EVALUATION OF D-PENICILLAMINE IN SCLERODERMA

PROEFSCHRIFT

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GENERAL INTRODUCTION

The aim of this study was to determine exactly what a treatment with D-penicillamine may achieve in scleroderma and which parameters or variables prove suitable as indicators of its activity in this disease.

Interest in this study was stimulated by the publication of HARRIS and SJOERDSMA[1] in 1966 and, partly, by the apparent lack of an adequate treatment for scleroderma. HARRIS and SJOERDSMA[1] found evidence that penicillamine treatment could increase the usual low ratio of soluble to insoluble collagen in the dermis of patients with scleroderma.

As suitable indicators of useful penicillamine activity, special attention has been paid to the measurement of finger function, to the ratio of soluble to insoluble collagen in the dermis of skin biopts, and to hydroxyproline metabolism.

The study mainly involved patients of the Department of Dermatology & Venerology of the Medical Faculty of the University of Rotterdam. Many of the costs relative to this study were indirectly borne by the producers of penicillamine in the Netherlands, the Koninklijke Nederlandse Gist & Spiritus Fabrieken (Royal Netherlands Fermentation Industries) at Delft, later merged into Gist-Brocades.

Chapter 1. SCLERODERMA AS A MULTIPLE ENTITY

Clinical types

Because the etiologies are unknown, the conditions or reaction types grouped under the name heading of scleroderma can only be defined by clinical description. All have in common the typical skin lesion presenting, in the fully developed case, as hardening, thickening and tightening of the skin, associated with atrophy.

The following subdivision into types seems to be generally accepted and is preferred in the classification for this study:

I. Progressive Systemic Sclerosis (PSS, Diffuse Scleroderma), a generalized disorder of connective tissue characterized by inflammatory, fibrotic and degenerative changes, together with vascular lesions in skin, synovium and some internal organs (notably the esophagus, intestinal tract, heart, lung and kidney).

II. Morphea, a skin disease limited to plaques and bands ("coup de sabre") which may affect any part of the body. It consists of thickened skin patches of insidious growth which feel like pieces of cardboard and are seemingly attached to the deeper structures. The affected area is usually of an ivory white color and is surrounded by a lilac ring (vasodilatation); it results in a depressed atrophic area.
Only in rare cases is an internal organ disturbance discovered.

III. Generalized Scleroderma or Generalized Morphea, an extensive case of morphea. Large areas are affected with multiple or large plaques, with internal organ involvement in some cases.

The diagnosis is usually confirmed by histological examination of a skin biopsy from an affected area. Histologically compatible features in all forms of scleroderma on simple HE-staining are:

- decrease in skin thickness;
- decrease in skin collagen, with an increasingly parallel orientation of the fibrils to the skin surface;
- loss of rete pegs;
- atrophy of dermal appendages;
- more widely separated normal elastic fibers, though apparently closer together when atrophy occurs;
- hypertrophy and glassy or hyaline degeneration of collagen,

without fibrinoid changes;

- small foci of lymphocytes perivascularly;
- in early stages, a cellular inflammatory reaction in the fat around sweat glands and in subcutaneous tissue with infiltrates of lymphocytes, plasma cells and histiocyte-type cells (fibrocytes, sometimes mast cells) and lymphoid follicles with germinal centres;
- in skin vessels, medial hypertrophy, fibrosis, mild hyaline changes and concentric intimal proliferation, the papillary layer being poor in capillaries.

Distribution

At Lille, HURIEZ[2] found an incidence of 0.5 per mille for all types of scleroderma, among the new patients of the dermatological clinic over a period of 30 years; there was a female preponderance of 60%. The ratio of localized to generalized forms was 2:1. Likewise, SEZE et al[3] observed an incidence of 1 per mille among all new patients, being one per 20 new patients with rheumatoid arthritis. KORTING and HOLZMANN[4] found an incidence of 0.247% among dermatological patients, with a ratio of 1:1.5 for generalized and localized forms; for the localized forms the sex ratio of male to female patients was found to be around 1:1.5 and for the generalized forms 1:3. OPPERSKALSKI[5] found an incidence of 0.166% in his clinic.

Apparently no race or people is exempt from scleroderma or especially predisposed to it[4, 6-11], though the predominant clinical phenomena may differ in importance.

In addition to morphea, progressive systemic sclerosis has also been observed in childhood[12, 13].

As to familial occurrence, BURGE et al[14] described two sisters with scleroderma, who had both demonstrated characteristic atopic manifestations at an early age; they found twelve cases mentioned in the literature of more than one family member being affected by scleroderma. CAVALIERI[15] reported on the occurrence in a mother and her daughter and RENDALL & McKENZIE[16] documented the occurrence in three sisters and a niece.

Pathogenesis

It seems probable that the various forms of scleroderma represent a standardized reaction pattern of the skin which can be triggered by a variety of mechanisms (e.g. microvascular lesions as suggested by FRIES et al[17]). The distribution pattern of the lesions may give some clues, such as trauma in the case of local scleroderma and light exposure in the case of progressive systemic sclerosis[18], as seems to be the case in dermatomyositis and some cases of porphyria cutanea tarda[19]. A case of multiple morphea following chicken pox is reported by SINGH & BECK[20].

WINKELMANN[21] considers true progressive systemic sclerosis as a disease of vascular fibrosis with secondary inflammatory phases, the primary pathology appearing to be a fibromucinous change of the vascular endothelium, with altered vessel reactivity to cold and serotonin.

The evidence does not seem to support inheritance[22] as a factor of importance.

The observation of FRIES et al[23] that clinically normal skin placed in a sclerodermatous bed becomes sclerodermatous and that sclerodermatous skin placed in a normal bed remains sclerodermatous can be interpreted as an indication for an active topical disturbance. From microscopic studies, it is evident that the main changes of a consequent nature are found in dermis and small arteries. WINKELMANN et al[24] demonstrated a hypersensitivity of cutaneous vascular smooth muscle to 5-hydroxytryptamine and epinephrine in progressive systemic sclerosis.

The value of positive autoimmunological and immunological findings in scleroderma cannot be properly judged as yet. They may be primary or secondary or they may be present for quite other reasons. The evidence is considered equivocal[25]. It is usually considered that there is no autoimmune basis for progressive systemic sclerosis[22,26,27]. ANGHELESCU et al[28] conclude that immunoglobulin alterations are rare and nonspecific. WINKELSTEIN et al[29] failed to demonstrate a deficiency in the cellular immune defense system in patients. WARD et al[30] suggest that the scleroderma-like vinyl chloride disease is an immune complex disorder in which the immune response is initiated by the adsorption of vinyl chloride or a metabolite to tissue or plasma protein; the non-organ-specific antitissue antibodies may well be related to tissue damage rather than a pathogenetic mechanism. ERASMUS[31] found

a higher prevalence of generalized scleroderma among male miners than is acknowledged for women in general, which could be confirmed by RODNAN et al[32]. In pulmonary asbestosis, the antinuclear antibodies and rheumatoid factor probably result from the pulmonary lesions instead of causing them[33]. STUART et al[34] found evidence of a monocyte-mediated immunity to collagen in progressive systemic sclerosis, and so did KONDO et al[35]. HUGHES et al[36] described a reduction of T-lymphocytes in progressive systemic sclerosis which correlated with the extent of visceral involvement.

The relatively high incidence of progressive systemic sclerosis in women in their forties and fifties suggests a correlation with hormonal changes (preclimacteric or climacteric).

Another pathogenetic mechanism in scleroderma might be found in the autonomic nervous system, as indicated by the pathognomonically increased chronaxy of the sensitive nerves in combination with a normal rheobase[37-39], which can be made more evident after adrenalin and azamethonium[40], and as suggested from the association of esophageal aperistalsis with Raynaud's phenomenon[11,41].

EMERIT et al[42] found an indication of a relatively increased number of acentric fragments, "minutes" and morphologically abnormal chromosomes in scleroderma patients, as well as an increase in hyperdiploid cells and mitoses with morphologically abnormal chromosomes in lymphocyte cultures, in comparison to their relatives.

Clinically, the progressive systemic sclerosis seems properly placed among the so-called collagen diseases, with as their main representatives rheumatoid arthritis and systemic lupus erythematosus[43,44]. Not infrequently, some of these syndromes occur in mixed forms, one usually presenting as the main illness[45-47].

One is led to state that, for practical/clinical purposes, morphea consists of the scleroderma lesion limited to the skin alone and that progressive systemic sclerosis consists of the scleroderma lesion not being limited to the skin and thus affecting other organs as well[47].

Prognosis

The prognosis of morphea is practically always good as far as survival is concerned, though uncertain cosmetically. In many cases, the skin may eventually soften and revert to normal thickness or atrophy[47].

What makes prognosis and evaluation of treatment difficult in progressive systemic sclerosis and some cases of generalized morphea, is the slowly progressive nature, the tendency to spontaneous improvement or even remission, the influence of psychophysiological factors on many symptoms and the limitation of the objective criteria for improvement[47] or deterioration. The frequency with which positive treatment results are mentioned in the medical literature is certainly not without consequence for the understanding of the natural course and prognosis of scleroderma.

In progressive systemic sclerosis MASI & D'ANGELO[8] found the mortality in Negro females to be about three times than in White females in the USA, the mortality increasing with age. Survival time after diagnosis was equal for males and females, but significantly shorter for Negroes than for Whites.

STAVA[48] described a follow-up study over a period of twenty years involving 157 patients with various types of scleroderma. He concluded that, in severe acrosclerodermа. periods of increasing impairment may alternate with periods without further progression or even of relative improvement.

MEDSGER et al[10] studied survival in 309 patients with progressive systemic sclerosis and observed a 7-year cumulative survivorship of 35%. Decreased survival was correlated with increasing age, male sex, black race and renal, cardiac or pulmonary involvement.

PERSITZ & ROSENTHAL[49] found mortality in progressive systemic sclerosis to be particularly high during the first 6 years after diagnosis, with relatively good survival after this.

RODNAN[7] studied 100 patients with progressive systemic sclerosis; 42 deaths occurred, mainly from 1 to 10 years after onset of the disease. Nine of 13 Negroes died, usually after three or fewer years of illness. Although the outlook is poor in cases with rapid progression of the dermal lesions, with severe malabsorption and with cardiac failure (renal involvement being always fatal), the disability may often be only moderate and not necessarily threatening to long life.

HODKINSON[50] observed 15 elderly patients with mild scleroderma of favorable prognosis and found a positive correlation with the presence of the CRST syndrome (calcifications, Raynaud's phenomenon, sclerosis, teleangiectasia).

SZCZEPANSKY[51] reviewed 186 cases of acrosclerosis and established calcifications and teleangiectasies to be present in 6.5% of these, which included cases with considerable visceral involvement and a severe course without improvement; he concludes that distinction of

the CRST syndrome for prognostic reasons is not useful.

ROWELL[52,53] observed 84 cases of progressive systemic sclerosis for up to 15 years; the prognosis was poorer in males than in females, but unpredictable in the individual case. A fulminating onset with gangrene can be followed by survival for many years and the prognosis is not linked to the presence or absence of the antinuclear factor, treatment or CRST syndrome.

Conclusion

Scleroderma is of unknown etiology and pathogenesis. It consists of several types: a systemic form, a topical form and mixed forms, with identical histology of the affected skin. It is a relatively rare disease and its prevalence is not confined to any particular population. Scleroderma is properly placed among the collagen diseases. The prognosis for the topical forms is favorable, but that for the systemic forms is unfavorable.

Chapter 2. TREATMENT MANAGEMENT IN MORPHEA AND PROGRESSIVE SYSTEMIC SCLEROSIS.

General remarks

When the patient with local or systemic scleroderma seeks medical advice, he or she will be automatically treated according to prevalent insight and the fashion of the time and place. The usual objective is to alleviate signs and symptoms and to support wellbeing as well as defensive mechanisms of body and mind.

It seems a good policy to combine those measures from the various treatment categories which may relieve the pronounced symptomatology of a case and to add some more basic treatment in the severe and progressive cases.

According to WINKELMANN et al[54] vasodilating drugs and physical medicine, together with a protected environment (air conditioning at 25°C, adapted work), may be all that is needed for a stable or slowly progressive acrosclerosis; however, the more severe forms may require anti-inflammatory, immunosuppressive and collagen-solubilizing treatment, the management of which should take into consideration such individual factors as occupation and the presence of other and related disease. BIRK[55] and WINKELMANN et al[54] recognized that there is no specific therapy and that patients with rapidly progressive disease had not been helped by any drug at the time of their reports.

Supportive treatment in progressive systemic sclerosis could consist of lubricating oils and creams, more frequent and smaller meals, elevating the head of the bed with blocks to prevent gastric acid reflux, antacids, pancreatic enzymes and/or antibiotics for malabsorption.

Reduction of edema as well as physical therapy will improve the cutaneous sclerosis[54].

Physical measures

Physical therapy measures have proven only partially effective in the prevention of contractures but can be of help in preserving muscle strength[47].

BRAITSEV et al[56] report on the favorable change in the indices of the state of the nervous system and in the outcome of immunobiological examinations in a group of 74 patients with progressive systemic sclerosis who underwent a spa treatment combined with drug

therapy at Pyatigorsk, as compared with a group of 30 such patients treated with drugs only.

Drying out of the horny layer, especially during the winter, should be prevented by moisturizing creams or a mixture of equal parts of glycerin and water as a hydrating agent; the patient with progressive systemic sclerosis should be kept warm, if necessary by the use of heated clothing such as gloves and jackets[57].

For Raynaud complaints, a very suitable measure suggested by MATHIES[58] is the use of gasoline heated pocket stoves such as are obtainable in sporting shops. Nursing the patient requires a stable, warm environment.

An increase in hand temperature in Raynaud's phenomenon, along with marked symptomatic improvement, has been accomplished by special trainig, making use of autohypnosis and operant conditioning via audible signalling thermal sensors[59].

WINKELMANN et al[54] consider physical medicine most important in progressive systemic sclerosis to maintain mobility and to aid the circulation; they suggest deep heat with paraffin baths and the use of active massage and exercise to tolerance; heavy petrolatum or lanolin lubricant should protect the skin during physical therapy.

Surgical measures

As surgical wounds heal normally or with only a slight delay in progressive systemic sclerosis[60], one should not be reluctant to consider surgical measures if these offer the prospects of relief from symptoms.

Sympathectomies are often performed to lessen peripheral vasospastic phenomena and their consequence[61], but the results are usually not permanent in cases of progressive systemic sclerosis[54,62]. Sympathectomy with skin grafting may be successful in treating the leg ulcers of progressive systemic sclerosis[63].

Further surgical possibilities in progressive systemic sclerosis may be the excision of calcium deposits from subcutaneous tissue[64], colectomy[65,66], esophageal reconstruction[67] and artrhodesis and joint prosthesis[68].

In morphea, surgical relief may be effected by skin excision[69] and liquid silicone injections[70] to correct cosmetic abnormalities, while physiatrics may minimize or prevent contractions if the lesion traverses joints[71].

Medication

A short survey will be given of the various medications reported in the literature to be of some value in the management of scleroderma, mainly the progressive systemic variety.

Vasodilating agents are used to improve Raynaud's phenomenon and related sympathetic signs and symptoms. As such are used among others: sympatholytic drugs (reserpine intra-arterially, methyldopa, guanethidine, ergotamine), ganglion blocking drugs (azamethonium, procaine intravenously), smooth muscle relaxants (nicotinic acid derivates like tetra-nicotinoylfructose), adrenergic drugs (isoxuprine), and cholinolytic or parasympathicolytic agents as anti-parkinson drugs and chlorpromazine. These medications may be useful as long as there are no serious organic vascular lesions. WINKELMANN et al[54] warn that circulatory problems may arise or aggravate in severe pulmonary and cardiac involvement.

Plasma expanding agents are used to improve the flow characteristics of blood and thereby bring relief in cases of vascular insufficiency from small vessel disease[72]. Low molecular weight dextran (dextran 40, Rheomacrodex) is used as a 10% solution in physiological saline, 2 litres intravenously over 48 hours at intervals of several weeks.

Antimicrobial agents are in use to treat or suppress infections of the finger tips and the gastrointestinal tract. There is also a small hope of influencing the underlying unknown etiological process (dormant microbes?) in progressive systemic sclerosis. Usual are penicillin G intramuscularly, also in cases of morphea, and broad spectrum antibiotics like tetracycline, especially for the gastrointestinal complications (pneumatosis cystoides intestinalis, blind loop syndrome). DOVER[73] reported good overall improvement in the symptomatology of progressive systemic sclerosis from the use of sulfasalazine over extended periods in 19 cases.

Antifungal medication is used to treat fungal infections, but oral administration of griseofulvin is mainly used for other effects, viz. its vasodilating and estrogenic actions.

Antimalarial agents are used as they proved of some value in the collagen diseases systemic lupus erythematosus and rheumatoid arthritis. Mainly chloroquine is used.

Non-narcotic analgesics are usually not given primarily for their analgesic effects in progressive systemic sclerosis, but for their other effects like mitosis inhibition or leukocyte disruption. From

this category of drugs have been used the common salicylates, colchicine, trimethylcolchicinic acid, and external applications of dimethylsulfoxide (DMSO).

Cytostatic drugs are used in progressive systemic sclerosis because of favorable experiences in the treatment of systemic lupus erythematosus and rheumatoid arthritis. They exert an indirect anti-inflammatory effect via suppression of the cellular immunological mechanisms. Used are mercaptopurine, azathioprine, chlorambucil, thioguanine and methylhydrazine.

Of the hormonal agents especially the progestational drugs deserve attention. Their use is based on the inhibition of uterine collagen formation in animal experiences and on the possible need for hormone replacement, as there is a relatively high incidence of progressive systemic sclerosis in preclimacteric and climacteric women. Mainly used are norethisterone acetate perorally and hydroxyprogesterone caproate intramuscularly; there is some experience with progestin. The therapeutic results are worthwhile[74-77].

Corticosteroids have an adverse effect on the typical skin affection in scleroderma and they may complicate the cardiorenal problems in progressive systemic sclerosis[54], but they are of use to suppress the accompanying myositis, arthritis and wasting. Corticosteroids are indicated in the treatment of mixed connective tissue disease (MCTD, Sharp syndrome).

Other hormonal treatments in progressive systemic sclerosis involved di-hydrotachysterol, thyroid extracts, sodium dextrothyroxine, testosterone, slow-release phenformin with ethylestrenol, ovarian extracts, estrogen preparations and relaxin. The results were meager.

In the category of chelating, demineralizing and lathyrogenic agents the idea is to catch away minerals which are essential for the functioning and anabolism of connective tissue, thereby impairing connective tissue enzyme activity, and obtain a wasting of this tissue and its deposits. Disodium etidronate (EDTA) is more specifically given to remove the calcium deposits in the CRST syndrome, with doubtful results. From this category, penicillamine is now mainly used, but at least in 4 patients experience has been gained with beta-aminopropionitrile (BAPN) by KEISER & SJOERDSMA[78].

Enzymes are either used orally as pancreatic extracts to relieve digestive problems in progressive systemic sclerosis, or as hyaluronidase by intravenous infusion to "loosen" connective tissue via an attack on mucopolysaccharides.

From the vitamins special attention has been given to Vitamin B_{15} or calcium pangamate, with which STRUKOV et al[79] obtained histological improvement in morphea. Nicotinic acid and Vitamin C have been used, and the negative results of a scorbutic diet explored[80].
Vitamin E has also been given attention.

Further drugs used are typhoid vaccine, epsilon-aminocaproic acid (to reduce edema), the insaponifiable parts of avocado and soybean oils according to Thiers (or Piasclédine), asiatocoside (or Madecassol), potassium para-aminobenzoate (POTABA), anti-lymphocyte globulins (a failure), and hyperbaric oxygen (2 atm.) in courses to relieve Raynaud's complaints in progressive systemic scleroderma[81].

Conclusion

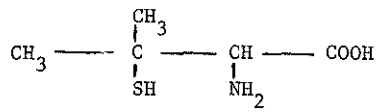
Most treatment methods in scleroderma are of doubtful value at long term. The choice should be led by the needs of the patient.

Chapter 3. THERAPEUTIC USE OF PENICILLAMINE

In all probability, penicillamine is a normal metabolite of penicillin G in man, as indicated by WALSHE[82], who demonstrated penicillamine in the urine of patients with liver insufficiency and treated with penicillin G injections; penicillinamine is not found in patients with a normal liver function under these circumstances.

Penicillamine (or beta-beta-dimethylcysteine) is commercially produced from penicillin G via a chemical degradation process followed by extraction via binding to mercury.
It is obtained as a racemic mixture; only the D-isomer is presently used clinically, as the L-isomer proved responsible for most of the bothersome side-effects and demonstrates a ten times stronger anti-vitamin B_6 effect than does the D-isomer[83]. CHLUD & LECHNER[84] and EGGENSCHWILLER[85] describe the analytical chemistry and assay methods for both isomers.

The structural chemical formula for penicillamine is:



Pharmacologically, penicillamine is primarily a chelating agent and it is used as such in the treatment of hepatolenticular degeneration (Wilson's disease, a hereditary insufficiency of copper transport leading to copper deposits in liver, basal ganglia and cornea), chronic lead intoxication, gold intoxication (as in the treatment of rheumatoid arthritis) and organic mercury intoxication. The advantage of penicillamine over other drugs used in these cases is its good absorption after oral administration. Its comparative affinity for metal ions in decreasing order is:
$Cu^{++} \rightarrow Pb^{++} \rightarrow Hg^{++} \rightarrow Au^{+} \rightarrow Co^{++} \rightarrow Zn^{++}$.

Penicillamine is also used in the treatment of cystinuria, replacing the cysteine-cysteine disulphides (cystine) in the urine by the 50 times more soluble cysteine-penicillamine disulphide, thus preventing the growth and further formation of urinary tract stones composed of cystine.

Attack on disulfide cross-linking bridges by the sulfhydril function

of penicillamine may account for the decreased cross-linking of collagen in the skin as observed by HARRIS & SJOERDSMA[1] during penicillamine treatment. It may also account for the improvement observed in rheumatoid lung disease by LORBER[86], pulmonary fibrosis[87-89], in cases of hyperkeratosis follicularis by BEER & LYLE[90] and in rheumatoid arthritis by JAFFE[91] and many others[92-97], in psoriatic arthritis[98] and the reduction of large protein molecules in serum, e.g. cryoprecipitable gamma M[99-102]. The reduction in the number of lymphocytes indicates an immunosuppressive effect in accord with the reduced serum IgG and IgM[103].

There are reports of a favorable influence in chronic progressive (cirrhotic) hepatitis[104-106], biliary cirrhosis[107] and in aortic sclerosis[108]. An indirect depressing effect on tyrosinase[109] may be responsible for the decreased degree of skin pigmentation observed by NICHOLSON et al[110] in some melanotic psychiatric patients on penicillamine. DANIELCZYK[100] treated multiple sclerosis with fair results. HUNTER & DONALD[111] describe successful treatment with increased porphyrin excretion in porphyria cutanea tarda. Penicillamine may prove useful in suppressing disturbing scar formation in surgery on sinews and biliary canals[112-114]. Its use in keratitis as eyedrops is based on the prevention of perforation via inhibition of collagenase and on its antiphlogistic properties[115,116].

Much experience has been gained with penicillamine in the still experimental therapeutic use in rheumatoid arthritis. DAY et al[96] summarize the observed positive clinical effects in rheumatoid arthritis as: a decrease in BSE, differential agglutination test, Rheumatic Factor titer and Latex slide test, C-reactive protein, serum M-globulins (IgM) and morning stiffness, as well as an increase in hemoglobin percentage and body weight. SCHMIDT & OTT[117] suggest from the correlations of decrease in BSE, IgM and coeruloplasmin that the latter two are at least partially caused by direct interference of D-penicillamine with these plasma proteins.

Conclusion

Penicillamine possesses several pharmacological and chemical properties which may be of therapeutic value in collagen diseases such as scleroderma.

Chapter 4. PENICILLAMINE IN SCLERODERMA

From the pharmacological properties of penicillamine, it appears that it has an effect on collagen and connective tissue which might predict its successful use in the group of collagen diseases and more especially in rheumatoid arthritis and scleroderma.

Interest in the experimental therapeutic use of penicillamine in scleroderma started with the publication by HARRIS & SJOERDSMA[1] of their findings on the effect of the drug on human skin collagen. Apart from observations in the skin of patients with other diseases, they studied three patients with scleroderma and found that the percentage of soluble collagen in skin biopsies increased and the cross-linking diminished on taking 2000 mg daily over a period of 2 weeks, while plasma and urinary hydroxyproline remained unchanged. The cross-linking was thought to be influenced via copper withdrawal from amino-oxidase.

FULGHUM & KATZ[118] published their observations on 5 scleroderma patients being treated with dosages of 1000 to 4000 mg of penicillamine daily for up to 11 months and with placebo periods. They found no influence on restricted joint movements, histologically stained skin collagen, the epidermal appendices, X-ray pictures of the gastro-intestinal tract and pulmonary function. However, they did note a high incidence of side-effects: proteinuria twice, leukopenia twice, anorexia five times, hypogeusia twice, an anaphylactic reaction to benzathine penicillin once and once a serum sickness-like picture on the 10th day of administration in a patient with a history of penicillin rash; all side-effects proved to be reversible on withdrawal of penicillamine.

KEISER et al[119] treated 4 cases of progressive systemic sclerosis with increasing doses of penicillamine ranging from 250 to 2000 or even 5000 mg daily within a period of about 10 days; all four suffered from loss of taste as a side-effect.

STEIN & SMYTHE[120] described the case of a female polyarthritis patient who, after 11 years, developed Raynaud's phenomenon followed by scleroderma. On daily treatment with 1000 mg of penicillamine and 75 mg of pyridoxine, she developed proteinuria with erythrocytes, leukocytes and leukocyte casts in the urinary sediment, a BSE of 108 mm, a decrease in serum albumin to 1.36 g/100 ml. Renal biopsy showed ischemic glomeruli with interstitial thickening and the assembly

of nuclei in the collecting tubules. Termination of penicillamine treatment led to recovery from the nephrotic syndrome.

BÖNI et al[121] treated 3 patients with progressive systemic sclerosis for several months with 1800 mg of penicillamine per day: a clear and measurable favorable effect was found on the pliability of the affected skin when using forceps to demarcate the maximal fold size at standard sites on the skin.

BLUESTONE et al[122] treated 11 patients with progressive systemic sclerosis with penicillamine. Nine continued the treatment. Though clinical benefit from this treatment was not obvious, skin tethering by a special suction cup apparatus showed improvement in six patients; palm prints indicated improvements in four. The improvement was maintained for up to 18 months after cessation of penicillamine-intake.

UITTO et al[123] studied the in vitro biosynthesis and the maturation of skin collagen in cases of untreated and penicillamine treated scleroderma. The eight untreated patients had a rate of ^{14}C-hydroxyproline synthesis and collagen maturation which did not differ from those in ten control subjects. In the five penicillamine treated patients, the biosynthesis of hydroxyproline and the conversion of soluble into insoluble collagen were reduced. Protocollagen proline hydroxylase activity was found to be elevated in two patients with untreated scleroderma, but penicillamine apparently did not affect this enzyme activity.

WINKELMANN et al[54] briefly mentioned their experiences with penicillamine treatment in 10 scleroderma patients at a dosage of 1000 mg per day (except for a boy, who was given 500 mg per day). Five patients improved subjectively, with skin softening and decreased stiffness; in two of these, the pulmonary tidal volume increased. As side-effects, urticaria with edema and vasculitis with features of erythema nodosum each occurred once.

LORENZEN et al[124] treated 17 patients with progressive systemic sclerosis with doses of 1200 mg of D-penicillamine per day for 4 weeks: analysis of skin biopsies before and after treatment revealed an increase in 0.14 M sodium chloride soluble collagen of 0.494, on the average. No changes were observed in the urinary excretion of hydroxyproline, serum proteins (increased alpha$_2$-globulins) or BSE; serum iron decreased. In two of the three patients under treatment with penicillamine and systemic corticosteroids, the soluble collagen content of the skin was found to be decreased.

BALDA[125] and ZIMMERMANN & BALDA[126] described their results with daily doses of at least 1800 mg of D-penicillamine for several years in 18 cases of progressive systemic sclerosis. They measured skin collagen solubility as the amount of dissolved collagen material obtained with the use of 8.0 M urea, which is about ten times that by other methods. Collagen solubility proved to be greater in the 18 patients than in 8 normal controls. D-penicillamine treatment was found to normalize this collagen solubility pattern to 50% of the initial value, which was already noticed shortly after the start of treatment. As side-effects were seen drug rashes - partly with fever, conjunctivitis, rhinitis and rarely otitis media - from the 8th to 11th day onwards; after several weeks, resumption of penicillamine treatment via slowly increasing doses was possible.

COLP et al[127] studied the serial changes in the pulmonary signs in patients, 16 of which had progressive systemic sclerosis, 13 interstitial fibrosis, 5 idiopathic diffuse interstitial disease and 2 peribronchial fibrosis. In only 3 cases was a progressive reduction in lung volumes and diffusing capacity seen. There was improvement with therapy in 2 cases (clearing of pleurisy and reduction in bronchial obstruction) and doubtful improvement in 1 case. Three of the scleroderma patients were treated periodically with penicillamine plus prednisolone and two with penicillamine alone; two of the patients with idiopathic diffuse interstitial disease were treated periodically with penicillamine. Three of these patients died. The authors thus could find little or no response to treatment.

DAROCZY & GYÖNGYVER [128] treated six cases of progressive systemic sclerosis and two of morphea with 600 to 1800 mg of penicillamine daily, to which were added 40 mg vitamin B_6 once weekly and a metal supplement in tablet form twice weekly. In the 5 cases which were treated for a long time (from 81 to 370 days), there was noticed: subjective improvement in skin induration, in skin pliability and facial expression, disappearance of Raynaud-like symptoms, improvement in palmar keratosis and an increase in the supporting surface of the hand (corresponding to improvement in the flexion contracture of the fingers). In three cases, skin biopsies before and during treatment were studied by polarization and electron microscopy. In scleroderma, a decreased negative collagen birefringence, an increase in ground substance and a quantitative increase in collagen fibers with a diameter of 200-300 Å could be detected before treatment.

The negative collagen birefringence became normal on penicillamine treatment. This action of penicillamine is probably based on depolymerization of the ground substance. As side-effects were seen: fever with maculo-papular rash three times, the rash alone once, hypogeusia twice and albuminuria three times (once as a reactivation of a pre-existing glomerulonephritis).

MOYNAHAN[129] reported on his experiences with low-dosage penicillamine treatment (300 mg plus 20 mg pyridoxine daily) in four cases of morphea in childhood. After one to three years of treatment, nearly all lesions had turned into normal skin again, except for the subcutaneous tissues of the forehead and scalp (coup de sabre) in one case and a foot lesion in another. There was a transient urticarial rash responding to antihistamine treatment in one boy. No soluble collagen was found to be present in the skin biopsies, according to the judgment of R.D.HARKNESS[130].

HERBERT et al[131] found penicillamine treatment to be clinically and biochemically effective in two cases with an active state of progressive systemic sclerosis, but not helpful in inactive forms of the disease. In 9 of 11 cases with active scleroderma skin collagen analysis revealed the presence of reducible aldimine bond cross-links, indicating that new collagen was being laid down. In the one case, at three months of treatment with 1500 mg daily, a subjective improvement in fine movements and a reduction in skin tightness and pigmentation with improvement of the range of movement and a decrease in the reducible cross-links in skin collagen were reported; at 6 months, the improvement was maintained and the skin collagen showed a further decrease in reducible cross-links; at 9 months there was a still further decrease in cross-links and striking clinical improvement. The other case, after one month of penicillamine treatment of 1500 mg per day, demonstrated decreased pigmentation and an increased range of movements; after three months there was a further reduction in pigmentation and skin tightness, with a decrease in reducible cross-links in skin collagen. In 3 out of 4 active cases, the amount of collagen synthesized in tissue cultures of skin was decreased in the presence of penicillamine; no such difference could be detected in an inactive case.

KREYSEL & KIMMIG[132] treated 30 patients with progressive systemic sclerosis with 1800 mg penicillamine daily and found an enormous reduction of the neutral salt soluble collagen fraction, a normalization of the acid soluble collagen fraction and a slight increase of the

insoluble collagen of the skin. They found a reduction of urinary hexosamine and hydroxyproline excretion, and an increase of collagen-like protein in serum (followed by a reduction after 40 weeks). They confirmed the reduction of high serum copper values and the increase of urinary copper values, and they found indications for a reduction of DNA and glycosaminoglycan biosynthesis in the skin.

JABLONSKA[133] treated 20 patients with penicillamine as they were unresponsive to other treatment. In some of the 12 cases of progressive systemic sclerosis (acroscleroderma) partial and transient improvement was obtained, and in localized scleroderma (8 cases) indurations regressed after several months of treatment with doses of 1.0 to 2.0 gram daily.

DAVIES & HOLT[134] describe the treatment with penicillamine in a case of generalized morphea, in which after 4 months of 250 mg t.d.s. an eruption developed which was clinically, histologically and immunologically consistent with pemphigus. After withdrawal of the drug the eruption resolved and two months later both direct and indirect immunofluorescence studies showed negative results.

DAVIS & BLEEHEN[135] review the treatment with D-penicillamine in scleroderma and find the situation confused because the natural history of especially progressive systemic sclerosis is unknown. They consider penicillamine to be of probable benefit, but it is quite possible that there is a clinical and biochemical response of dermal collagen and not of visceral collagen, as this contains a different crosslink.

UITTO & LICHTENSTEIN[136] summarized the relation between scleroderma and D-penicillamine as follows: in active scleroderma the defect is in the rate of collagen synthesis and degradation, while D-penicillamine induces a defect in the formation and stabilization of crosslinks.

Conclusion

In general, the experiences of other investigators with a variety of techniques confirm a favorable activity of penicillamine on sclerodermatous skin.

Chapter 5. MATERIAL, METHODS AND PROGRAM OF THE EVALUATIVE STUDY.

Introduction

In this study 51 patients with scleroderma and related diseases were available for investigation. Of these 44 were on penicillamine for short or long periods. Only 38 cases were sufficiently followed up to allow some judgment on the efficacy of the treatment.

From the onset of the study, it was considered that the changes brought about by the administration of penicillamine to the chronic stabilized cases of uncomplicated progressive systemic sclerosis should be of decisive significance. Changes in the course of acutely progressive cases cannot be judged properly. Cases of localized scleroderma (morphea), with its intrinsically favorable prognosis and very limited clinical symptomatology, offer comparatively few parameters and hardly justify the risks involved in penicillamine treatment.

As possibly interesting and suitable indicators of efficacy, attention was especially paid to measurement of 1. finger validity, 2. ratio of soluble to insoluble collagen in skin biopsies and 3. hydroxyproline metabolism (see Chapters 9, 8 and 7 respectively).

Approximately 15,000 finger joint flexions were measured in this study and a total of 131 hydroxyproline determinations in blood and urine were done in 11 patients.

Gist-Brocades contributed the greater portion of the 11,130 grams of penicillamine taken by the patients under study, as well as expertise for all of the 124 chemical analyses of skin biopsies (15.71 sq. cm of skin area) and statistical help. Statistical advice was also obtained in a later stage from R.van Strik of the Rotterdam Medical Faculty.

The duration of the study was from the end of 1968 to the beginning of 1974 or, depending on the patient, 1975.

In the course of this study, some papers which gave evidence of the generally aroused interest in the evaluation of penicillamine in scleroderma were published [54, 118, 119, 121-129, 131].
In general, the findings of these investigators correlated with those of this study; experiences with two of its initial cases were earlier reported by TIO et al in 1973[137].

Methods

In order to establish the extent of local and systemic involvement and to have a baseline for follow-up, the following diagnostic approach of the patients participating in this study was decided upon:

I. General examination

1. history and physical examination
2. routine urine examination (specific gravity, glucose, albumin, biliary salts, urobilin, sediment)
3. routine blood examination (BSE, hemoglobin, white blood cells and differential count, red cell count, MCV, MCHC, platelet count, plasma/cell volume)

II. Specific clinical examination

1. finger flexion (see Chapter 9)
2. skin folding and mouth opening
3. X-ray of hands and feet, affected joints, chest, esophagus (barium swallow), stomach and intestines (barium meal)
4. pulmonary function tests: total lung capacity, vital capacity, expiratory reserve volume, respiratory frequency during performance, lung compliance, oxygen diffusing capacity at rest and during performance
5. motor nerve conduction time and chronaxy
6. intestinal absorption (xylose test, Schilling test, fecal fat)
7. blood chemistry (urea, creatinine, cholesterol, bilirubin, alkaline phosphatase, zinc sulphate turbidity, lactic dehydrogenase, glutamic oxaloacetic and pyruvate transaminases, creatine phosphokinase, sodium, potassium, chloride, bicarbonate, all according to laboratory routine)

III. Histological examination

1. skin histology by light microscope
2. skin histology by electron microscope (only in a few cases)
3. chromosome examination (only in a few cases)

IV. Immunological examination

1. immunoelectrophoresis (total serum protein, albumin, alpha$_1$ globulin, alpha$_2$ globulin, beta globulin, gamma globulin, paraproteins, complement, haptoglobulin, immunoglobulins A, D, E, G, M)

2. Coombs test
3. Rose-Waaler and Latex test
4. anti-streptolysin O test
5. antinuclear factors
6. LE-cells
7. tuberculin sensitivity (Mantoux and von Pirquet test)
8. cold agglutination, cryoglobulins, cold antibodies
9. C-reactive proteins
10. antibodies to penicillin and penicillamine (only in few cases)
11. cross-over autotransplantation of affected and healthy skin areas (only in few cases)

V. Biochemical examination

1. soluble/insoluble collagen ratio in skin (see Chapter 8)
2. serum hydroxyproline (see Chapter 7)
3. serum collagen-like protein (see Chapter 7)
4. urinary hydroxyproline (see Chapter 7)

Treatment programs

At the beginning of the study, the maximum tolerated dosages were used. This decision was based on a lack of information concerning the effective dose in scleroderma on the one hand, and on the proven acceptability of daily dosages as high as 1500 to over 3000 mg in the treatment of Wilson's disease over many years on the other.

From the information obtained with various dosages, it was concluded that even daily dosages of up to 300 mg of penicillamine had an effect. New patients were therefore given higher dosages only if there was no clear response to the daily dose of 300 mg.

Because of the observed high incidence of allergotoxic reactions to penicillamine at about the 10th day of administration, it became routine to start treatment with minimal daily doses and to increase these much more gradually than had been done before under these circumstances; 300 mg daily were often not even reached on the 10th day of penicillamine administration[138].

Survey of patients involved in the study

Appendix A summarizes the essential data on the patients involved in this study in the sequence of their participation. These data concern their sex, birth date, clinical examination, biochemical investigation, disease course, follow-up period and notes on dosages used and their effects.

Chapter 6. CLINICAL EFFECTS OF PENICILLAMINE

Introduction

Axiomatically, all effects of drugs are toxicity phenomena. Some of these effects are more or less desirable under certain circumstances and so are considered therapeutic.

Most effects, however, are undesirable and thus are seen as side-effects. The less specifically a drug acts, the more side-effects can be expected. Toxicity in the strict sense represents the effects of overdosage of a drug. A very special type of undesirable, toxic, effect is the allergic reaction.

Methods

The effects of penicillamine in the patients under study were closely followed and measured, where possible, by the clinical and laboratory methods mentioned in Chapter 5.

Results (see also Appendix A)

a. Desirable effects.

During penicillamine treatment, finger flexion and dermal collagen ratio were altered in the desired direction as mentioned in Chapters 9 and 8 respectively. In some of the patients, the penicillamine treatment also resulted in the apparent disappearance of Raynaud's phenomenon (cases B, S) or myopathy (cases E, GG, KK) and in the improvement in facial expression (cases A, E, I), inflammatory reaction around superficial calcium deposits (cases F, LL) or various serological parameters (cases D, E, K, CC, FF, GG, KK).

Especially intriguing were the observed improvements, though minor, in esophagus and gastrointestinal passage in 3 patients with progressive systemic sclerosis (cases A, B, LL; out of the 10 which could be followed up properly after the establishment of prior functional impairment) as well as in some pulmonary function tests in 5 patients (cases B, J, P, GG, KK; out of the 11 which could be followed up properly after the establishment of some prior functional impairment).

Of course it is not possible to ascribe these latter effects to the penicillamine treatment with any degree of certainty, but pharmacological studies do suggest that, e.g., fibrotically impaired pulmonary function may improve as a result of penicillamine administration[54,86,127,139-141].

Some improvements were seen in morphea patients while on penicillamine treatment, presenting as a decrease in induration and possibly in erythema (cases J, O, P, BB, HH, JJ) and resulting in complete cure in two cases (J, JJ).

b. Indistinct effects.

During penicillamine treatment the condition of the patient may deteriorate:
progression of the underlying disease process (marked in cases K and EE), the typical pruritus of the affected skin (cases I, EE, KK), tendinitis (cases E, K), myopathy (case LL), cardiac arrhythmia (cases E, K), cardiac death (cases E, W, II), and unfavorable alterations in (auto) immune serological parameters (cases E, I, FF, GG, HH, II, LL, MM). These all belong to the normally expected course of events in progressive systemic scleroderma, but it is difficult to make certain that they could not be due to penicillamine at times.

In the patients under study, the penicillamine treatment did not influence co-existing diabetes mellitus (cases O, Q, GG) or rheumatoid arthritis (cases C, D, G, V, FF, KK) and it did not interfere in the healing of an appendicitis with abscess formation followed by an appendectomy 'à froid' (case I).

No definite influence of penicillamine treatment could be established on maximum mouth opening distance, maximum skin fold by forceps pick-up, teleangiectasia, osteoporosis and subcutaneous calcifications on X-ray photography of the hands, and on the fatigue in progressive systemic sclerosis.

In progressive systemic scleroderma, a high leukocyte count (cases C, E, L, K, W and BB) as well as a high platelet count (cases E, K) may be seen; the decrease in these counts as a result of penicillamine treatment could therefore be interpreted as of partial therapeutic value. A decrease in leukocyte count was observed in four cases (A, W, X and EE); 3 of these patients even developed leukopenia (cases A, X and EE). Five cases (A, B, E, CC and FF) demonstrated a decrease in platelet count, which developed into thrombocytopenia in two patients (CC and FF).

Some indication of a decrease in BSE was found in 3 cases (B, K, L).

c. Undesirable effects.

Loss of taste, hypogeusia or ageusia occurred in 7 cases (C; E; F, twice; K; Q; KK; NN).
Allergototic reactions presenting a rash and fever were seen in 8

cases (B, twice; C, twice; D; I; L, three times; O; S, twice; AA, twice).

Leukopenia and thrombocytopenia occurred as mentioned above. Albuminuria as a side-effect from penicillamine was not seen.

Taste-loss and allergotoxic reactions proved to be hindrances, as it took some time before penicillamine could be restarted, in lower doses of course. It was about six weeks before the taste-loss disappeared completely after stopping penicillamine. The allergotoxic reactions were usually dose-dependent and could be prevented from recurring if penicillamine was restarted in slowly increasing doses not exceeding 300 mg per day by the 10th day of administration. Such a reaction, which is allergic in character but only appears at higher dosages of a drug, could be considered as a "high-dose" allergy according to NIEWEG[142,142].

Penicillamine was not always well tolerated by the stomach, resulting in pyrosis, stomach pains - and even vomiting - often combined with taste-loss. Lowering the dose and taking antacids were usually helpful; in some cases (E, EE, KK), rectal administration of penicillamine base (less acid than penicillamine hydrochloride) by suppository was helpful and apparently also effective[144].

In some women (cases P and LL), an increase of appetite and consequent gain in body weight was noted when penicillamine was taken orally. This can probably be explained by the well-known effect of acids as appetite inducers.

Not seen were the following recognized side-effects of penicillamine: impaired wound healing, friable skin, pyridoxine deficiency, nephritis, LE-reaction, pemphigus, myasthenia[145].

Discussion

Not all of the patients with progressive systemic sclerosis or morphea accepted the proposal of an experimental treatment with penicillamine, usually because they did not yet suffer badly from their scleroderma. And none of the suitable (viz. positively responding) patients accepted the proposal of a temporary treatment with a placebo (dummy) preparation, usually claiming that they could well sense that penicillamine was of benefit to them. This attitude obviated

the possibility of a doubleblind study like that claimed by ALARCÓN-SEGOVIA et al[146] for colchicine (interacting with the microtubules of the fibroblast which secrete collagen[147]) with a favorable outcome.

Because of technical limitations and the storage times of over 6 months before analysis, timely follow-up or repeat biopsies for dermal collagen ratio determinations were not possible after interesting results became known.

As indicators for the short-term judgment of effectiveness, apart from the measurement of finger flexion and the determination of dermal collagen ratio, the further clinical and laboratory parameters appear to be vague, variable or poorly standardizable. Of course, improvement in such parameters does support the credibility of a therapeutic effect of penicillamine in the individual case.

Conclusion

No really new properties of penicillamine have come to light in this study involving 38 patients. Taste loss and gastric intolerance could be avoided by the preferential use of the penicillamine base over the hydrochloride salt and the eventual use of rectal suppositories containing penicillamine base. Allergotoxic reactions should be prevented by using slowly increasing daily doses initially and only to exceed a daily dose of 300 mg after the 10th day of administration if, e.g., finger flexion does not seemingly respond to that dosage.

Penicillamine treatment does not seem to influence the basic phenomena of progressive systemic sclerosis to any great extent and though it certainly is not curative, the improvement in finger flexion is a real asset to the patients[131].

Chapter 7. HYDROXYPROLINE EXCRETION AND ITS RELATION TO PENICILLAMINE TREATMENT.

Introduction

Collagen has a specifically high content of hydroxyproline of about 1 in every 3 amino acid units in its molecular structure. The total amount of collagen in the body is very large and any noticeable increase in hydroxyproline metabolism has to be considered as evidence of an increase in collagen metabolism, usually as a result of bone or endocrine disease[148]. As such can be mentioned hyperthyroidism, acromegaly, burns, bone-destroying lesions such as Paget's disease of bone, rickets of vitamin D-deficiency, metastatic carcinoma and extensive fractures[149]. Collagen is probably the most abundant protein in the human body, comprising about $\frac{1}{4}$ to 1/3 of its total protein weight, half of which is contained within bony structures, and it is the major constituent of most connective tissues; in skin and tendon, collagen accounts for over 70% of the dry weight and for about 23% in whole bone[150]. The dermis contains 77.7% collagen[151].

With progesterone treatment, HOLZMANN & KORTING[76] observed a two-fold increase in the urinary excretion of hydroxyproline in their patients (ten with progressive systemic scleroderma and one with generalized morphea). They interpreted this as most probably due to an increase in the catabolism of salt-soluble collagen. Concomitantly, they observed an increase in the normally low level of collagen-like protein in the serum of their patients.

From their studies in patients treated with penicillamine, HARRIS & SJOERDSMA[1] concluded that the urinary excretion of hydroxyproline remained unchanged, even though the skin collagen clearly changed; hydroxyproline levels in plasma protein also remained normal. KREYSEL & KIMMIG[132] found a reduction in urinary hydroxyproline excretion and an increase in serum collagen-like protein, with a reduction after 40 weeks.

The first two groups of investigators mentioned as their normal ranges for hydroxyproline excretion in adults 14.9 to 33 mg per 24-hour urine and 15 to 45 mg per 24-hour urine, respectively.

The normal range for plasma hydroxyproline is considered to be 15 to 25 mcg/ml (by the colorimetric method of PROCKOP & UDENFRIEND[152]), 7.8 to 9.7 mcg/ml of which are protein bound[153].

It was considered attractive and desirable to check these findings

relative to hydroxyproline metabolism during penicillamine treatment in the patients of this study.

Methods

In two patients (A, B), a series of determinations were made over 20 months concerning 24-hour hydroxyproline excretion and serum hydroxyproline concentration. In five other patients (C, E, F, L, and one untreated, alpha), incidental determinations of these values were made, usually during the induction period of penicillamine treatment. The data are given in Appendix B.

The determinations were made at the Laboratory for Clinical Chemistry of the University Hospital Rotterdam (head: Professor Dr.B.Leijnse), using the procedure of PROCKOP & UDENFRIEND[152] as modified by KIVIRIKKO et al[154].

For urine, 1 ml is autoclaved or treated on a water bath with 1 ml concentrated hydrochloric acid; after hydrolysis it is neutralized with potassium hydroxide and by dilution with water, followed by filtration. The sample is then oxidized with chloramine and this is halted after 20 minutes with thiosulfate solution saturated with potassium chloride and toluene is added. After shaking, centrifuging, cooling and heating, Ehrlich's reagent is added to the toluene phase for coloration and the optical density is read after 30 minutes.

Serum is first deproteinized with trichloroacetic acid, removed from the clear supernatant with ether and then treated as for urine.

Results

The data obtained all remained below the upper normal limits and could not be interpreted as indications of elevating or descending trends.

Creatinine excretion determinations, as a check for the completeness of the 24-hour urine collections, yielded contradicting results, so probably confirming the findings of EDWARDS & BAYLISS[155] as to its intrinsic unreliability, apart from the possibility of sampling errors.

The serum levels for hydroxyproline were all rather on the low side, as is usual in progressive systemic sclerosis.

Discussion

No special low hydroxyproline diet was given at the time of the hydroxyproline studies; this may easily explain the fluctuations in the data obtained. Despite the fact that these determinations were unusual for the laboratory concerned and the samples therefore had to be stored over varying periods until the next series of determinations could be made, not much of a laboratory influence is evident. For practical reasons, the control periods could only be regrettably short. The overall results, however, support the findings of HARRIS & SJOERDSMA[1] that penicillamine treatment does not materially increase hydroxyproline metabolism.

Conclusion

In 127 samples of urine and blood from 6 patients with progressive systemic sclerosis no evidence for an increased hydroxyproline excretion as a sign of increased collagen catabolism was found among the fluctuating data during treatment with penicillamine.

Chapter 8. INFLUENCE OF PENICILLAMINE ON THE COLLAGEN RATIO OF THE SKIN IN SCLERODERMA.

Introduction

As HARRIS & SJOERDSMA[1] had suggested that penicillamine treatment led to an increase in the ratio of soluble to insoluble collagen in the dermis (contrary to the pathological trend in scleroderma), it was decided to confirm this by follow-up analysis in skin biopsies from the patients treated in this study. The ratio determinations were made at the General Research Laboratory of Gist-Brocades at Delft under the supervision of E. de Haan.

Methods

The chemical analytical method used was the following. The stored deep-frozen skin specimen was defrosted and cleared of subcutaneous fat by scalpel; it was then kept overnight at a relative humidity of 80% in order to obtain a constant water content. The specimen was then refrozen by a mixture of acetone and carbon dioxide to -75°C and ground in a metal block by the use of a precisely fitting metal rod. The quantities of soluble and insoluble collagen were then determined in the pulverized skin by extraction of the total sample with 0.5 M acetic acid at a maximum temperature of 5°C[156,157] and by extraction of the remaining material with 0.3 M trichloroacetic acid at 90°C[158,159]. Collagen was measured in terms of its hydroxyproline content. The extracts were evaporated to dryness, redissolved in 6 N hydrochloric acid and subsequently heated in a closed vessel in an oven at 120°C for more than 16 hours. The contents were then dried by evaporation with an airstream and the residue dissolved in water. Hydroxyproline was determined in the solution obtained according to the method of BERGMANN & LOXLEY[158], but without warming[160,161].

Small sized skin biopsies (3 or 5 mm bore) were obtained before, during and sometimes after a course of treatment with penicillamine, always from one clearly affected area in such a way that successive biopsies were taken from a spot as close to the previous one as possible.

The fresh biopsy material was immediately placed in a small glass or plastic flask; it was then frozen in a thermos flask with solid carbon dioxide for t ansport to the laboratory, where it was kept in a freezer until a sufficient number of biopsies had been collected

for a worthwhile determination series.

As a check, the laboratory first practised this method of determination on the skin of rats.

Two control series of determinations were made with human skin to establish the standard deviation and coefficient of variance for the circumstances and method used in this clinical study. The results for one control series, consisting of rather large biopsies from the ablated normal breast skin of an elderly woman, are given in table 1.

Table 1. Soluble and insoluble collagen in biopsies from normal human skin (breast).

sample	weight in mg	% soluble collagen	% insoluble collagen	ratio soluble/insoluble collagen
I	6.32	4.47	37.6	0.119
II	14.20	3.58	31.4	0.114
III	23.18	2.39	23.9	0.100
IV	8.26	4.74	40.0	0.119
V	21.63	3.77	37.7	0.119
VI	8.00	4.14	32.9	0.126
VII	13.26	3.76	29.0	0.130
mean:		3.83	33.2	0.118

with a standard deviation of the mean ratio equal to 0.011, and thus a coefficient of variation of 9.3%.

The other control series, consisting of the usual small biopsies (4 mm diameter) from normal skin of the amputated lower leg of an elderly man, gave the results shown in table 2.

Table 2. Soluble and insoluble collagen in biopsies from normal human skin (lower leg).

sample	weight in mg	% soluble collagen	% insoluble collagen	ratio soluble/insoluble collagen
L	2.93	2.29	29.12	0.0787
M	3.21	2.15	33.99	0.0633
N	2.91	2.88	34.61	0.0832
O	3.32	2.47	39.18	0.0630
P	3.93	2.14	41.00	0.0522
Q	3.44	(1.46)	(43.17)	(0.0338)
R	4.04	2.40	34.90	0.0688
S	5.40	2.66	40.06	0.0664
T	2.46	-	36.69	-
U	3.90	2.10	41.51	0.0506

	including Q	excluding Q	including Q	excluding Q	including Q	excluding Q
standard deviation	0.403	0.276	4.32	4.10	0.015	0.0114
coefficient of variation	17.7%	11.5%	11.5%	11.5%	24.2%	17.3%
mean	2.28	2.38	37.42	36.78	0.0622	0.0657

Table 3. Time correlations of penicillamine treatment and collagen ratio in patients with progressive systemic sclerosis. Collagen ratio is given as percentage (control = 100%) and composing laboratory results (soluble %/insoluble %).

	A*	B*	E*	F*	H*	I*	K*	L*
	1500 mg	300 mg	100 mg	100 mg	1600 mg	2800 mg	1200 mg	150 mg
	100% (0.58/11.0)	100% (2.43/19.1)	100% (4.34/18.8)	100% (2.29/17.5)	100% 2.58/26.3)	100% (1.91/27.2)	100% (1.59/19.4)	100% (1.89/47.2)
	back	left wrist	right wrist	back	sternum	sternum	right forearm	back
nt-eat-	increase	no change	no change	no change	increase	increase	no change	increase
						170% (2.46/20.6)		
			50% (2.57/22.6)		150% (3.10/21.1)			
	140% (1.52/19.5)	99% (2.91/22.7)			stop			
						135% (2.06/21.9)	90% (1.61/22.5)	
	400% (4.71/21.4)	107% (3.22/23.5)					stop	
								133% (2.08/39.2)
						85% (1.53/25.3)		
	200% (2.62/23.9)	84% (1.82/16.9)						
			80% (2.86/15.6)					
		200% (3.25/13.0)						221% (2.80/31.5)
	240% (3.68/28.5)							
					113% (3.12/28.1)			
				104% (2.89/21.4)				
			71% (3.74/22.9)					
		95% (2.68/22.0)						
	600% (7.44/21.3)							
	stop		stop			500% (6.71/18.7)		
			96% (7.65/34.7)					
		stop						
	122% (2.33/35.25)							
		93% (2.66/22.7)						

* treated with penicillamine base.

)[1] after a short course of penicillamine from 17/10/1968 to 17/11/1968, patient L was re-started on penicillamine from 1/6/1970 onwards; the biopsy of 14/6/1969 is taken as the control in this table.

S	T*	V	W	X	Z*	EE*	KK*	LL
50 mg	1800 mg	800 mg	300 mg	200 mg	1400 mg	2000 mg	600 mg	300 mg
100%	100%	100%	100%	100%	100%	100%	100%	100%
(3.95/25.9)	(1.25/39.5)	(1.30/21.1)	(2.37/10.7)	(1.26/15.3)	(5.59/25.8)	(4.24/12.0)	(2.40/40.6)	(2.05/17.7)
left wrist	left breast	left wrist	left 3rd finger	right 3rd finger	right forearm	left forearm	sternum	left 3rd finger
no change	no change	increase	increase	no change	no change	no change	increase	increase
135%								
(5.12/24.5)								
			188%	106%				
			(0.98/1.54)	(0.65/7.47)				
	120%							
	(0.97/24.5)							
				stop		77%		209%
						(4.48/16.6)		(2.91/12.0)
				844%				
				(1.19/1.72)				
	stop			123%			210%	
				(1.64/16.3)			(3.31/26.0)	
						76%		
						(5.80/22.3)		
74%								
(2.83/25.2)								
			stop		125%			
					(7.24/26.5)			
		stop						
		175%						
		(1.60/14.7)						
					95%			
					(5.89/28.7)			
140%								
(3.59/16.7)								

Table 4. Time correlations of penicillamine treatment and collagen ratio in patients with morphea. Collagen ratio is given as percentage (control = 100%) and composing laboratory results (soluble%/insoluble %).

patient	J*	O	P	Q*	BB	JJ	(M*)
daily dose	1600 mg	1200 mg	400 mg	300 mg	200 mg	300 mg	1600 mg
control ratio	100% (0.76/36.1)	100% (1.99/30.0)	100% (2.64/33.0)	100% (2.76/37.1)	100% (3.73/22.0)	100% (1.52/22.0)	100% (1.35/29.0)
biopsy site	left thigh	left breast	left thigh	right breast	back	left forearm	left breast
final judgment	increase	no change	increase	increase	no change	increase	no change
months of treatment 1/4	350% (2.38/32.6)						
1		75% (1.73/34.9)	100% (2.79/37.6)		77% (3.76/28.7)		
3						150% (2.36/22.0)	
4				198% (3.22/23.0)			
5							111% (1.26/24.5)
7			217% (3.27/18.8)				
8							113% (1.36/26.0)
9						stop	stop
10					stop		
11		stop					
13							245% (2.53/22.3)
16	stop			630% (8.29/17.8)			
17			stop				

* treated with penicillamine base

Results and interpretation

The two tables 3 and 4 group the cases of progressive systemic sclerosis and of morphea, respectively, in such way that the ratios of soluble/insoluble collagen (and their composing laboratory results) per patient can be correlated in the course of time with the period(s) of penicillamine treatment and, roughly, with the dosage of penicillamine. Here, 100% denotes the control value of the collagen ratio, usually derived from a biopsy taken before the start of penicillamine treatment , and the follow-up ratios are accounted for in relation to the respective control values.

Both tables allow a judgment with regard to the possible increase in the collagen ratio as a result of penicillamine treatment: taking an increase of 50% (about 2 to 3 times the coefficient of variance in control series L to U inclusive) or over as decisive for a positive influence of the treatment, the following overall results are obtained

Progressive systemic sclerosis		morphea	
decrease	0 times	decrease	0 times
no change	9 times	no change	2 times
increase	8 times	increase	4 times

This roughly indicates a 50% chance of a positive effect (increase). If an equal distribution of chance over three possibilities (decrease, no change, increase) is assumed to occur in the untreated natural course of both diseases, the chi-square test on comparison with the results of penicillamine treatment yields 6.74 for progressive systemic sclerosis alone ($0.025 < p < 0.050$), 2.66 for morphea alone ($0.20 < p < 0.30$), and 9.14 for both diseases together ($0.01 < p < 0.02$); this is considered statistically significant in favor of a penicillamine effect for progressive systemic sclerosis alone and for progressive systemic sclerosis and morphea together[162].

Another way of examining the results is by the use of only two categories, viz. increased ratio and decreased ratio, and testing for statistical significance by means of the sign test[163]. For the collagen ratio in progressive systemic sclerosis, this yields an increase 14 times against a decrease 3 times (E, K, EE), with a one-sided $p = 0.05$; if one leaves out the equals according to the 50% criterion, this yields an increase 8 times against a decrease 0 times ($p = 0.005$). For morphea, the collagen ratio distribution then yields an increase 4 times against a decrease 2 times (O, BB), numbers too low for the sign test. Taking the ratios of progressive systemic sclerosis and mor-

phea together yields an increase 18 times against a decrease 5 times, with a one-sided $p = 0.05$; leaving out the equals according to the 50% criterion yields an increase 12 times against a decrease 0 times, with a one-sided $p < 0.005$.

The assumption of an equal distribution of the chances for improvement, stabilization and progression of the disease under the natural course of events is in all probability a too favorable one. Really expecting a skew distribution of these chances, however, with stress on disease progression and stabilization, the positive influence of penicillamine on the collagen ratio would be all the more striking.

Discussion

Interindividual differences and intraindividual variations of soluble and insoluble dermal collagen content between sites were quite noticeable and could not be accounted for other than being due to inheritance and possibly topical factors, including pathological influences, as well as age.

The values found for the ratios of soluble to insoluble collagen in the patients in this study do not indicate a particularly low content, or a low percentage, of soluble collagen in sclerodermic skin.

HARRIS & SJOERDSMA[1] reported the following collagen ratio values for the forearm skin of their scleroderma patients:

case 5	0.0090	case 10	0.0121	(0.0225 after penicillamine)
case 6	0.0090	case 11	0.0101	(0.0549 after penicillamine)
case 7	0.0131	case 12	-	(0.0471 after penicillamine)
case 8	0.0080			

The influence from penicillamine is compatible with the findings of this study.

SCHEINBERG[165] and KUEPPERS & DANIELS[166] found clinical evidence of skin changes with atrophy after penicillamine treatment in Wilson's disease, the soluble collagen content being increased up to 18% instead of the 2.5% usually found for atrophic skin.

Corroborative evidence for a positive effect of penicillamine on the ratio of soluble to insoluble collagen is also presented by LORENZEN et al[124] and UITTO et al[125].

In the case of patient M, suffering from porphyria cutanea tarda, is illustrated how closely the ratios for the unaffected skin of the buttocks and for the scleroderma-like skin of the breast run

parallel and follow the improvement in the clinical picture of teh diseased skin (not necessarily due to penicillamine in this case):

05-12-1969	1.73/34.1 = 0.0507	100%	11-12-1969	1.35/29.0 = 0.465	100%
28-05-1970	1.67/29.3 = 0.057	115%	28-05-1970	1.26/24.5 = 0.051	111%
21-08-1970	1.62/23.3 = 0.070	140%	21-08-1970	1.36/26.0 = 0.052	113%
14-01-1971	3.57/28.1 = 0.127	255%	14-01-1971	2.53/22.3 = 0.113	245%

This can also be considered as a confirmation of the exactness and validity of the determinations.

For the sake of completeness, a frequency distribution for the control values for the collagen ratio (100% values) of the skin biopsies over the patients involved is given as Table 5.

0.01	pat.L	0.13	pat. B, F
0.02	pat.J	0.15	pat. S
0.03	pat.T	0.17	pat. BB,D
0.04	pat.X	0.20	pat. beta
0.05	pat.M, A	0.22	pat. Z, W
0.06	pat.KK,V	0.23	pat. E
0.07	pat.O,JJ, I, Q	0.24	pat. AA
0.08	pat.delta,K,P	0.35	pat. EE
0.10	pat.alpha,epsilon	0.37	pat. C, Y
0.11	pat.N	0.44	pat. gamma
0.12	pat.DD,LL, U, R		

Table 5. Frequency distribution for control values of collagen ratio over the patients studied. (morphea cases are underlined; Greek letters indicate patients with progressive systemic sclerosis who did not participate in the treatment study).

Conclusion

Based on 124 determinations in the 23 patients studied, the results concerning the action of penicillamine treatment on the collagen ratio of sclerodermatous skin are compatible with the findings of other investigators (see Chapter 4) and indicate a corrective influence on the scleroderma lesion.

Chapter 9. INFLUENCE OF PENICILLAMINE ON FINGER FLEXION.

Introduction

At the start of the experimental treatment with penicillamine in scleroderma, it was decided to determine the degree of incapacity of the affected fingers in the more serious cases of progressive systemic sclerosis.

As finger flexion proved to be the limiting factor in the functioning of the hand in two pilot cases (patients A and B), as was soon confirmed in others, goniometric measurements of metacarpophalangeal and interphalangeal joint flexions were included in the follow-up of patient treatment[167].

In the two pilot cases, it was evident that the finger flexion improved rapidly on the oral administration of penicillamine; this was most evident and statistically significant in the metacarpophalangeal joints, evident and still statistically significant in the proximal interphalangeal joints but much less so in the distal interphalangeal joints[137].

It seems reasonable to assume that penicillamine may, at least partially, restore the sliding capacity of the skin over the underlying structures[121,122], which is so characteristically lost in scleroderma. So, a greater freedom of flexion for the finger joints can be reestablished when more "sliding" skin length becomes available proximally. In this way the metacarpophalangeal joints have the whole back of the hand to stretch (some 10 centimeters in length), whereas the distal interphalangeal joints have available only the dorsal skin over the middle phalanges (3 to 4 centimeters).

Methods

The results of the goniometric measurements were recorded as the number of degrees over which the joints could flex spontaneously from the hypothetical straight line of the proximal bony lever (taken as 0^{o}). The goniometer has always been applied over the dorsal side of the joint, with its "Origin" or "sharing point" lying over the joint to be assessed (see Figure I). An unimpaired joint can usually flex about 90^{o} or more; in scleroderma, this is often much less.

All of the finger joints (normally 28) of both hands of a person were measured for maximal spontaneous flexion in each session.

Figure I. Illustration of method for goniometric finger flexion measurement.

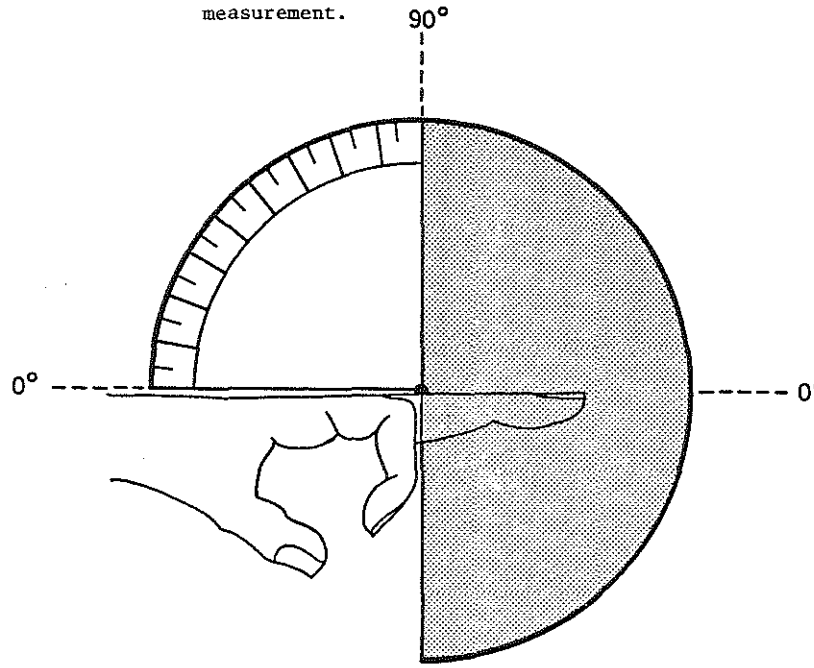


Table 6. Results of finger flexion measurements in normal persons. Means of the three joints and S.D. are given.

control person	metacarpophalangeal flexion	S.D.	proximal interphalangeal flexion	S.D.	distal interphalangeal flexion	S.D.
1.male						
at 39yrs	97.1°		103.7°		77.8°	
at 42yrs	91.1°, 94.9°	1.90°	98.0°, 99.8°	0.98°	72.7°, 74.1°	0.70°
at 44yrs	91.1°		100°0°		72.7°	
2.female						
at 41yrs	92.5°, 90.4°	1.05°	100.2°, 99.6°	0.30°	76.7°, 73.2°	1.75°
at 44yrs	87.5°		99.4°		68.0°	
3.female at 55yrs	69.9°, 68.6°	0.65°	92.4°, 90.1°	1.15°	58.2°, 48.8°	4.70°
4.male at 14yrs	93.0°, 97.2°	2.10°	107.3°, 109.7°	1.20°	80.6°, 80.1°	0.25°
at 16yrs	92.9°		108.0°		89.0°	
5.male at 11yrs	85.9°, 87.6°	0.85°	102.4°, 102.7°	0.15°	76.6°, 77.3°	0.35°
at 13yrs	86.0°		103.6°		75.1°	

Such sessions were initially repeated at intervals of several days to a week and later in the follow-up at longer intervals (every 2 weeks, every month, every 2 to 3 months).

Some double or duplicate measurements of finger flexions were made in five control persons in order to estimate standard deviations of the obtained mean values per group of joints per person. The results are summarized in table 6.

These data are also slightly suggestive for an age-dependent evolution of maximum flexion capacity. Taking into consideration only the intra-individual differences in mean metacarpophalangeal flexion to evaluate the result of treatment, it will be clear that the lower limit of 10^{o} difference in measurement (over 3 times the maximum standard deviation, S.D., observed) for a judgment on improvement or deterioration is on the safe side. When cumulative sum techniques[168] are applied to the flexion data of the patients, a standard deviation of 2.1^{o} is found in those who apparently do not respond to the treatment, while a standard deviation of, e.g., over 4^{o} indicates a significant shift in the patient's flexion capacity (see Figure IV).

Results

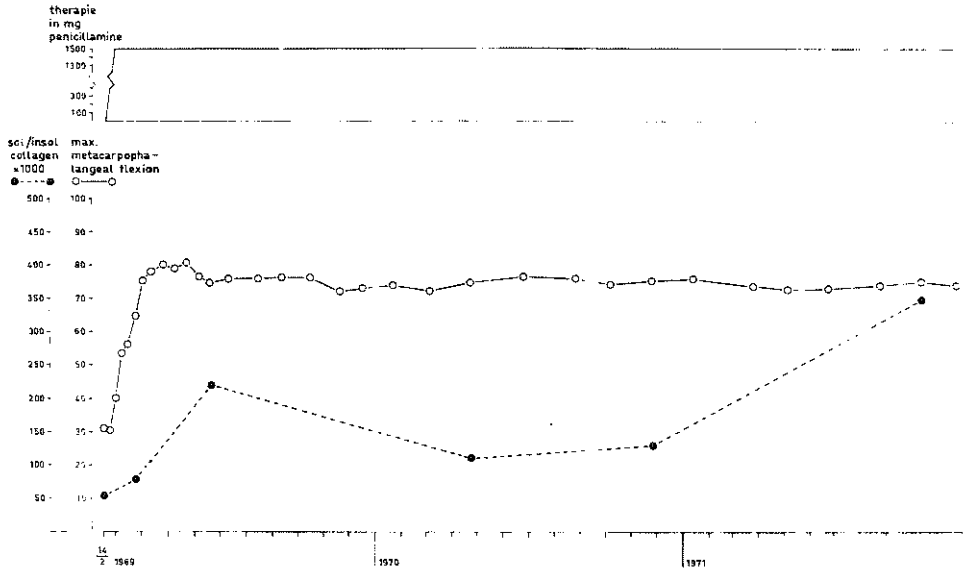
As a typical illustration, the values obtained for mean metacarpophalangeal flexion during the follow-up of two treated patients are given in time relation to penicillamine treatment and dosage along with the collagen ratio determinations. The flexions were always measured without knowledge of previous results. The data obtained for patients A and B are shown in Figures IIa and b.

The most important data regarding the mean metacarpophalangeal flexion of the patients with progressive systemic sclerosis studied will be given in table 7 and Figures IIIa, b, c and d.

The five patients coded SA to SE were treated and followed up by professor Pinol Aguadé from the Escuela Professional de Dermatologia y Venereologia at Barcelona, who expressed finger flexion as the mean of the degrees of flexion of the two interphalangeal joints of the index and middle fingers of both hands, and thus not as the mean metacarpophalangeal flexion.

In Figure IIIa, b, c and d the patients are grouped according to the same four categories as in table 7, viz. a. cases with rapid improvement, b. the spanish cases from Barcelona, c. cases with slow improvement, and d. cases demonstrating no improvement or deterioration (patients E and EE are each represented twice, under different categories). The graphs are projected with a logarithmically shortened time axis.

Patient A Figure IIa.



Patient B Figure IIb.

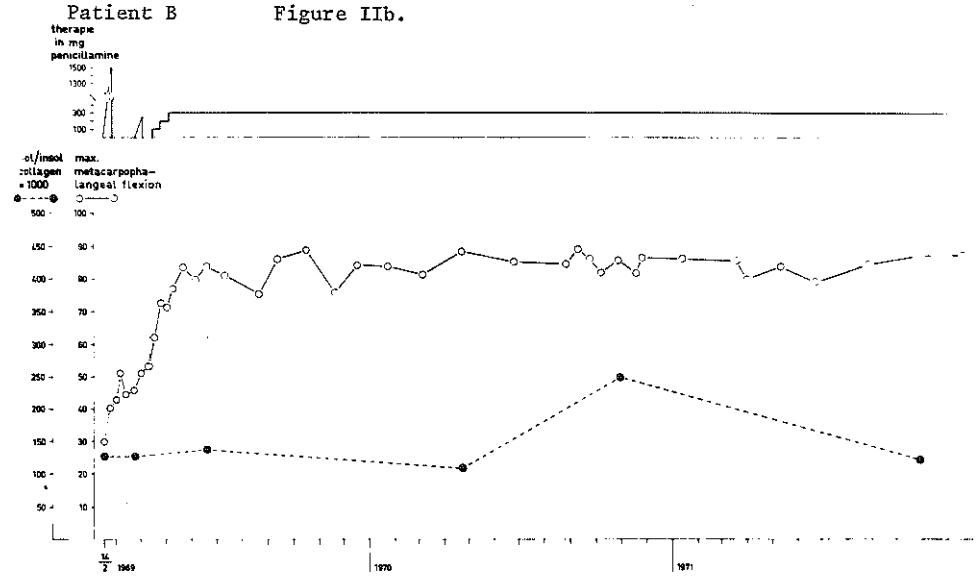


Figure II.
Graphical presentation of penicillamine dosage, mean maximum metacarpophalangeal flexion, and soluble/insoluble collagen ratio in successive biopsies of the affected skin from one area. (patient A and B).

Table 7. A summary of the most important mean metacarpophalangeal)* flexion data for each patient with progressive systemic sclerosis studied.

Patient code	flexion before treatment	flexion during treatment with time indication	flexion at end of treatment or later
A	31.0°	80.0° after 2 months	72.7° after 3 years
B	30.0°	83.8° after 4 months	87.4° after 3½ years
D	69.8°	77.7° after 10 days	80.4° after 8 months
E	63.1°	77.8° after 1 month	59.0° after 3 years
I	54.7°	77.1° after 10 days	
		66.1° after 4 years	
AA	50.1°	59.1° after 5 days	62.4° after 2 weeks
KK	27.3°	67.2° after 2 months	
SA	60°	70° after 2 months	
SB	55°	65° after 3 weeks	
		55° after 4 months	
SC	55°	85° after 8 months	
SD	35°	80° after 4 months	
SE	75°	100° after 5 months	
L	64.1°	69.9° after 1 year	
		72.3° after 3 years	
W	78.4°	86.2° after 1 month	
X	77.0°	79.8° after 4 months	81.4° after 4 3/4 months
Z	67.8°	78.7° after 2½ years	
EE	55.8°	64.3° after 2 months	
		9.2° after 1½ years	
GG	52.1°	66.9° after 6 months	
LL	75.5°	80.1° after 6 months	
MM	38.0°	59.9° after 8 months	
E	63.1°	77.8° after 1 month	59.0° after 3 years
F	80.2°	80.2° after 4 years	
K	70.6°	59.0° after 4 months	60.0° after 9 months
EE	55.8°	64.3° after 2 months	
		9.2° after 1½ years	

)* except for cases SA, SB, SC, SD and SE.

Figure IIIa.

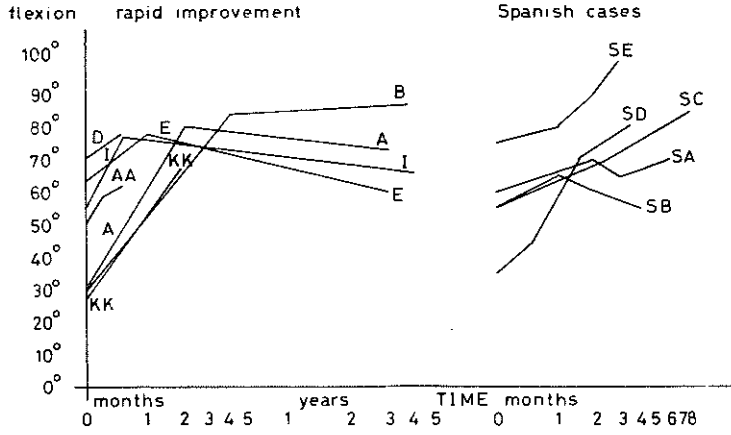


Figure IIIb.

Fingerflexion changes under the influence of penicillamine intake in relation to duration of treatment

Figure IIIc.

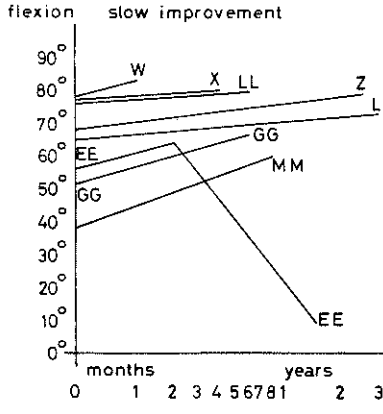
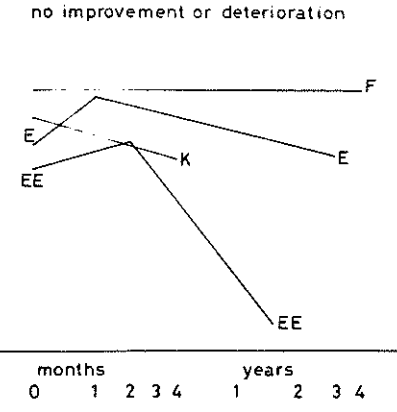


Figure IIId.



Fingerflexion changes under the influence of penicillamine intake in relation to duration of treatment

Interpretation

From the data obtained, it is clear that penicillamine treatment tends to increase the flexion possibilities of the affected fingers in patients with progressive systemic sclerosis. The flexion increases can, however, only be statistically significant, viz. 10^{o} or more, in the more severely affected fingers of patients whose mean metacarpophalangeal flexion measures 75^{o} or less. The latter was the case in 17 patients (A, B, D, E, I, KK; SA, SB, SC, SE; L, Z, EE, GG, MM, K). Among these 17 patients, flexion was increased significantly in the underlined 12 cases, decreased significantly in 2 (K, EE) and increased nonsignificantly in the other 3 (D, L, Z) on exposure to penicillamine.

It will now be attempted to interpret the results statistically, as a frequency distribution. If an equal distribution over three possibilities (increased, unaltered, and decreased flexion) is assumed to occur by chance in the untreated natural course of the disease, the chi-square test on comparison with penicillamine treatment results yields 4.86 ($0.05 < p < 0.10$), which is on the borderline of being statistically significant with respect to improvement in flexion[162]. The assumption for the frequency distribution of the natural course in progressive systemic sclerosis may, however, be too optimistic.

Another way of examining the results is by the use of only two categories, viz. increased finger flexion and decreased finger flexion and testing for statistical significance by means of the sign test[163]. Taking into consideration all of the cases with progressive systemic sclerosis, finger flexion shows an increase 18 times (or 19 times if patient D is also taken in consideration) against a decrease 3 times (K, EE, F), with a one-sided $p < 0.005$ (without the five spanish cases $p = 0.05$); if one leaves out of consideration the equals according to the 10^{o} criterion, this gives an increase 12 times against a decrease 2 times (K, EE), with a $p = 0.025$.

Discussion

One could object to using the means of all 10 fingers to judge changes in finger flexion, as the thumb and little finger (1st and 5th finger) are atypical and may therefore disguise or exaggerate changes in the more typical and important fingers.

For this reason, and in order to assess the validity of professor Pinol's restricted measuring method (used in cases SA, SB, SC, SD, SE), the means of the 6 typical fingers were compared with those of all 10 in patients B and K. This did not result in any differences in the

interpretion of the flexion changes in the course of time; the means of both series ran parallel.

The patients usually first notice a subjective easing of finger flexion soon after the start of the penicillamine treatment. Later an objective improvement in maximum finger flexion may be found.

Some of the patients are not thought suitable for a proper judgment of the efficacy of penicillamine: in one (C), corticosteroid treatment for rheumatoid arthritis parallelled penicillamine treatment and only finger extension was impaired and measured at follow-up; in another (AA) penicillamine was given only for a few days at a daily dose of only 50 mg.

In one case (L), penicillamine was added to the long-term treatment with oral prednisone and norethisterone at low dosages earlier instituted; this patient was therefore thought suitable to judge the possible extra influence of penicillamine on the clinical course of her progressive systemic sclerosis.

The possible use of the cumulative sum (cusum) method[168] of evaluating the fluctuations of mean metacarpophalangeal flexion per patient, is illustrated as an example in Figure IV. It represents the graph of the cumulative sum of differences from the means of the flexion measurements of patient A. From this,one can determine whether finger flexion shows just the deviations of a steady state or a genuine shift in level.

In one patient (E), the only disfigured finger nail started growing into normal shape again from the start of penicillamine treatment, with a clearly shifting demarcation groove for several months.

In another patient (I), maximum spontaneous elbow extension was followed-up by measurement, as the impairment of this joint function presented a special problem of incapacitation here. From the data in Table 8 it will be apparent that a gradual and almost complete recovery was seen during penicillamine treatment.

Table 8. Gradual improvement in maximum spontaneous elbow extension during penicillamine treatment in patient I.

	ELBOW EXTENSION (normally 0°)						
date	right	left	daily dose	date	right	left	daily dose
29-10-70	43°	23°	1000 mg	24-11-71	33°	19°	2400 mg
10-11-70	42°	25°		19-11-72	30°	16°	
19-11-70	44°	20°	1200 mg	02-03-72	28°	10°	
26-11-70	44°	20½°		28-04-72	22°	9°	
02-12-70	42°	18°	1600 mg	09-06-72	24°	9°	
17-12-70	42°	19°		11-08-72	16°	9°	1600 mg
14-01-71	41°	25°		06-10-72	15½°	8°	2400 mg
25-02-71	46°	28°		22-12-72	16°	9°	2000 mg
11-03-71	49°	31°	2000 mg	02-03-73	14°	9°	
01-04-71	50°	28°	2800 mg	04-05-73	15°	10°	1600 mg
22-04-71	45½°	30°		29-06-73	13°	10°	1200 mg
27-05-71	47°	26°	1800 mg	31-08-73	12°	5°	
24-06-71	45°	27°	1400 mg	26-10-73	11°	6°	
19-08-71	40°	24°	2800 mg	07-06-74	12°	7°	
06-10-71	37°	18°		16-08-74	10½°	8°	

Finger extension, as opposed to flexion, gradually became impaired in most patients during the course of time. This happened also under penicillamine treatment.

That finger exercises did not materially contribute to the improvement in finger movements was apparent in 5 patients (C, I, E, L, EE) during the periods of supervised forced exercises which they were subjected to.

There were also no clear effects apparent from periods of progrestagen treatment (D, K, L, AA), prednisone or tetracosactrin treatment (C, L, X), or Madecassol treatment (A, E, AA).

Conclusion

Goniometric follow-up of finger flexion in this clinical study was done in 22 patients. The findings are consistent with a normalizing effect of penicillamine in progressive systemic sclerosis on the scleroderma impaired finger flexion.

Figure IV. Patient A

```
N    X*GEM.-
     CUMSOM
 1   -44.5
 2   -89.5
 3   -125.1
 4   -147.1
 5   -166.6
 6   -177.5
 7   -177.5
 8   -175.1
 9   -170.6
10   -167.1
11   -162.1
12   -161
13   -162.2
14   -161.7
15   -161.3
16   -160.5
17   -159.6
18   -162.9
19   -165.3
20   -166.9
21   -170.1
22   -171.2
23   -170.3
24   -169.8
25   -171.1
26   -171.3
27   -170.9
28   -172.7
29   -175.6
30   -178.4
31   -179.9
32   -180.1
33   -181.8
34   -184.6
35   -185.1
36   -186.7
37   -184.4
38   -186.8
39   -190.8
40   -192.8
41   -192.8
42   -192.2
43   -192.9
44   -192.7
45   -190.4
46   -186.7
```

```
-180      -160      -140      -120      -100      -80       -60       -40       -20        0         20
...X.....X.....X.....X.....X.....X.....X.....X.....X.....X.....X.....X.....X.....X.....X.....X.....X
```

IN HØEVEEL GEBIEDEN WENST U DE GRAFIEK TE VERDELEN? 3
GEBIED 1 LØØPT VAN X T/M Y
? 1,6
GEBIED 2 LØØPT VAN X T/M Y
? 7,12
GEBIED 3 LØØPT VAN X T/M Y
? 13,46

GEBIED VAN	GEBIED TOT	GEMIDD.	STANDD.	UITBIJTERS
1	6	45.9	14.2	0
7	12	78.27	1.95	0
13	46	74.76	1.76	0

Chapter 10. FINAL EVALUATION

A summary of the quantifiable changes established in this study is given in the table 9. It presents the correlation between the degree of improvement in finger flexion and the degree of change in collagen ratio per patient. With the exception of patient E a parallellism of both changes is clearly seen.

Using a rank correlation method on the numerical data for flexion and collagen ratio, a statistically significant correlation is obtained $(0.02 < p < 0.05)$[164].

Table 9. Correlation between degree of improvement in finger flexion and collagen ratio.

patient code	flexion change	collagen ratio change	patient code	flexion change	collagen ratio change
A	ii	ii	AA	i	ii
B	ii	i	EE	dd	d
D	i		GG	ii	
E	ii	d	KK	ii	ii
F	=	=	LL	i	ii
I	ii	ii	MM	ii	
K	dd	d	SA	ii	
L	i	ii	SB	ii	
U	i		SC	ii	
W	i	ii	SD	ii	
X	i	ii	SE	ii	
Z	i	i			

i	not significantly improved
ii	significantly improved
=	unchanged
dd	significantly decreased
d	not significantly decreased

One is finally led to conclude from all the assembled data in this study that, though penicillamine does not seem to influence the basic phenomena of progressive systemic sclerosis to any great extent, the improvement in finger flexion coupled with an altered collagen ratio in the skin is indeed a real asset to the patients[131].

It is therefore advisable to try penicillamine in cases of progressive systemic sclerosis for at least several weeks and search for a therapeutic response by measuring the finger flexion.

EVALUATION OF D-PENICILLAMINE IN SCLERODERMA

Summary of contents.

Chapter 1. Scleroderma as a multiple entity.

A survey is made of the different types of scleroderma and their most outstanding characteristics, prevalence, pathogenesis and prognosis. It is evident that there is not much information of a basic nature available and that the clinical manifestations may vary widely.

Scleroderma is apparently a disease of structures derived from the middle germ layer and may well represent a syndrome or reation pattern, the pathogenesis of which is triggered by a range of causes.

Chapter 2. Treatment management in morphea and progressive systemic sclerosis.

The treatment of scleroderma is reviewed from the literature. It is clear that there is no such thing as a specific treatment. A number of symptomatic measures are taken aimed at the manifestations of the disease.

Chapter 3. Therapeutic use of penicillamine.

A review of the chemical and pharmacological properties of penicillamine and of its various applications in medicine is given. It possesses properties which may be of therapeutic value in collagen diseases such as scleroderma.

Chapter 4. Penicillamine in scleroderma.

The literature on the use of penicillamine in scleroderma is reviewed. Interest in such treatment dates back to 1965-1966. The studies are all of a preliminary nature; they in general indicate a fair degree of influence of penicillamine on the collagen abnormality in scleroderma, and so confirm the findings reported in this thesis.

Chapter 5. Material, methods and program of the evaluative study.

Set-up, diagnostic approach and dosage schemes used are given, along with short descriptions of the observed patients and the course of their disease (Appendix A).

Chapter 6. Clinical effects of penicillamine.

Desirable and undesirable effects of penicillamine in the 38 patients under study are reported. Allergotoxic effects and loss of taste were particularly bothersome due to the frequency of their occurrence.

It is concluded that penicillamine has a small but clinically relevant symptom-relieving action in scleroderma, apparently not affecting the basic phenomena of progressive systemic sclerosis. The improvement in finger flexion is a real asset to the patients.

Chapter 7. Hydroxyproline excretion and its relation to penicillamine treatment.

The data from 127 urine and blood samples of 6 patients with progressive systemic sclerosis on the relationship between penicillamine treatment and the hydroxyproline content of serum and urine proved to be variable, but they were in no way indicative of a possible increase in hydroxyproline excretion via the urine.

Chapter 8. Influence of penicillamine on the collagen ratio of the skin in scleroderma.

The 124 data on the ratio of soluble to insoluble dermal collagen in the skin biopsies from 23 patients with progressive systemic sclerosis and morphea obtained in the course of this study indicate a positive effect of penicillamine, which is in accordance with the findings of others.

Chapter 9. Influence of penicillamine on finger flexion.

The data obtained from the goniometric follow-up measurements of pathologically impaired finger flexion in 22 patients with progressive systemic sclerosis are interpreted as an indication of a positive, normalizing effect of penicillamine treatment. The measurement of the metacarpophalangeal flexion of the fingers is a very simple and straightforward parameter.

Chapter 10. Final evaluation.

The effect on skin collagen ratio correlates reasonably well with that on metacarpophalangeal flexion on the fingers. The improvement in finger flexion is therefore coupled with an altered skin collagen ratio and is a real asset to the patients.

It is advisable to try penicillamine in cases of progressive systemic sclerosis for at least several weeks and search for a therapeutic response by measuring the finger flexion.

Nederlandse samenvatting:

DE WAARDE VAN D-PENICILLAMINE BIJ SCLERODERMIE.

Hoofdstuk 1. Sclerodermie als een veelvuldige eenheid.

Een overzicht wordt gegeven van de verschillende typen sclerodermie en hun belangrijkste kenmerken, voorkomen, vorming en verwacht verloop. Het is duidelijk dat het hier ziektebeelden betreft waarover nog zeer veel onbekend blijft. De ziekten gaan uit van het middelste kiemblad en vertegenwoordigen wellicht een syndroom of reactie-patroon dat door verscheidene oorzaken in gang wordt gezet.

Hoofdstuk 2. Behandelingsaanpak van morfea en progressive systeemsclerose.

Behandelingswijzen voor sclerodermie worden aangegeven uit de literatuur, waaruit het duidelijk wordt dat er geen specifieke therapie bestaat, doch dat een aantal op de ziekteverschijnselen gerichte symptomatische maatregelen worden genomen.

Hoofdstuk 3. Therapeutisch gebruik van penicillamine.

Een kort overzicht wordt gegeven van de chemische en farmacologische eigenschappen van penicillamine en van de verschillende toepassingsgebieden in de geneeskunde. Het bezit eigenschappen die van therapeutische betekenis kunnen zijn bij collageenziekten zoals sclerodermie.

Hoofdstuk 4. Penicillamine bij sclerodermie.

De in de literatuur beschreven ervaringen met het gebruik van penicillamine bij sclerodermie worden weergegeven. De belangstelling begint in 1965/1966 op te komen. De behandelingen zijn alle van proefondervindelijke aard. In het algemeen wordt een invloed op de collageenafwijking bij sclerodermie gevonden en zijn de uitkomsten verenigbaar met de ervaringen vermeld in dit proefschrift.

Hoofdstuk 5. Wijze van opzet en uitvoering van het onderzoek.

Opzet en gebruikte methoden van onderzoek en behandeling worden weergegeven, alsmede enkele korte beschrijvingen (in bijlage A) van de in de beoordeling betrokken patiënten met hun ziektebeloop.

Hoofdstuk 6. Klinische effecten van penicillamine.

De gewenste en ongewenste uitwerkingen van penicillamine bij de 38 bestudeerde patiënten worden genoemd. Vooral toxische overgevoeligheidsverschijnselen en smaakverlies bleken lastig door hun veelvuldig optreden.

Als conclusie wordt gesteld dat penicillamine een gering doch klinisch betekenisvol effect heeft op een symptoom van sclerodermie en dat het niet de indruk wekt het eigenlijke ziekteproces aan te grijpen. De verbetering van de buigmogelijkheid van de vingers is voor de patiënt belangrijk.

Hoofdstuk 7. Hydroxyproline-uitscheiding met betrekking tot penicillamine-behandeling.

De bepalingsuitkomsten van 127 urine- en bloedmonsters van 6 patiënten met progressieve systeemsclerose betreffende de invloed van penicillamine op het hydroxyprolinegehalte van serum en urine bleken een enigszins grillig resultaat op te leveren, doch in elk geval geen aanwijzing op te leveren voor een eventuele toename van de uitscheiding via de urine.

Hoofdstuk 8. De invloed van penicillamine op de collageenverhouding van de huid bij sclerodermie.

De 124 gegevens inzake de ratio van oplosbaar tot onoplosbaar collageen in de huidbiopsie uit de lesies bij 23 patiënten met progressieve systeemsclerose en morfea wijzen op een effect in de normaliserende richting van de penicillamine-behandeling, zoals ook door andere onderzoekers gevonden.

Hoofdstuk 9. De invloed van penicillamine op de vingerbuiging.

De flexiemetingen, in het beloop van de behandelingen met penicillamine verricht bij 22 patiënten met progressieve systeemsclerose ten aanzien van de ziekelijk beperkte vingerbuigingen, worden geduid als een aanwijzing voor een gunstig, normalizerend effect van deze behandeling. De metingen van de maximale spontane buiging van de metacarpophalangeaalgewrichten blijken een eenvoudige en directe maatstaf te vormen.

Hoofdstuk 10. Eindwaardering.

Het effect op de collageenverhouding in de huid correleert redelijk met dat op de metacarpophalangeaalbuiging van de vingers. Derhalve betekent deze aan de veranderde huidcollageenverhouding ge-

koppelde vingerbuiging een werkelijke aanwinst voor de patiënten en kan als maat dienen voor het vaststellen van de zinvolheid van een ingestelde proefbehandeling.

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Curriculum vitae

De auteur werd geboren op 3 december 1929 te Eindhoven, alwaar hij het gangbare onderwijs volgde tot en met het eindexamen lyceum-gymnasium beta 1948. De hierop volgende studie der geneeskunde aan de Rijksuniversiteit te Leiden werd op 9 november 1956 met het artsexamen afgesloten.

Voor, tijdens en na het vervullen van de dienstplicht werd ervaring opgedaan in ziekenhuis en algemene praktijk. Van 1958 tot 1973 volgden functies in de klinische farmacologie bij farmaceutische bedrijven in Nederland (ACF-Nedchem, Philips-Duphar, Kon.Ned. Gist & Spiritus Fabrieken - Brocades).

Sinds 1973 is de auteur als medisch wetenschappelijk medewerker werkzaam bij het Bureau van de Raad voor Gezondheidsresearch TNO te 's-Gravenhage.

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Gedurende de vele jaren die het onderzoek vergde hebben opeenvolgende generaties assistent-geneesheren hun sporen mede verdiend met de moeizame begeleiding van de patiënten welke betrokken waren in dit onderzoek, en dat verdient zeker vermelding.

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De Afdeling Documentatie en Bibliotheek van Gist-Brocades leverde belangrijke bijdragen op haar terrein.

APPENDIX A

BRIEF INFORMATION ON PATIENTS INVOLVED IN THE STUDY

BRIEF INFORMATION ON PATIENTS INVOLVED IN THE STUDY.

Patient sex birth date	known as . PSS since	affected areas & organs on entering study	disease course	period under study	conclusive remarks
A. female 16/04/1949	1962 Raynaud	fingers, hands, forearms, face, mouth, forehead, neck, back, chest, esophagus, intestines, ANF dubious, low thrombocyte count (100, to 200,000), low leukocyte count (3,700).	stable	december 1968- january 1975	gastric intolerance to high doses of penicillamine, leukopenia after 3 years treatment; finger flexion, skin & esophagus improved; then Madecassol inj. without result; amputation of fingertips after penicillamine.
B. female 11/01/1915	1946 Raynaud fingers & toes (sympathectomy); 1956 PSS.	fingers, hands, forearms, face, mouth, forehead, lower legs. (malleolar ulcers), esophagus, intestines, lungs, ANF+, CPR+ once only, cold agglutinins+ 1/16, finger amputation 1952. (1972 bilateral lumbar and thoracic sympathectomy).	stable	december 1968- january 1974	hypersensitivity to penicillamine (rash & fever, fever & joint pain), gastric intolerance;300 mg/day penicillamine improved finger flexion, healing of ulcer, skin, Raynaud possibly, infected fingertips, esophagus & intestines possibly, lung function.
C. female 26/08/1914	1948 Raynaud, 1966 PSS, 1968 RA.	fingers, hands, arms, face, mouth, esophagus, liver, lungs, cor pulmonale, ANF+, LE-cells class IV, leukocytosis (20,000), Rose+ 1/32, cold agglutinins + 1/32, RA fingers, hands, shoulders, hips, knees.	stable	november 1969- may 1970	several reactions to penicillamine (rash & eosinophilia, rash & fever, ageusia), no clear effect; corticosteroids for RA.
D. female 03/12/1904	1959 Raynaud & finger ulceration, 1963 RA, 1967 dysfagia & tight skin.	fingers, face, mouth, esophagus, intestines, lungs, kidneys? fatigue, ANF weakly +, abnormal lymphocyte chromosomes; RA knee, wrist.	slow progression	december 1969- january 1975	penicillamine hypersensitivity (fever, rash, eosinophilia, leukocytosis, recurrence of knee arthritis & facial nerve paralysis); progestagen, thrombophlebitis lower legs; then 160 mg/day penicillamine; no clear effects; ANF became doubtful.

patient sex birth date	known as PSS since	affected areas & organs on entering study	disease course	period under study	conclusive remarks
E. male 20/02/1911	summer 1969.	fingers, hands, forearms, face, mouth, forehead, liver, myopathy, ANF neg., ASOT 600U, thrombocytosis (to 600,000), leukocytosis (+ 10,000), fatigue; pericarditis before, tachycardia later; later tenditis of wrist and malleoli; CPK elevated.	rapid progression, regression on treatment, followed by progression	november 1969- january 1975	immediate regression on 800 mg/day penicillamine, taste loss & gastric intolerance reducing and interrupting administration, thus by suppositories; Madecassol injections, then penicillamine again; weight loss; myopathy cleared; ASOT normalized, ANF+; BSE increased, cryoglobulin trace; calcium deposits; death from cardiac failure june 1975.
F. female 10/01/1914	1966 Raynaud.	fingers (subcutaneous calcium deposits), old interscapular patch, impaired shoulder movement, lungs; ANF pos.	slow progression	november 1969- january 1975	2x taste loss on penicillamine, but 100mg/day well tolerated; calcium deposits less troublesome, skin more supple, shoulder movement improved, Raynaud disappeared, fingers slimmed on penicillamine.
H. male 16/05/1898	1966 Raynaud.	fingers, chest; Rose+ 1/128, agglutinins+, serum IgM increased & paraprotein line.	very slow progression	march 1970- january 1974	treated for 5 weeks with 1600mg/day penicillamine in a very early phase, till gastric intolerance; skin collagen improved.
I. female 24/08/1924	spring 1969.	fingers, hands, arms, face, neck, mouth, shoulders, chest, sacral area, esophagus, stomach, intestines?, lungs, lower legs; itch; serum IgM elevated; gamma globulin lowered; LDH elevated.	rapid progression	march 1970- january 1975	hypersensitivity reaction to penicillamine, immediate improvement of finger flexion & skin collagen; only stabilized with up to 2800mg/day, again able to do housekeeping, Raynaud less severe, elbow flexion improved.
K. male 24/02/1917	spring 1968 Raynaud, end 1968 PSS; (1959/1960 rheumatic fever).	fingers, hands, forearms, face, forehead, neck, back, chest, upper abdomen, legs, feet, esophagus, stomach, lungs, heart, leukocytosis (10,300), eosinophilia (21%), thrombocytosis (430,000), ANF+; later tendinitis, arthritis.	rapid progression	december 1969- december 1970 (death)	neither penicillamine up to 1200mg/day nor progestagen halted the rapid progression; cardiac death?

patient sex birth date	known as PSS since	affected areas & organs on entering study	disease course	period under study	conclusive remarks
L. female 23/11/1920	1966 Raynaud, june 1967 PSS, 1968 esophagus & lung fibro- sis.	fingers, hands, arms, face, mouth, neck, shoulders, back, chest, lower legs, esophagus, li- ver, lungs, later kidneys, ANF+, leukocytosis (13,000).	clearly progres- sive	november 1968- july 1975	hypersensitivity reactions to penicillamine (shivering & fever); later added to proges- tagen & corticosteroid treat- ment as 150mg/day resulting in stabilization, although kidney affection progressed.
M. female 01/05/1911 porphyria CT	1966.	chest; elevated serum copper.	stable	december 1969- january 1971	1600mg/day penicillamine over 40 weeks improved skin, colla- gen ratio, and possibly light sensitivity.
N. female 05/05/1924	1939 Raynaud, 1962 PSS, 1964 sympa- thectomy 3x.	fingers acrosclerosis with sub- cutaneous calcium deposits, feet, lungs, ANF+, mitochondrial an- tibodies+.	progression	january 1971- january 1974	300mg/day penicillamine gave some improvement but amputa- tions not prevented, skin more supple.
S. female 20/11/1928	mid 1970 skin tight (hands & face).	acrosclerosis; leukoderma pat- ches on hands & wrists; ASOT+ 500U; direct Coombs test+; slight dysphagia.	stable?	march 1971- january 1974	2x hypersensitivity reaction; symptom free on 50mg/day pe- nicillamine; skin much softer.
U. female 02/04/1930	1966 Raynaud.	acrosclerosis, fingers, hands, forearms, feet, lungs; subcu- taneous calcium deposits.	slow pro- gression	january 1971- may 1971	evaded follow-up of treatment.
V. female 25/11/1909	1933 Raynaud, 1937 PSS, 1960 rheumatic fever or RA.	fingers, hands, face, mouth, trunk, extremities, feet, eso- phagus, stomach, intestines, liver?, lungs, coup de sabre lesion on forehead, ANF+, LE- cells class VA, CRP+, Rose+ 1/64; RA cervical vertebrae, shoulders, elbows, hands, hips, knees, feet.	slow pro- gression	january 1971- january 1972	600mg/day penicillamine im- proved skin and possibly RA.

patient sex birth date	known as PSS since	affected areas & organs on entering study	disease course	period under study	conclusive remarks
W. male 22/07/1922	1944.	fingers, hands, face, mouth, neck, feet, malleolar ulcer, esophagus, stomach, intestines, liver, heart, lungs, ANF+, LE-cells class III, ASOT elevated, leukocytosis (11,100).	slow progression	august 1971- june 1972 (death)	initial improvement of finger function but progression not halted by 300mg/day penicillamine; cardiac death.
X. female 15/08/1915	1954.	fingers, hands, arms, face,mouth, lower legs, ANF++.	stable to slow progression	august 1972- january 1974	penicillamine up to 200mg/day repeatedly decreased the already low leukocyte count to leukopenia; further treatment by prednisolone; no clear positive effects.
Y. female 12/12/1948	1970 Raynaud, 1972 PSS.	fingers, feet, lungs, heart (AV block), cold agglutinins+.	very slow progression	january 1973- january 1975	300mg/day penicillamine did not influence Raynaud complaints or halt progression.
Z. male 12/12/1936	july 1971.	hands, forearms, lower legs, feet, liver, itch, stomach & duodenum?	rapid progression	december 1971- july 1975	some gastric intolerance to 700-1400mg/day penicillamine, combined with vasodilators & topical corticosteroids, resulting in clear regression of skin & liver signs , not of Raynaud phenomenon.
AA. female 29/12/1919	1962.	fingers, hands, arms, face, mouth, forehead, shoulders, lower legs; subcutaneous calcium deposits in hands, elbows, knees; myopathy; ANF weakly+.	stable to slow progression	january 1972- january 1975	hypersensitivity reaction to penicillamine on 3rd day; treatment by progestagen and then Madecassol inj., exercises; then 160mg/day penicillamine; no clear effects.
CC. female 17/02/1930	invalid since 1945? 1954 Raynaud.	fingers, hands, face, feet, toes, intestines, fatigue, syphilis serology+; ANF weakly+ to +; antibodies to smooth muscle; lungs fibrotic from Vim* inhalation.	stable	july 1972- january 1975	progestagen gave no clear effect; 300mg/day penicillamin stopped after 6 months because of thrombopenia, no clear effect; ANF doubtful.

* household polishing powder.

patient sex birth date	known as PSS since	affected areas & organs on entering study	disease course	period under study	conclusive remarks
DD. female 25/02/1920	1970.	fingers, lungs, intestines, stomach?	progression	july 1972	apparently no improvement on penicillamine, which was stopped after 3 months because of erythrocyturia.
EE. female 08/12/1923	1970.	fingers, hands, arms, face,mouth, forehead (1970); shoulders, neck, chest, back, abdomen, lower legs, itch (1972 spring); esophagus, lungs.	rapid progression	november 1972-january 1975	no clear evidence of effect on progression from up to 2800mg/day penicillamine over 20 months, partly by suppository; stopped because of leukopenia.
FF. female 29/03/1902	1959 RA, 1964 Raynaud, december 1972 PSS.	acro-osteolysis; fingers, hands, scleredematous; coup de sabre patch on forehead; toes, lungs, fatigue, ANF++, cryoglobulins+; cervical ribs; RA of hands, feet, shoulders, fingers, back. Gold for RA.	stable	november 1972-january 1975	under 200-300mg/day penicillamine scleredema improved, and possibly pulmonary function; ANF+, thyroid antibodies doubtful; IgA elevated, IgM slightly elevated; thrombopenia.
GG. male 16/12/1898 diabetic	mid 1971 swollen hands & feet.	fingers, hands, arms, face, forehead, legs; lung; esophagus; myopathy, ANF weakly+, cryoglobulin trace, thyroid antibodies weakly+, M paraprotein; CPK elevated; old myocardial infarction.	slow progression	january 1973 august 1974	walking ability improved, and finger flexion slightly, on 300mg/day penicillamine; lung function improved, myopathy disappeared; ANF doubtful, IgA & M increased.
KK. female 10/09/1013	end 1971 Raynaud, march 1973, PSS.	fingers, hands, arms, face, forehead, eyelids, shoulders, back, chest, abdomen, lower legs, toes, intestines?, lungs, fatigue, itch, myopathy (EMG), ANF weakly+; RA of hands, left shoulder; basalmembrane fluorescence; serum IgA slightly elevated, IgM slightly lowered.	slow progression	march 1973-january 1975	300-600mg/day penicillamine poorly tolerated in stomach, with taste loss, thus administration by suppository; resulted in ANF-;improvement in finger flexion, walking ability, and pulmonary compliance; myopathy cleared.

patient sex birth date	known as PSS since	affected areas & organs on entering study	disease course	period under study	conclusive remarks
LL. female 01/12/1927	1959 Raynaud, january 1973 PSS.	fingers, hands, face, neck, shoulders, esophagus, stomach, intestines; subcutaneous calcium deposits in fingers, hands, knees, left shoulder, elbows.	very slow progression	march 1973- january 1975	subjective improvement and less trouble with calcium deposits on 300mg/day penicillamine; esophagus possibly improved; weakly positive ANF and myopathy arose during treatment.
MM. male 15/03/1915	november 1972 initial complaint, september 1973 PSS.	fingers, hands, arms, shoulders, lower legs; trophic disturbances of hand palms & lower legs, decreased power of finger muscles & ankles; ANA doubtful; eosinophilia; ASOT 350U; IgA & IgG slightly elevated, IgM decreased; serum copper elevated; zinc in urine elevated.	progression	september 1973- january 1975	some initial improvement in finger flexion and arms on penicillamine, but legs did not respond even to high doses (300mg/day); ANA positive.

patient sex birth date	known as morphea since	affected areas	disease course	period under study	conclusive remarks
J. female 16/06/1946	1962.	coup de sabre like patch on frontal side of left thigh; lung.	stable	april 1970- september 1971	1600mg/day penicillamine influenced skin collagen ratio, lesion, lung function favorably.
O. female 07/12/1920 diabetic		several patches on breasts; LE-atrophy on hands.	slow progression	february 1971- january 1974	no clear effect from penicillamine 300-1200mg/day, still progression, ulceration may 1972.
P. female 10/03/1947	february 1970.	multiple patches on back, large patch on left thigh; lungs; kidneys?	slight progression	february 1971- january 1975	skin collagen, pigmentation of lesions, lung functions improved on penicillamine up to 400mg/day.
Q. female 21/02/1909 diabetic		one confluent area on breasts, abdomen, sternum.	slight progression	february 1971- january 1974	taste loss from penicillamine 100mg/day improving skin collagen, but no clear clinical improvement, nor from Madecassol ointment.
R. female 04/10/1943		small coup de sabre like area on left side of abdomen.	stable	april 1971- january 1972	300mg/day penicillamine led softening but no complete cure.
T. female 26/05/1913	may 1970.	chest, shoulders, abdomen, upper arms, back, microstomia, lungs, kidney?, liver?, itch, ANF very weakly+, mitochondrial antibodies weakly+; hot nodule hyperthyroidism.	progressive	july 1970- may 1971	penicillamine up to 900mg/day resulted in less tight skin and improved collagen ratio.
BB. female 05/08/1956	1966.	right arm & shoulder, back, 'dead' fingers, leukocytosis (10,000).	stable to slight progression	january 1972- january 1974	200mg/day penicillamine resulted in less pigmentation & increased suppleness of affected areas.
HH. male 04/06/1946	mid 1971.	patches on abdomen & forearms, later left loin; coup de sabre lesion on right loin.	slow progression	july 1972- february 1974	300mg/day penicillamine resulted in softer & whiter lesions.
II. male 08/02/1899	begin 1973.	interscapular lichen sclerosis; diverticulosis of duodenum & jejenum; myocardial infarction 1972; BSE elevated; slight increase of IgA, IgM, IgG.	stable	january 1973- october 1974 (death)	questionable improvement on 300mg/day penicillamine; ANF doubtful; cardiac death.
JJ. male 20/02/1940	april 1972.	patches on arms, legs, trunk, 'dead' fingers, cardiac conduction disturbances; esophagus?	progression	february 1973 january 1975	300mg/day penicillamine led to disappearance of patches by march 1974; recurrence in 1975.

APPENDIX B

ALL DATA COLLECTED ON HYDROXYPROLINE CONTENT OF URINE AND SERUM IN PATIENTS INVOLVED IN THE STUDY.

Appendix B

Table Ba. Hydroxyproline content of urine and serum in relation to penicillamine administration.
(creatinine as gram/Liter placed between parentheses).

date	ml/24 hr urine	mcg/ml urine hydroxyproline	mg/24 hr urine hydroxyproline	mcg/ml serum hydroxyproline	penicillamine daily dose
Patient A					
14/02/1969	1050	11	11.6	3.3	started in increasing doses
21/02/1969	800	12	9.6	4.2	
28/02/1969	920	13	12.0	4.1	1400 mg
07/03/1969	850	12	10.2	4.4	
04/03/1969	700	14	9.8	4.0	
21/03/1969	1750	13	22.8	4.1	
02/04/1969	1600	14	22.4	3.9	
25/04/1969				9.2	
11/04/1969	920	15	13.8		
09/05/1969	700	15	11.4		
22/05/1969	810	40	32.4	3.2	
06/06/1969	1000	36	36.0	2.7	
19/06/1969	920	34	31.3	2.6	
11/07/1969	840	20	16.8	1.8	
11/07/1969	820 (0.25)	9	7.4	4.8	
15/08/1969	460 (0.15)	21	9.7	2.8	
16/10/1969	600 (1.77)	34	20.5	2.8	
21/11/1969	740	39.6	29.3		
14/12/1969				5.1	
19/12/1969	990	33.4	33.0		
23/01/1970	770	28.7	22.1		
06/03/1970	1140	29.6	33.7	10.1	
24/04/1970	570	53.6	30.5		
26/06/1970	800	34.6	27.6	10.3	
28/08/1970	570	48.8	27.8	9.5	
24/09/1970				10.3	
08/10/1970	500	43.3	22.6	10.3	
26/11/1970	880	40.5	35.6		1400 mg
Patient F					
28/09/1969	600 (1.02)	10.0	6.0		
29/10/1969				4.7	
05/11/1969	1220 (0.89)	23	28.0		
21/11/1969	1720 (0.58)	10	17.2		
19/12/1969				2.5	
Patient E					
28/09/1969	860 (1.30)	24	20.6		
15/10/1969	960 (0.86)	19	18.2		
05/11/1969	1680 (1.00)	22	37.0		
21/11/1969	1470 (0.78)	19	27.9	2.5	600 mg
05/12/1969	900 (0.78)	17	15.3	3.1	
19/12/1969				3.4	600 mg

penicillamine

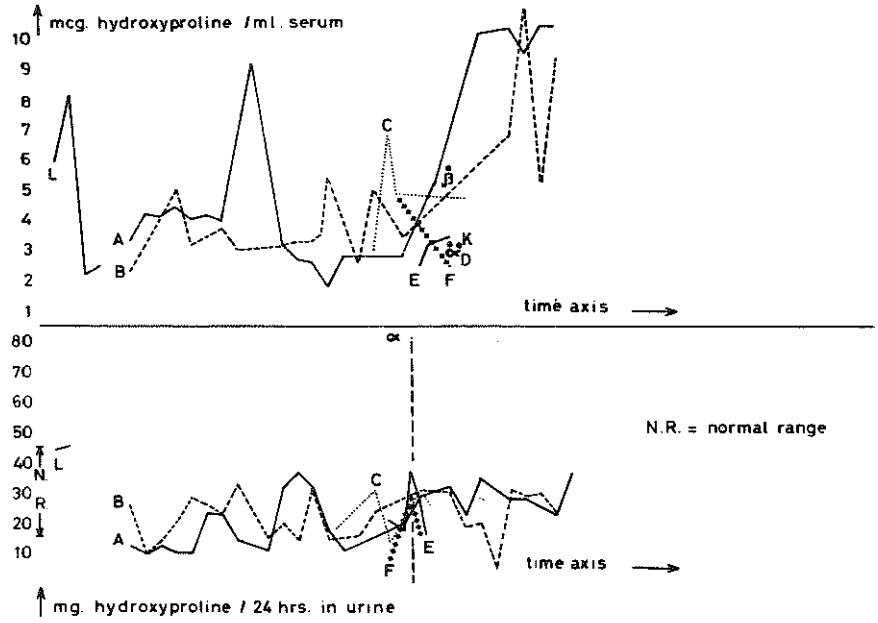
Table Bb. Hydroxyproline content of urine and serum in relation to penicillamine administration.
(creatinine as g/L placed between parentheses).

	date	ml/24 hr urine	mcg/ml urine hydroxyproline	mg/24 hr urine hydroxyproline	mcg/ml serum hydroxyproline	penicillamine daily dose
	Patient B					
	14/02/1969	2000	13	26.0	2.3	started in
↑	21/02/1969	850	12	10.2	3.2	increasing
↓	28/02/1969	1080	13	14.0	4.1	doses
						1500 mg
	07/03/1969	1430	14	20.0	5.0	stop
	14/03/1969	1640	17	27.9	3.1	
	21/03/1969	1600	16	25.6	3.4	
↕	02/04/1969	1500	15	22.5	3.7	250 mg
						stop
	11/04/1969	2190	15	33.0	3.0	
↑	25/04/1969	1720	14	24.0		100 mg
	09/05/1969	1040	14	14.6		200 mg
	22/05/1969	1640	13	21.4	3.2	300 mg
	06/06/1969	1620	9	14.6	3.3	
p	19/06/1969	1980	16	31.7	3.3	
e	25/06/1969				3.5	
n	11/07/1969	850	17	14.5	5.4	
i	22/08/1969	880 (0.56)	18	15.8	2.6	
c	12/09/1969	1580 (0.71)	15	23.8	5.0	
i	26/09/1969				4.1	
l	16/10/1969				3.4	
l	21/11/1969	1840 (0.57)	17	31.3		
a	19/12/1969	1900	16.5	31.4		
m	23/01/1970	730	26.3	19.2		
i	06/03/1970	590	33.8	19.9	5.9	
n	24/04/1970	1520	3.3	5.0		
e	26/06/1969	2310	13.6	31.4	6.8	
	28/08/1970	1630	17.8	29.0	10.7	
	24/09/1970	1900	16.0	30.4	5.3	
↓	08/10/1970	1630	14.8	24.2	9.3	300 mg
	Patient L					
↑	23/10/1968	2000	22	44	5.9	520 mg
↓	06/11/1968	1650	27.2	45	8.1	1560 mg
						stop
	04/12/1968				2.2	
	09/12/1968				2.5	
	Patient G					
	06/08/1969	1030 (0.31)	18	18.5		
	12/09/1969	1420 (0.32)	22	31.2	3.0	
	26/09/1969	1000 (0.35)	14	14.0	6.8	started
↕	10/10/1969	1020 (0.40)	17	17.3	3.8	to 1600 mg
						stop
	05/11/1969	1790 (0.52)	14	25.1		
↑	21/11/1969	1610 (0.51)	19	30.6		1200 mg
↓	05/12/1969	1680 (0.40)	16	26.9		
						stop
	19/12/1969				4.7	

date	ml/24 hr urine	mcg/ml urine hydroxyproline	mg/24 hr urine hydroxyproline	mcg/ml serum hydroxyproline	penicillamine daily dose
Patient alpha					
30/10/1969	2940 (0.48)	30	78.2		
06/11/1969	1000 (0.46)	24	24.0		
19/12/1969				3.2	

Further data collected:

19/12/1969	3.0 mcg/ml serum	pat. D
19/12/1969	5.5 mcg/ml serum	pat. G
29/12/1969	3.1 mcg/ml serum	pat. K
19/12/1969	5.7 mcg/ml serum	pat. beta



Graph correlating hydroxyproline determination results of Tables Ba. and Bb. relative to time of sample collection and to patient.