from a strain experiment on the skin of the calf in the direction of the tibial axis

'$\perp$' : derived from an experiment in the direction perpendicular to the tibial axis

p_v-V relation: venous pressure-limb volume relation

SVP: standing venous pressure in the foot

WVp: walking venous pressure fall, due to tiptoe movements (see 2.4.3)

C_o : venous capacity, as measured from the initial slope of the p_v-V relation

p_o : resting venous pressure

Addendum II: Meaning of the used elasticity parameters

Purely elastic process:

$E_{sd//}$: measure for the amount of elastin and collagen fibres, which are involved in the strain experiment along the tibial axis.

$E_{sd\perp}$: measure for the amount of elastin and collagen fibres, which are involved in the perpendicular direction to the tibial axis.

A_{sd} : measure for the angular distribution of the fibres.

$k_{sd\perp}$: measure for the fibre-fibre interactions of the collagen fibre meshwork.

Delayed elastic process:

$E_{pu//}$: measure for the amount of collagen fibres bundles involved in the orientation process of the bundles in the direction of stress.

$E_{pd//}$: measure for the amount of elastin fibres and orientated collagen fibres, involved in the process after deformation of the skin, trying to restore the collagen fibre meshwork in its original position.

A_{pd} : measure for the angular distribution of the fibres involved in the delayed elastic process.

$k_{pd\perp}$: measure for the relaxation of the collagen fibre meshwork after deformation of the skin, depending fibre-fibre interactions and fibre-fluid interactions of the meshwork.

$\tau_{u\perp}$: time constant related to the velocity of shifting of the bulk of the collagen fibres through a viscous medium of glycosaminoglycans.

$\tau_{d\perp}$: time constant related to the velocity of shifting of the bulk of the collagen fibres, after removal of the load, in the direction of the original position of the fibre meshwork, depending on the tightened elastin fibres and on the collagen fibre-fibre interactions of the meshwork.

Summary

The present study was initiated to investigate the supposed skin elasticity deficit in patients with phlebologic disorders. Skin elasticity is determined by the dermal fibre meshworks of collagen and elastin, embedded in a viscous medium of glycosaminoglycans.
In chapter 1, a survey is given on the anatomical and biochemical characteristics of these components of the dermis. In the second chapter the method is described by which skin elasticity has been measured in this investigation.
Skin elasticity can be represented by means of stress-strain relationships. In mechanical terminology, stress means force divided by the cross sectional area of the piece of skin involved and strain is the relative increase in length of that piece of skin, caused by the stress.
With a new version of an uni-axial strain device, earlier developed by Wijn, the strain of a piece of skin between two 'tabs' (figure 2.5), which are attached to the skin, can be measured during and after loading of the skin by six stepwise loads with increasing amplitudes. Plotting the stresses against the corresponding strain values, results in a stress-strain relationship (figure 2.11).
In this thesis the stress-strain relationship of the skin was investigated only for small loads. This stress-strain relationship is non-linear. From the slope of the stress-strain relationship for zero stress, the so-called initial coefficient of elasticity can be calculated (cotgα in figure 2.3). The degree of curvature of the stress-strain relationship can be described with an alinearity coefficient. A preferential direction for collagen fibres exists in the skin (Langer's lines); the skin is said to be anisotropic. The anisotropy parameter of the skin of the calf, is the ratio between the coefficient of elasticity as found in the direction of the tibial axis and the coefficient perpendicular to that axis. The deformation of the skin as a result of the applied force, appears to be different during loading and after loading. Therefore the fore-

mentioned parameters have been calculated both during loading and after loading. Besides the respons of the skin as a function of time, has been subdivided into three parts (figure 2.9) corresponding to: a purely elastic process, a delayed elastic process and a purely viscous process. In this way 34 parameters can be calculated. For practical reasons and because some of the parameters are highly correlated and other parameters are difficult to measure, a set of ten parameters was selected for further investigations.

The skin of the calf has been choosen as measuring place, to be able to compare skin elasticity data with the venous capacity of the calf.

The venous capacity of the calf and a calf muscle pump function parameter were determined plethysmographically (see 2.4). Skin elasticity parameters, as a measure for the state of the dermal connective tissue, were compared with the venous capacity, as a measure for the vascular wall connective tissue.

Normal values for the elasticity parameters were determined in 92 normal subjects and for the venous capacity in 33 normal individuals. Sex did not influence the parameter values. Ageing influences some skin elasticity parameters considerably (chapter 3). In the chapters on skin elasticity in disease, the parameters values of the patient groups were compared with the normal values (Mann Whitney U test).

In the chapter 4 and 5 skin elasticity measurements in patients with phlebologic disorders are described. Skin elasticity was found to be impaired in patients with reticular varicose veins, but not in patients with truncal varicosities. In the truncal varicosity group a higher venous capacity was found, on the other hand in the reticular varicose vein group a normal venous capacity was found. A correlation between elasticity data and the venous capacity could only be demonstrated in the male subgroup of the truncal varicosity group.

By means of skin elasticity measurements an estimation of the supposed contribution of the skin to the calf muscle

pump could be made. From these calculations it seems highly unlikely that the skin contributes to the calf muscle pump mechanism, as suggested by some authors.
The skin does not behave as an elastic stocking. Obviously the suggestion of a skin elasticity loss as a contributing factor to the development of chronic venous insufficiency must be rejected. Actually the clinical impression of a skin elasticity loss in patients with chronic venous insufficiency is correct; we did find a skin elasticity loss in such patients. However the observed skin elasticity loss in these patients is not the cause of the chronic venous insufficiency, but the secondary result of the chronic dermal oedema, causing elastin fibre fragmentation. Such an elasticity loss could be found in all kinds of oedema not due to CVI (chapter 6).
During the investigations the need was felt for a more adequate interpretation of the determined elasticity parameters. Therefore skin elasticity measurements were performed in two generalized connective tissue disorders, viz. pseudoxanthoma elasticum, an elastin disorder (chapter 7) and Ehlers-Danlos syndrome, a collagen disorder (chapter 8). On the same day venous capacity was determined in these patients plethysmographically. In pseudoxanthoma elasticum lower initial coefficients of elasticity and an increased venous capacity were found; however these two quantities were not correlated. Skin elasticity parameters which reflect the condition of the dermal connective tissue, did not seem to be an appropriate measure for the state of the vascular wall connective tissue, as measured by the venous capacity.
A comparison between the skin elasticity data of patients with Ehlers-Danlos syndrome and the data obtained on the skin of patients with pseudoxanthoma elasticum, showed lower initial coefficients of elasticity (elasticity loss) in both disorders and lower alinearity coefficients only in patients with Ehlers-Danlos syndrome. From such observations it was hypothesized that the initial part of the stress-strain relationship is not only determined by the dermal elastin fibres but also by the collagen fibre meshwork (chapter 9).

The alinearity coefficient of the purely elastic process is related mainly to dermal collagen meshwork properties, presumably to interactions between the collagen fibres of the meshwork. For theoretical reasons it is not likely that the elasticity parameters determined with small loads reflect properties of the collagen fibre itself.
Suggestions for practical purposes are given in the last chapter (chapter 9).

Het onderzoek dat beschreven wordt in deze dissertatie werd aangevangen om een eventueel bestaand huidelasticiteitsverlies bij patienten met flebologische aandoeningen aan te tonen.
De elasticiteit van de huid wordt bepaald door de dermale collageen en elastine vezelnetwerken, die zijn omgeven door een visceuze substantie van glycosaminoglycanen.
In het eerste hoofdstuk wordt een overzicht gegeven van de anatomische en biochemische eigenschappen van de verschillende bestanddelen van de dermis.
In het tweede hoofdstuk wordt de methode beschreven waarmee de huidelasticiteit werd onderzocht. De elasticiteit van de huid kan worden beschreven met behulp van zogenaamde 'stress-strain' relaties. In de mechanica van de huid betekent stress (σ): kracht per oppervlakte van de doorsnede van een stukje huid en strain (ε): de relatieve lengtetoename van het betreffende stukje huid ten gevolge van een belasting (stress). Met een nieuwe versie van een rekapparaat dat eerder door Wijn werd ontwikkeld, wordt de strain van een kleine huidstrip tussen twee op de huid geplakte 'voetjes' ('tabs', zie figuur 2.5) gemeten, tijdens en na belasting van de huid met zes stapvormige, in grootte toenemende krachten. Het uitzetten van de stress-waarden tegen de corresponderende strain-waarden resulteert in een stress-strain curve (figuur 2.11). In ons onderzoek werden alleen stress-strain relaties voor kleine belastingen gemaakt. Het blijkt, dat een dergelijke stress-strain relatie niet lineair is. Uit de hoek die de raaklijn aan de stress-strain curve in het punt $\sigma = 0$ maakt met de stress-as wordt de initiële elasticiteits-coëfficiënt berekend (cotgα in figuur 2.3). De mate van kromming van de stress-strain relatie kan beschreven worden met een alineariteitscoëfficient. Er bestaat een voorkeursrichting voor de collageen vezels in de huid (Langer lijnen): dit betekent dat de huid anisotroop is. Een anisotropieparameter kan worden berekend door het quotiënt te nemen van de initiële elas-

ticiteitscoëfficiënten van de huid van de kuit, respectievelijk bepaald in de richting van de tibia-as en in de richting loodrecht op deze as. De vervorming van de huid tijdens belasting blijkt anders te zijn dan direct na belasting. Dit houdt in dat voornoemde parameters berekend moeten worden voor de vervormingen van de huid tijdens en na belasting. De tijdsafhankelijke responsie van de huid op een kracht (figuur 2.9), kan in drie delen worden verdeeld; overeenkomend met een puur elastisch proces, een vertraagd elastisch proces en een puur visceus proces. Voor ieder van deze processen kunnen voornoemde parameters berekend worden, in het totaal 34 stuks. Omdat een dergelijk aantal parameters praktisch niet hanteerbaar is en omdat een aantal parameters sterk gecorreleerd is en weer andere parameters moeilijk te meten zijn, werd een set van 10 parameters geselecteerd voor het verdere onderzoek. Als meetplaats werd genomen de huid van het midden van de kuit, om de elasticiteitsparameters te kunnen vergelijken met de veneuze capaciteits parameter van de kuit.
Met behulp van plethysmografie werden de veneuze capaciteit van de kuit en een pompfunctie parameter bepaald (2.4). Huidelasticiteitsparameters, als uiting van de toestand van het bindweefsel van de huid werden vergeleken met de veneuze capaciteit als maat voor de toestand van het bindweefsel van de veneuze vaatwand.
Normaal waarden voor de elasticiteitsparameters werden berekend bij 92 normale personen en voor de veneuze capaciteit bij 33 normale proefpersonen. Het geslacht bleek niet van invloed op de parameter waarden. De leeftijd beinvloedt enkele elasticiteitsparameters aanzienlijk (hoofdstuk 3). De normaal waarden werden gebruikt als vergelijkingsmateriaal voor de waarden van de patientengroepen (Mann-Whitney U toets).
In de hoofdstukken 4 en 5 worden de resultaten van het huidelasticiteitsonderzoek bij patientengroepen met flebologische aandoeningen beschreven. Er werd een huidelasticiteitsverlies gevonden in de groep met reticulaire varices, maar niet in de groep met stamvaricose (hoofdstuk 4). Plethysmo-

grafisch werd bij de stamvaricose groep een grotere veneuze capaciteit aangetoond. De veneuze capaciteit van de patientengroep met reticulaire varices was normaal. Een correlatie tussen de huidelasticiteitsparameters en de veneuze capaciteit kon alleen worden aangetoond in de groep mannelijke patienten met stamvaricose.
Met behulp van de huidelasticiteitsmetingen kon ook een schatting gemaakt worden van de veronderstelde bijdrage die de huid zou leveren aan het kuitspierpompmechanisme (hoofdstuk 5). Uit deze berekingen blijkt dat een dergelijke bijdrage aan de spierpomp te verwaarlozen is. De huid van de kuit functioneert kennelijk niet als een elastische kous, zoals beweerd wordt door bepaalde auteurs. De hypothese die stelt dat een huidelasticiteitsverlies bijdraagt aan de ontwikkeling van een idiopatische diepe chronische veneuze insufficiëntie kan niet worden gehandhaafd. De klinische indruk dat er een huidelasticiteitsverlies bestaat bij patienten met een chronische veneuze insufficiëntie (CVI) is inderdaad gegrond, een dergelijk elasticiteitsverlies werd in het onderhavige onderzoek aangetoond. Dit elasticiteitsverlies heeft geen pathogenetische betekenis voor het ontstaan van een CVI, maar is het gevolg van fragmentatie van elastine vezels door het dermale oedeem. Een dergelijke afname van de huidelasticiteit kon ook worden aangetoond bij oedemen die niet het gevolg waren van CVI (hoofdstuk 6).
Tijdens het onderzoek werd de behoefte gevoeld om de interpretatie van de elasticiteitsparameters, hoofdzakelijk in elastinevezeleigenschappen zoals voorgesteld door Wijn (3.1.4), te toetsen. Voor dit doel werden elasticiteitsmetingen verricht bij patienten met gegeneralizeerde bindweefselziekten, namelijk pseudoxanthoma elasticum, een elastine ziekte (hoofdstuk 7) en het Ehlers-Danlos syndroom, een typische collageen ziekte (hoofdstuk 8). Op dezelfde dag werd tevens de veneuze capaciteit bepaald met behulp van plethysmografie. Bij pseudoxanthoma elasticum werden lagere initiële elasticiteitscoëfficiënten (elasticiteitsverlies) en een grotere veneuze capaciteit gevonden; de grootheden waren echter niet gecorreleerd. De elasticiteitsparameters,

die afhankelijk zijn van het dermale bindweefsel, zijn kennelijk geen goede maat voor de toestand van het vaatbindweefsel, zoals dat beoordeeld kan worden op grond van de veneuze capaciteit.
Zowel de patienten met pseudoxanthoma elasticum als de patienten met Ehlers-Danlos syndroom vertoonden lagere initiële elasticiteitscoëfficiënten; alleen de Ehlers-Danlos patienten hadden lagere alineariteitscoëfficiënten. Ondermeer door dergelijke waarnemingen werd geconcludeerd dat het intiële deel van de stress-strain curve, niet alleen wordt bepaald door de elastine vezels van de dermis, maar ook door het collagene vezelnetwerk van de huid (hoofdstuk 9).
De alineariteitscoëfficiënt van het puur elastische proces is waarschijnlijk gerelateerd aan eigenschappen van het collageenvezelnetwerk, en wel vermoedelijk aan de interacties tussen de vezels. Uit theoretisch oogpunt is het onwaarschijnlijk dat de parameters die met kleine krachten zijn bepaald, eigenschappen van de collagene vezels zelf reflecteren.
Suggesties voor praktische toepassingen van de methode worden gegeven in het laatste hoofdstuk (hoofdstuk 9).

Dankwoord

Het proefschrift dat voor U ligt, is natuurlijk niet alleen de verdienste van de auteur. In de eerste plaats heb ik het aan mijn ouders te danken, dat ik een universiteire opleiding heb kunnen volgen.
De uitvoering van het onderzoek werd mogelijk gemaakt door een goede samenwerking tussen de sectie Flebologie van de afdeling Dermatologie (Prof. Dr. J.P.Kuiper) en de werkgroep Perifere Circulatie van het Laboratorium voor Medische Fysica en Biofysica (Dr. A.J.M.Brakkee en Dr. Ir P.F.F.Wijn). Ik heb veel geleerd van het constructieve commentaar van voornoemde personen bij het schrijven van deze dissertatie. Zonder de medewerking van vele personen op de afdelingen Dermatologie en Medische Fysica, had ik dit proefschrift nooit kunnen voltooien. Ik wil hen allen hier nog eens van harte bedanken. Speciale vermelding verdienen Dr. Ir. P.F.F. Wijn, die een aanzienlijke bijdrage heeft geleverd ten aanzien van het optimaal functioneren van de apparatuur en mevr. T.Overmeer-Langezaal, zonder wiens hulp ik nooit in staat geweest zou zijn om bij 380 mensen de huidelasticiteit te meten.
De statistische bewerking van het materiaal geschiedde op de Mathematisch-Statistische Adviesafdeling van de K.U.N. door Ir. H.J.J.van Lier.
De omslag werd ontworpen door mevr. A.Th.Ackermans-de Leeuw, de figuren werden getekend door de heer J.J.A.Linssen en het typwerk is verricht door mevr. B.van Toledo.
Tot slot mag ik niet vergeten een woord van dank te richten aan al die patienten en proefpersonen die zo vriendelijk waren om zich door mij aan de huid te laten trekken.

Berend Jagtman werd geboren op oudejaars avond te Breda in 1951. Na het behalen van het eindexamen HBS-B aan het Onze Lieve Vrouwe Lyceum aldaar in 1970, ging hij medicijnen studeren aan de Katholieke Universiteit te Nijmegen.
In 1978 werd het artsexamen afgelegd. Daarna volgde zijn opleiding tot dermatoloog aan de afdeling dermatologie van het St. Radboudziekenhuis te Nijmegen (hoofd: Prof. Dr. J.W.H.Mali), welke in juli 1982 voltooid werd.
Sinds 1 september 1982 is hij als wetenschappelijk medewerker verbonden aan de afdeling dermatologie van de Rijksuniversiteit Limburg (hoofd: Prof. Dr. W.J.B.M.van de Staak).

STELLINGEN

1. De vorm van de 'toe-part' van de stress-strain curve is niet alleen afhankelijk van de dermale elastine vezels.
   Dit proefschrift

2. De alineariteitscoëfficiënt $k_{sd|}$ is geen maat voor de elastinevezelkwaliteit, maar is gerelateerd aan de eigenschappen van het dermale collagene vezelnetwerk.
   Dit proefschrift

3. De huid levert geen bijdrage aan het kuitspierpomp mechanisme, de zogenaamde huid(pees)pomp bestaat niet.
   Dit proefschrift

4. Het huidelasticiteitsverlies bij patiënten met chronisch veneuze insufficiëntie heeft geen pathogenetische betekenis voor deze aandoening.
   Dit proefschrift

5. De aetiologie van stamvaricosis en reticulaire varices is verschillend.
   Lechner W. Phlebol. u. Proktol. 11: 125 (1982)

6. Elasticiteitsmetingen van de huid kunnen een bijdrage leveren aan de vroegtijdige detectie van systemische sclerodermie.

7. Zweetklieren dienen niet om te zweten, maar om water en zout te recycleren.
   Mali J.W.H. Huidziekten in de praktijk. p.280 (Dekker & v.d.Vegt, 1983)
   Thiele F.A.H. Measurements on the surface of the skin (proefschrift, Nijmegen 1974)

8. Het meten van de subcutane weefseldruk met behulp van subcutaan geïmplanteerde capsules is onjuist.
   Wiederhielm C.A. in: Tissue fluid pressure and composition. p.25 (Williams, Baltimore, 1981)

9. Periorale dermatitis is niet alleen het gevolg van het gebruik van gefluorideerde lokale corticosteroiden of gefluorideerde tandpasta.
   Steigleder G.K. Der Hautarzt 33: 347 (1982)

10. Bij cryochirurgische behandeling van ongecompliceerde huidmaligniteiten kan worden volstaan met doseringsschema's, gebaseerd op gestandaardiseerde vries-dooi cyclustijden.
    McLean D.I. J. Dermatol. Surg. Oncol. 4: 175 (1978)

11. De initiële conservatieve behandeling van lymfoedeem dient geleidelijke decongestie van de oedemateuze extremiteit te beogen; te drastische (machinale) reductie van het oedeem in korte tijd moet worden vermeden.
    Földi M. Med. Welt 31: 801 (1980)

12. Het feit dat,
    a) de Maastrichtse fakulteit gericht zal moeten zijn op de eerste lijnsgezondheidszorg (Deetman c.s. diverse publicaties),
    b) 10% van de consulten in de huisartsenpraktijk huidaandoeningen betreffen (Klokke c.s., Compendium huidziekten, p.21, Stafleu, 1982),

    zou moeten betekenen dat aan de afdeling dermatologie van de Rijksuniversiteit Limburg meer formatieplaatsen zouden moeten worden toegekend.

13. De kostenbesparing van 13.10^7 gulden in de dermatologie, door verschuiving van klinische naar poliklinische patientenzorg, kan alleen worden gerealiseerd, wanneer aanzienlijk meer financiën en ruimte beschikbaar komen om poliklinische dagbehandeling mogelijk te maken.
    Van de Staak W.J.B.M. Oratie, p.13 Maastricht, 29 april 1983

14. Het verdient aanbeveling om bij internationale conventie te bepalen, dat boektitels op boekruggen voortaan nog maar in één richting gedrukt mogen worden.

Stellingen behorende bij het proefschrift: Clinical investigation of skin elasticity.

Nijmegen, 13 oktober 1983 B.A. Jagtman