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# Epidermolysis bullosa in Finland

## Clinical features, morphology and relation to collagen metabolism

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*To Mikko, Milka and Ville*

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II Niemi K-M, Kero M, Kanerva L and Mattila R. Epidermolysis bullosa simplex. A new histologic subgroup. Arch Dermatol; 1983; 119: 138–141.

III Kero M, Niemi K-M, Kanerva L. Pregnancy as a trigger of epidermolysis bullosa acquisita. Acta Derm Venereol (Stockh) 1983; 63: 353–356.

IV Kero M, Peltonen L, Foidart JM, Savolainen E-R. Immunohistological localization of three basement membrane components in various forms of epidermolysis bullosa. J Cutan Pathol 1982; 9: 316–328.

V Kero M, Palotie A, Peltonen L. Collagen metabolism in two rare forms of epidermolysis bullosa. Br J Dermatol, in press.

VI Savolainen E-R, Kero M, Pihlajaniemi T, Kivirikko KI. Deficiency of galactosylhydroxylysyl glucosyltransferase, an enzyme of collagen synthesis, in a family with dominant epidermolysis bullosa simplex. N Engl J Med 1981; 304: 197–204.

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## Abstract

The inheritance and occurrence of various subtypes of epidermolysis bullosa (EB), the clinical and ultrastructural features of the disease (I) and its connections with possible defects in collagen metabolism were studied in the population of Finland.

Information was obtained on 40 families with 121 diseased members alive. Genealogical analysis of these revealed 55 additional cases of probable EB. The series probably includes all the subjects affected by the recessively inherited types living in Finland in 1971–1980. Subjects with dominant types often have such a mild disease that they cannot all be included in a retrospective study. Eleven of the subtypes of epidermolysis bullosa were represented, and two families were found not to belong to any previous subgroup and were regarded as so far unclassified.

The largest morphological group among the intraepidermal types, with seventeen families altogether, represented the pattern of cytolysis, acantholysis and dyskeratosis corresponding to EB simplex Köbner and Weber-Cockayne. In six families tonofilament clumping was the main morphological finding and the clinical picture in these cases was EB herpetiformis Dowling-Meara (I, II). Tonofilament deficiency, found in one family with congenital skin defects, was classified as EB Bart, and in one family a deficiency in one enzyme of collagen synthesis, galactosylhydroxylysine glucosyltransferase activity in skin and serum, was found to correlate significantly with severity of EB simplex Köbner (VI). In three other EB simplex families no such deficiency could be shown, suggesting that it may be of aetiological significance in some but not all EB simplex cases.

In the junctional group three families with six dead infants were classified as EB atrophicans gravis-Herlitz and one live member with a hemidesmosome defect as EB atrophicans mitis. There were also two unclassified cases with junctional splitting and normal hemidesmosomes. This suggests greater heterogeneity in this group than has hitherto been thought.

Among the dermal forms of EB there were eight families with dominant EB dystrophica Cockayne-Touraine and Pasini with 55 patients altogether. In one family both of these clinical expressions were found, suggesting a common gene source. Two families represented recessive EB dystrophica and one also contained a patient with acquired EB (III). In this last case the disease broke out in connection with parturition.

Active collagenase and compensatory collagen production were increased in the cases of recessive EB dystrophica and in that of recessive EB atrophicans mitis (V). One of the recessive EB dystrophica cases was treated with an inhibitor of collagenase production, phenytoin, with a favourable response.

An immunohistochemical staining method with antibodies against basement membrane components was used successfully to show the levels of the blisters (IV). Moreover, the lamina lucida in EB herpetiformis Dowling-Meara, especially the proteoglycan component, showed a surprising looseness in structure.

The results show that most of the EB types exist in Finland, and the recording of the occurrence of the rare subtypes furnishes essential information for the grouping of these heterogeneous diseases. The structural and biochemical alterations found in these mechanobullous diseases are also analysed.

*Key words: mechanobullous diseases, histology of EB, collagen metabolism.*

# Abbreviations

| | |
|---|---|
| EB | epidermolysis bullosa |
| D-EB | dominant epidermolysis bullosa |
| R-EB | recessive epidermolysis bullosa |
| A-EB | acquired epidermolysis bullosa |
| EBS | epidermolysis bullosa simplex |
| EBH | epidermolysis bullosa herpetiformis |
| EBA | epidermolysis bullosa atrophicans |
| EBD | epidermolysis bullosa dystrophica |
| FEB | Finnish epidermolysis bullosa |
| GGT | galactosylhydroxylysyl glucosyltransferase |

# Contents

## Introduction

The term epidermolysis bullosa (EB) was introduced by Köbner (1) almost ninety years ago. Today it applies to a heterogeneous cluster of diseases, the common feature of which is blistering or separation of the skin after minimal trauma. At least nine genetically separate diseases are known to exist (2) and morphologically EB comprises seventeen types, sixteen of which are inherited and one acquired (3).

The blisters may be formed intraepidermally, junctionally or intradermally. Figure 1 presents a scheme for the seventeen types of EB. This classification is based mainly on microscopic findings and may not be the final one, because new diagnostic methods, especially those capable of revealing the structural and biochemical alterations in the skin, will continue to provide new data, but it does form a good basis for both epidemiological and morphological investigations into mechanobullous diseases. The study of genodermatoses is nowadays on the point of stepping from the era of morphology into that of biochemistry and recombinant DNA techniques. The recent progress achieved in the study of the biochemistry of basement membranes has also given impetus to research into EB.

Apart from certain case reports, there are no studies on EB inheritance in Finland. The present study was started four years ago, with the purpose of investigating the occurrence of various subtypes and also the morphological and metabolic alterations taking place in cases of EB.

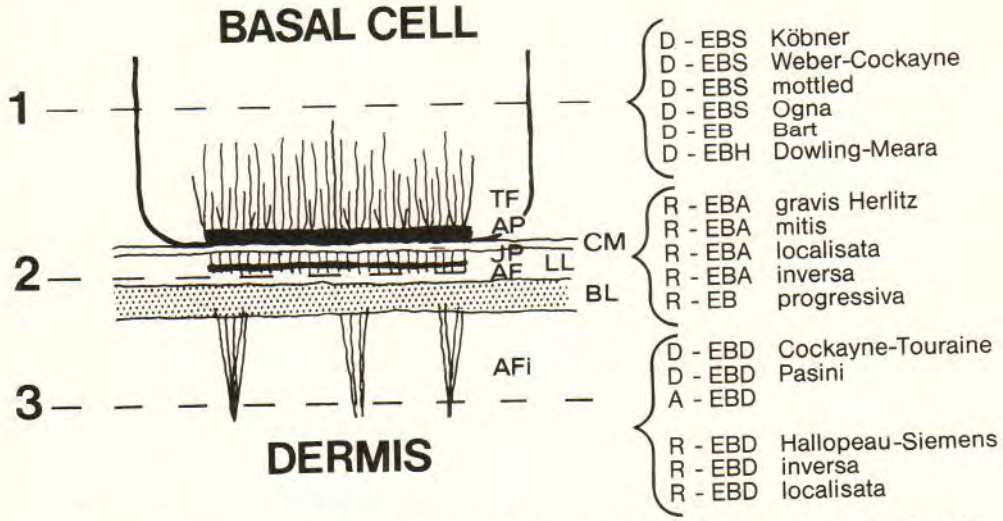



*Fig. 1. Structural components of the basement membrane zone. TF = tonofilaments, CM = membrane of basal cell, LL = lamina lucida (lamina rara), BL = basal lamina (lamina densa), AF = anchoring filament, AFi = anchoring fibril, AP = attachment plate, JP = junction plate.*

*The three broken lines symbolize the splitting levels:*

*1. intraepidermal 2. junctional 3. dermal.*

*D = dominant, R = recessive, A = acquired, EBS = epidermolysis bullosa simplex, EBH = epidermolysis bullosa herpetiformis, EBA = epidermolysis bullosa atrophicans, EBD = epidermolysis bullosa dystrophica.*

# Review of the literature

## Morphology of the epidermal-dermal junction

Our increased knowledge of the ultrastructure of the epidermal-dermal junction and its biochemical composition has been significant for progress in understanding the pathomechanism of different forms of EB (4, 5, 6, 7).

The narrow epidermis-dermis line observed earlier by light microscopy has broadened under the electron microscope into a complicated structure which can be divided into four zones (Fig. 1):1. the basal cell plasma membrane, 2. the lamina lucida, 3. the basal lamina, and 4. the sub-basal lamina with fibrous elements. The plasma membrane of basal keratinocytes is seen as a 7–9 nm trilaminar structure with regular electron-dense thickenings called hemidesmosomes. These consist of an electron-dense 20–40 nm thick attachment plaque upon which the tonofilaments converge. Hemidesmosomes are important for the classification of certain recently recognized subtypes of EB.

The lamina lucida (lamina rara) is an amorphous electron-translucent zone of thickness 20–40 nm between the basal cells and the basal lamina. The anchoring filaments initiating from the outer leaflet of the plasma membrane traverse the lamina lucida and mesh with the basal lamina. The basal lamina (lamina densa) is an electron-dense zone of 30–50 nm linking the epidermal cells with the dermal structures. The fibrous elements beneath the basal lamina, anchoring fibrils, collagen fibres and dermal microfibril bundles constitute an interlinking meshwork from the basal lamina to the dermis.

## Connective tissue components

### *Collagen structure*

The connective tissue matrix consists of fibrillar structures, mainly collagen, but also elastine and an amorphous ground substance (8, 9, 10). The collagen fibrils are formed by an ordered aggregation of collagen molecules. The molecules are formed of three polypeptide alpha-chains, which are coiled around one another into helical rod-like structures, measuring 280 x 1,4 nm, with a molecular weight of 300 000 daltons. The characteristic features of collagen amino acid sequences include glycine in every third position and the presence of hydroxyproline and hydroxylysine. Galactose and glucosyl-galactose are linked to the hydroxylysine residues.

Many structurally and genetically distinct collagen types have been characterized in different tissues. All of these possess the general structure of collagen, having the typical amino acid sequence and being susceptible to collagenase digestion, but they differ in their precise amino acid sequence and in the extent of posttranslational modifications.

The different collagen types may be grouped into:

- interstitial collagens, types I, II and III
- basement membrane collagen, type IV

– other collagens, type V and several types not yet fully characterized

All three interstitial collagen types form electron-microscopically distinct fibres. The major type of skin collagen, accounting for 70 % of the collagen in human skin, is type I, but there is also some type III collagen, accounting for 10–20 %, which is responsible for the thin fibres, often seen as a reticular network. Collagen type II is mainly found as a component of cartilage.

Collagen type IV forms a three-dimensional network in the lamina densa and is responsible for the rigidity and a certain flexibility of the basement membranes (11, 12, 13, 14, 15). This tertiary structure is composed of four type IV collagen molecules which are linked together at one end by disulphide bonds and at the other by an extremely strong structural domain termed the 7-S fragment. Type IV collagen forms triple helical molecules like the other collagens, but the amount of glycine is lower due to frequent interruptions of the collagenous amino acid sequence and the carboxyhydrate content is 20-fold higher than in the interstitial collagen types. The function of type IV collagens in the skin is to serve as a filtration barrier and as an attachment matrix for the epidermal cells.

Collagen type V is thought to be closely associated with basement membranes, but recent studies have suggested that it may also be found in the epidermis (16).

### *Biosynthesis and degradation of collagen*

A number of post-translational biochemical processes are needed to modify collagen molecules before they are able to aggregate into stable fibrils. These post-translational steps in collagen synthesis and their biological importance are listed briefly in Table I (8). The following enzymes have been shown to participate in the synthesis of the final collagen fibril (8, 17):

– two prolyl hydroxylases
– lysyl hydroxylase
– galactosyltransferase
– glucosyltransferase
  = galactosylhydroxylysyl glucosyltransferase (VI)
– procollagen aminoprotease
– procollagen carboxyprotease
– lysyl oxidase

Inborn errors at the level of post-translational regulation have been known to lead to genodermatoses, e.g. some types of the Ehlers-Danlos syndrome (18–26), of which type VI is known to be due to a deficiency in lysyl hydroxylase, type VII results from a lack of procollagen aminoprotease and type IX and the Menkes syndrome are related to a decrease in lysyl oxidase activity.

*Table I. Intracellular and extracellular steps in collagen biosynthesis.*

| Process | Biological importance |
|---|---|
| Transcription and translation | primary structure |
| Intracellular modifications | |
| Removal of signal sequences of preprotein | uncertain |
| Hydroxylation of proline residues | essential for triple helix |
| Hydroxylation of lysine residues | sites for glycosylation<br>essential for stable cross-links |
| Glycosylation of hydroxylysine residues | correct fibril assembly and stability |
| Glycosylation of propeptides | uncertain |
| Chain association and disulphide bonding | essential for triple helix formation |
| Triple helix formation | essential for rapid secretion and fibril formation |
| Translocation and secretion of procollagen | transport |
| Extracellular modifications | |
| Removal of procollagen propeptides | necessary for normal fibril formation |
| Ordered aggregation of collagen molecules | normal structure |
| Cross-link formation | tensile strength of fibrils |

The enzyme principally responsible for collagen degradation is collagenase. Collagenase appears in tissues and tissue fluids in at least three molecular forms: a latent inactive enzyme, a free active enzyme and a collagen-bound form. The process of enzyme activation in vivo is an unsolved problem. The active form cleaves collagen molecules into two fragments at a specific locus, causing denaturation of the triple helix. The fragments are then broken up by unspecific hydrolytic and proteolytic enzymes (27). Specific collagenases have recently been identified for collagen types IV and V (28, 29, 30, 31). In addition, gelatinase, an enzyme splitting denatured collagen, has been purified from human skin tissue culture medium (32). The urinary excretion rates of hydroxyproline, hydroxylysine and associated glycosides have been used as indicators of collagen breakdown in vivo (33, 34).

### *Other components*

L a m i n i n is the main non-collagenous glycoprotein in basement membranes, being located in the lamina lucida (35). The cross-like tertiary structure of laminin is formed of one long and three short arms. Its function remains partly an open question, but in view of its location it is thought to be a specific attachment protein operating between the epithelial cells and collagen type IV (36, 37).

P r o t e o g l y c a n is a gigantic molecule aggregate with a 300 nm long core protein to which the glycosaminoglycans such as keratin sulphate, chondroitin sulphate,

dermatan sulphate and heparan sulphate are attached (38, 39). Heparan sulphate is a predominant component of glycosaminoglycans in basement membranes, being situated in the lamina lucida. These proteoglycan aggregates are responsible for the viscous and elastic properties of connective tissue. They obviously have a role in stabilizing the cell-fibronectin-collagen connection and play an essential part in regulating the permeability of the basement membranes.

Bullous pemphigoid antigen consists of two glycoproteins with molecular weights of 20000 and 92000 daltons. It reacts with bullous pemphigoid antibody in the lamina lucida immediately beneath the basal cell plasma membrane (4, 40). It probably regulates the production of other basement membrane zone components and maintains the proliferation of the basal cells while preventing their terminal differentiation.

Fibronectin is a disulphide-linked dimer consisting of two glycosylated polypeptide chains (41). It binds to collagen and mediates cell attachment through this fibronectin-collagen complex (42, 43). No studies have been found on its significance for mechanobullous diseases.

Basement membrane also contains elastic fibres (44), which are responsible, at least in part, for the elasticity of the skin, and a recently discovered glycoprotein, entactin (45), the exact function of which is still unclear at present.

## Experimental blister formation studies on the skin

Unlike immunological blistering diseases, the inborn errors leading to bullous skin disease are thought to be caused either by mutation in structural proteins or by destructive enzymes in situ. In addition to mechanical friction, the blisters may be caused experimentally by suction (46, 47). In vitro experiments suggest that the proteolytic enzymes may play a role in inducing splitting of the skin (48). Trypsin has been routinely used to separate the epidermis from dermis for tissue culture purposes (49). Human skin chymotrypsin-like protein in a physiological concentration in vitro causes a split of the skin into the epidermis and dermis at the lamina lucida level (50), while elastase has been demonstrated to cleave the skin at the basement membrane level and produce a destruction of the lamina densa (51). In addition, recent studies demonstrate that pemphigus IgG induces the synthesis and secretion of a plasminogen activator, which activates plasminogen to plasmin. This plasmin then degrades the surface proteins and causes the acantholytic separation of keratinocytes (52).

## Historical and clinical review of the seventeen types of EB

Von Hebra (53), in 1870, was the first to draw attention to a hereditary traumatic blistering disease called "Erblichen pemphigus", and many reports of similar symptoms of the skin were presented in the following decades (54, 55, 56). The scarring forms described by Hertzfeld (57) and Hallopeau (58) and the non-scarring form presented by Köbner (1) already provided a hint of the great clinical variety of the disease.

*Intraepidermal forms*

The non-scarring intraepidermal form of EB is nowadays divided into six subtypes: EBS Köbner, EBS Weber-Cockayne, EBS mottled, EBS Ogna, EBS Bart and EBS Dowling-Meara (Fig 1).

Siemens (59, 60) demonstrated an association between the non-scarring form of EB and dominant inheritance in 1922, and local variants of non-scarring EB were presented in reports by Weber in 1926 (61) and Cockayne in 1938 (62) under the description "recurrent bullous eruptions of the feet". In 1957 Wesener (63) proposed a morphological division of EB simplex (EBS) into a local form on the hands and feet known as EBS Weber-Cockayne and a general form known as EBS Köbner.

Friction and heat are precipitating factors for both types of EBS. In the generalized form the blisters appear soon after birth on areas typically predisposed to friction such as the palms, soles, axillae and waist but in EBS Weber-Cockayne the blistering may begin at any time up to school age (64, 65). Erosions of the mucous membranes are rare. The symptoms are aggravated during summer time, the patient often becoming incapacitated (64, 65, 66, 67). The nails grow normally in both EBS Köbner and EBS Weber-Cockayne, but they may be destroyed secondarily as a consequence of blistering of the nailbeds (66). Hyperhidrosis of the hands and feet is a frequent symptom, especially in EBS Weber-Cockayne (65, 68).

EBS Weber-Cockayne and Köbner are evidently manifestations of the same gene defects (2, 64). Light and electron microscopy both show disintegration of the basal cells (69, 70, 71, 72), although the cell organelles remain largely intact. Haneke and Anton-Lamprecht (72) have shown that the friction-induced blister in D-EBS Weber-Cockayne always develops in the basal layer, while spontaneous blisters are found at a higher level in the epidermis. The cause of blister formation is unknown. At first Pearson presented a hypothesis of activation of cytolytic enzymes. On the other hand Gedde-Dahl (64) proposes that the polypeptide coded for by the mutated gene in each instance may involve an amino acid substitution which leads to an abnormal conformation of the protein at a critical temperature level in the skin. This results in disturbed functioning of the protein. Recently Takamori et al demonstrated the blister fluid from patient with EBS to contain some specific factor to be able to induce an intraepidermal blister (74). Decreased gelatinolytic protease activity in fibroblast cultures has been described as a phenotypic marker for one type of dominant EBS in a preliminary report (75).

EBS Ogna is a type described from Norway (76). The patients show a tendency for skin bruising, with the post-traumatic haemorrhagic blisters favouring the acral parts of the extremities. The genetic linkage to GPT (glutamic-pyruvic transaminase) polymorphism justifies its classification as a separate subgroup of EB (2, 77).

Blistering of a dirty-looking skin with hypopigmented and hyperpigmented spots and longitudinally curved nails are typical characteristics of EBS mottled (78). Electron microscopy suggests that the blister may result from cytolysis in hyperpigmented basal cells with lipid vacuolization (79).

The main diagnostic criteria for EB Bart are congenital ulcerations of the skin, usually on the extremities, and erosion of the mucous membranes. The lesions tend to show spontaneous improvement with age. This syndrome was first reported by Bart (80, 81) under the heading "EB and congenital localized absence of the skin". This latter symptom has also been described in connection with other types of EB and therefore the specific verification of EB Bart is inadequate (82). Its status as a separate entity of EB

seems to be dependent upon further ultrastructural investigations using an electron microscope (3).

D-EBH Dowling-Meara was first pointed out in 1954 (83), but was then forgotten for some time. In the late 1970's it was rediscovered because of its typical clinical features (2, 3, 84) and specific electron microscope findings with cytolysis resulting from clumping of the tonofilaments in the basal cells (84, 85).

*Dermal forms*

Dominant EB dystrophica: The diagnostic clinical features of D-EBD were established some fifty years ago (86, 87), although according to the review of Touraine in 1942 (88) terminological instability existed between the French and Anglo-Saxon usage until D-EBD Cockayne-Touraine was uniformily accepted in the 1960's (89, 90). In localized D-EBD Cockayne-Touraine blisters and scars with miliae are seen only on the flexor aspects of the extremities, the sites of preference being the dorsa of the hands and feet and also the forearms, legs, knees and elbows.

Pasini (91) and Maschkilleisson (92) had both drawn attention in 1928 to a dominant type of EB with atrophic scars and flesh-coloured papules affecting larger areas of skin than that described by Cockayne and Touraine. This has later come to be regarded as a subtype of the dominant dystrophic forms and is known as D-EBD Pasini (albopapuloidea) (89, 90).

About a fifth of the patients have erosion of the mucous membranes and some have enamel defects on the teeth and a tendency for dystrophic nails. The symtoms may vary greatly from infancy to adolescence, however (3, 64, 65, 66, 93, 94).

The cleavage in these subtypes takes place beneath the basement membrane, and there is a 40 % decrease in the number of anchoring fibrils (95), leading to a weakened fibril network between the basement membrane and the structures of the upper dermis. In generalized dominant EB dystrophica Pasini this defect of the anchoring fibrils can also be shown by electron microscopy in apparently normal skin (96).

There are some studies which demonstrate that a superfluous tissue accumulation of mucopolysaccharides and glycoproteins may alter collagen fibril deposition and impair the firmness of the skin in cases of D-EBD (97, 98, 99, 100).

Recessive EB dystrophica: The recessively inherited forms of EB have long been an object of some confusion. Some twenty years after the first case reports of Hallopeau (58), Siemens (59) found that the blisters were formed subepidermally. R-EBD is now divided into three subtypes on morphological grounds: mutilating or generalized R-EBD Hallopeau-Siemens, R-EBD inversa, located on the bend regions, especially those of the lower body, and R-EBD localisata, found only in restricted areas (3, 76, 101). Gedde-Dahl considers the generalized and localized subtypes to be quantitative rather than qualitative variations of the same gene mutation, while R-EBD inversa is probably the expression of a mutation in a different gene locus (64).

Large scarring blisters appear shortly after the birth, most commonly on the hands, feet, elbows, knees and face. In the most severe cases the fingers and toes may be fused into bags and the nails may be completely destroyed (76). The lesions on the skin heal with scars and miliae, causing contractions, and strictures are formed on the mucous

membranes. The scars are predisposition sites for both basal and squamous cell carcinomas (102, 103). The teeth are usually destroyed early by severe caries, and the hair may be sparse. The patients are frequently anaemic and small in size, and in the most severe cases the disease will shorten the life span (64, 65, 66).

Electron microscopy has revealed lytic changes in collagen to be responsible for the splitting of the skin in R-EBD (70), and later the anchoring fibrils were found to be diminished in number or even lacking (7, 104). R-EBD has no association with HLA antigens (105), but there are contradictory reports on the occurrence of carcinoembryonic antigens in R-EBD patients (106, 107).

The next step forward in the study of the pathogenesis of R-EBD was achieved with the detection of a significant increase in the amount of immunoreactive collagenase, which would cause an excessive destruction of the collagen fibrils (108, 109, 110, 111, 112). Subsequent studies showed a structurally altered form of collagenase in skin fibroblast cultures (113) and an increased concentration of the enzyme caused by enhanced translation of collagenase messenger RNA (114). Bauer (115) proposed that increased synthesis, reduced thermal stability and diminished affinity for calcium ions could serve as in vitro markers of R-EBD. These findings have not yet been confirmed by other groups, however.

Acquired EB dystrophica: The clinical features of A-EBD are very similar to those of D-EBD Cockayne-Touraine, first published by Kablitz in 1904 (116), and there was some confusion in diagnosing this disease in subsequent years (76). Although Siemens (60) proposed the name "Bullosis mechanica tarda", and Gedde-Dahl (76) "EB dystrophica idiopathica tarda" the term "acquired" has been generally accepted. In spite of its clinical similarity to D-EBD Cockayne-Touraine (3, 117), recent immunofluorescence findings (118, 119, 120, 121) have confirmed that it is an independent variant of EB. Its histology shows destruction of the anchoring fibrils (122), and it has some association with systemic diseases such as diabetes mellitus, Crohn's disease, multiple myeloma, systemic lupus erythematosus and lymphoma and also with the use of drugs such as penicillamine and sulfonamine (64, 65, 123, 124, 125).

The diagnostic criteria for A-EBD (126) are: 1. bullae induced by trauma and leaving atrophic scars similar to those found in D-EBD Cockayne-Touraine, 2. adult onset, 3. negative family history, 4. exclusion of other bullous diseases, 5. the finding of IgG in the basement membrane zone by direct immunofluorescence microscopy, 6. demonstration of blister formation beneath the basal lamina, with a zone of amorphous material, 7. deposition of IgG in the amorphous zone beneath the basal lamina demonstrable by immunoelectron microscopy.

In addition to IgG, complement and in some cases IgA, IgM and fibrin have been found below the basal lamina (118, 119, 120). The amorphous substances to which the IgG antibodies bind may be secreted by the basal cells (119). Speculations that antianchoring fibril antibodies may cause the destruction of fibrillar structures could well be correct, suggesting that A-EBD may be an autoimmune skin disease (126).

*Junctional forms*

In 1935 Herlitz (127) described a lethal type of EB in which the blisters healed without prominent scars but left permanent atrophy without miliae. There arose much confusion

in subsequent years, however, because this lethal disease was considered to be a more difficult form of R-EBD Hallopeau-Siemens. Later the studies of Pearson (128) showed the splitting to take place between the plasma membrane of the basal cells and the basement membrane, and the dissolution of the basal cells to be secondary to the action of lytic enzymes (70, 129).

Hypoplastic hemidesmosomes with a numerical reduction in anchoring filaments have been regarded as typical ultrastructural hallmarks of lethal junctional EB (130, 131), and Gedde-Dahl (64) proposes that the Herlitz-Pearson gene locus may code either for an enzyme necessary for the maturation of hemidesmosomes or for structural components of types with normal hemidesmosomes. Recent reports (132, 133) have shown junctional types which have normal hemidesmosomes.

Typical clinical features are nail deformities and anaemia, together with hoarseness and dysphagia due to injuries of the mucous membranes (3, 64, 97, 129). In a report by Roberts (134), only three out of 54 junctional EB cases reached the age of one year, but additional non-lethal cases of EBA have been presented later (131, 135, 136).

The biochemical background to EBA is unknown. Increased collagenase activity has been observed in vivo (137), but not in vitro. Until we know more, the subgrouping of EBA must be based on the morphology of the lesions, and the following types are thus regarded as disease entities: 1. lethal R-EBA gravis Herlitz, 2. non-lethal R-EBA mitis (131, 136), 3. R-EBA inversa (3, 138), and 4. R-EBA localisata (3, 76, 94, 135).

In addition, R-EB progressiva, initially called neurotrophica (76), forms an entity of its own among the junctional EB-types with normal hemidesmosomes. Gedde-Dahl has described R-EB progressiva linked to hypoacusis (76) in Norway and Sweden.

Linkage studies have shown associations between EBA and gastrointestinal anomalies such as atresia pylori and volvulus intestini (139, 140, 141, 142, 143). A linkage between junctional EB and muscle dystrophy was found in one Dutch family (144) and Gedde-Dahl later proposed that the diagnosis should be R-EBA inversa (3).

## Treatment and prevention of EB

Since 1969 vitamin E, an antioxidant, has been used as a treatment for different forms of EB (145) both with success (146, 147, 148) and without any marked effect (149). There are also interesting reports of good responses to gold in cases of A-EBD (150) and of the beneficial effect of seaside baths in a hot climate upon D-EBH Dowling-Meara (2, 3). Corticosteroids have been used in emergencies and also to prevent strictures, but whether or not they improve the prognosis is uncertain (141, 152).

The increased collagenase activity noted in R-EBD suggests the use of inhibitors of collagenase production (153). In the first experiments about a half of the patients experienced some benefit from the use of phenytoin (154). Preliminary reports have been published on the use of retinoids to regulate collagenase activity (155), but no patient studies have been forthcoming so far.

An interruption of pregnancy is indicated on eugenic grounds in the most severe R-EBD and R-EBA gravis Herlitz cases (156). The introduction of foetoscopy and foetal skin biopsy techniques has made prenatal diagnosis possible after only 16–21 weeks of gestation (157,158, 159, 160). A reduction in the number of hemidesmosomes to a half of the normal has been described in a foetus with R-EBA (159), and prenatal diagnosis of EBS-Ogna via foetoscopy and blood GPT typing is also possible (2).

## Outlines of the present study

The purpose was to gather material comprising as many cases of EB as possible in order to gain an impression of the occurrence of the various types in Finland (I) and to enable specialized investigations to be carried out (IV, V, VI).

A number of heritable diseases with skin affections are known to result from inborn errors in collagen metabolism, one of the best examples being the varieties of the Ehlers-Danlos syndrome. The biochemical errors in EB subtypes are not so well known, but the known connection between R-EBD and increased collagenase activity is a finding which has led to a search for connections with impaired collagen metabolism in other subgroups of EB.

The particular problems approached in the present work may be summarized as follows:

1. to map out the occurrence of the various types of EB in Finland (I)
2. to study biochemically the metabolism of collagen in selected cases of EB (V, VI)
3. to investigate the use of immunofluorescence of basement membrane components as a diagnostic tool (IV)
4. to determine the special clinical and ultrastructural features required for exact subtyping (II, III)

## Material and methods

### Patients (I)

A search for probands of EB families was made on the basis of the files of thirteen dermatological units and five university paediatric clinics in Finland during the ten-year period 1971–1980. Eight Finnish central hospitals had no dermatology unit of their own at that time, however, so that families from their districts were reached via the registers of the university skin clinics. The author investigated all the living probands personally during the years 1980–1981, paying special attention to the location and appearance of blisters, scars, miliae and pigmentations, the age of onset and changes in the nails, teeth, hair and eyes. Inquiries were also made concerning the occurrence of the disease in the patients'families. The results of the clinical, genealogical and histological studies (I) were used to classify the patients into subtypes according to the scheme presented by Gedde-Dahl (3).

### Genealogical studies (I)

The EB families were traced 4–7 generations back through the parish records in order to get information on possible earlier occurrences of EB. Pedigrees were drawn for the dominantly inherited forms to illustrate the occurrence of the disease in the families, while for the recessive forms the purpose of the genealogical analysis was to identify any cases of consanguinity between the paternal and maternal branch.

### Morphological studies (I, II, III)

Biopsy specimens for light microscopy had been taken from at least one patient in each family affected, either in connection with the personal examination or some time earlier.

Biopsy specimens for electron microscopy were obtained from patients representing 33 different EB-families. These were taken from the edges of fresh blisters under local anaesthesia with 1 % lidocaine before clinical healing had begun. Light friction was used in some cases to provoke the blister, but this did not succeed in the cases of D-EBD. Specimens were fixed in 2,5 % cacodylate or phosphate-buffered glutaraldehyde at 4°C, postfixed in osmium tetroxide, dehydrated in a graded alcohol series and embedded in Epon. The ultramicrotome sections were stained with lead citrate and examined on a Jeol 100 S electron microscope (I, II, III).

## Immunohistochemical studies (IV)

Specimens for indirect immunofluorescence examination (161) were taken from fresh blisters in eight cases of different subtypes of EB (IV) and frozen with liquid nitrogen. 7 μm cryostat sections were prepared.

Antisera against collagen of types IV and V and heparan sulphate proteoglycan were prepared in rabbits and their specificity was tested as described by Timpl et al (162). Type IV collagen and proteoglycan were purified from murine EHS sarcoma tissue (163) and type V collagen from human placental membranes (9) raised by Dr. Foidart at the University of Liège, Belgium (IV).

Immunofluorescence stainings were performed using fluorescein-conjugated goat IgG to rabbit IgG (Dako, Copenhagen, Denmark) in order to demonstrate the location of the immuno-complexes formed between the antibodies and the antigens in the tissue. The specimens were examined by epi-fluorescence microscopy (Orthoplan, Leitz).

## Studies on collagen metabolism (V, VI)

### *Samples*

After a screening study (164), serum samples from twelve affected and ten unaffected members of one family with D-EBS Köbner (FEB 3) were studied for galactosylhydroxylysyl glucosyltransferase (GGT) and immunoreactive prolyl hydroxylase activity (VI). Skin biopsies were taken from six family members. Part of each biopsy was used to determine skin GGT, prolyl hydroxylase, lysyl hydroxylase and hydroxylysyl galactosyltransferase. The remainder was used to grow skin fibroblasts, from which GGT and prolyl hydroxylase were analyzed.

Hydroxylysine glycosides, hydroxyproline and creatine were determined in 24 h urine samples from eleven affected and eight unaffected family members. The patients had observed a low-gelatin diet for at least 16 h before urine collection. Serum GGT and immunoreactive prolyl hydroxylase were also determined in three other EBS families with eight members and in two cases of R-EBD. Control serum samples (VI) were taken from 42 healthy persons and control skin biopsy specimens were obtained from eleven persons at the excision of naevocytic naevi.

Collagenase activity in the medium of skin fibroblast cultures was determined for two patients with R-EBD and one with R-EBA mitis (V). The assays were performed both before and after trypsin activation of latent collagenase. The activities of the intracellular enzymes of collagen biosynthesis and the total collagen production per cell were also determined.

### *Cell culture procedure*

Fibroblast cultures (V, VI) were established routinely from each biopsy specimen and were grown until the fifth passage in Dulbecco's modification of Eagle's medium supplemented with 10 % foetal calf serum, ascorbate 50 ug/ml, L-glutamine 290 ug/ml and a mixture of penicillin 50 U/ml and streptomycin 50 ug/ml.

*Assays (VI)*

GGT activity (VI) was assayed by the method of Myllylä et al (165), modified for skin specimens as described by Kuutti-Savolainen and Kero (164), and for serum after Anttinen (166) and Kuutti-Savolainen (167), and in cultured skin fibroblasts according to Risteli et al (168). The other intracellular enzymes participating in the synthesis of collagen which were assayed were serum immunoreactive prolyl hydroxylase (169), skin prolyl hydroxylase (170), hydroxylysyl galactosyltransferase (171) and lysyl hydroxylase (172). Urine samples (VI) were assayed for hydroxylysine glycosides (173), hydroxyproline (174) and creatine.

Collagenase activity was determined using $^{3}H$ proline-labelled collagen made in freshly isolated chicken fibroblasts as a substrate (175) and following the method of Ryhänen et al (176) in which the fragments from collagenase digestion are further degraded with a mixture of trypsin and alpha-chymotrypsin. The degradation products were separated by precipitating the remaining intact collagen with additional trichloroacetic acid. Total collagen production per cell was determined by measuring the formation of hydroxyproline (177).

## Treatment trials

7 mg/kg/day of diphenylhydantoin (Enkefal$^{R}$) was given to one patient with R-EBD (FEB 39) once a day for a trial period of one year. The serum concentration of the drug was maintained above 8 nmol/ml, as suggested Bauer in 1980 (154).

Four EBS Köbner patients had been given oxychloroquine sulphate (Oxiklorin$^{R}$) 200–300 mg per day for three months according to the suggesting of Schnyder (178).

The response of different EB types to vitamin E treatment was evaluated from the patient files, since many of the patients had been treated with dosages of 50–400 mg per day.

In one case of EBH Dowling-Meara (FEB 22) it was possible to study the response to sea-side baths in a warm summer climate. Successful treatment of this kind has earlier been reported by Gedde-Dahl (3).

## Results and comments

### Epidermolysis Bullosa Subtypes in Finland (I)

Forty families with EB were found, the genealogical analysis of which included nearly 700 people and gave reliable information on 176 members with EB, 121 of whom were alive at the time. In thirteen families the proband was the only case of EB. Eleven out of the seventeen types of EB, sixteen inherited and one acquired, were represented, and there was no areal segregation. Two further patients were regarded as unclassified cases. A more detailed division of the patients into subtypes is presented in Table II and the Appendix.

*Table II. Distribution of EB families into subtypes. D-EBS mottled, D-EBS Ogna, R-EBA localisata, R-EBA inversa, R-EB progressiva and R-EBD localisata were not diagnosed in this study. The families without verification by electron microscopy are marked with an apostrophe.*

| | Families | Number of cases |
|---|---|---|
| D-EBS Köbner | FEB 3, 4, 5, 7, 10, 12′, 14′, 15′, 16, 25, 26 | 54 |
| D-EBS Weber-Cockayne | " 6, 17′, 18, 19, 20′, 21 | 40 |
| D-EBH Dowling-Meara | " 8, 9, 11, 13, 22, 23 | 10 |
| D-EB Bart | " 1 | 3 |
| R-EBA gravis Herlitz | " 2′, 28′, 29′ | 6 |
| R-EBA mitis | " 29 | 1 |
| D-EBD Cockayne-Touraine | " 31, 32, 33, 34, 35, 36, 37, 38 | 52 |
| D-EBD Pasini | " 31′, 32 | 3 |
| R-EBD Hallopeau-Siemens | " 40 | 1 |
| R-EBD inversa | " 39 | 1 |
| A-EBD | " 27 | 1 |
| unclassified cases | " 24, 30 | 4 |

### Intraepidermal forms

Twenty-four families with 107 affected members, of whom 74 were still alive, had intraepidermal types of EB. Seven of the cases were sporadic.

### *Dominant EB simplex Köbner and dominant EB simplex Weber-Cockayne*

Eleven families with 54 members, of whom 37 were still alive, had the generalized EBS Köbner type of disease. In two families there was only one case of this disease. Six families with 40 members, of whom 28 were alive, had EBS Weber-Cockayne. Three patients were sporadic cases.

Nail deformities, observed in 28/65 EBS cases, were interpreted as secondary post-traumatic changes which had led to a diagnosis of a dystrophic subtype in some instances. Light microscopy showed cytolytic intraepidermal splitting both in EBS Köbner and in EBS Weber-Cockayne in all biopsied cases. Common features revealed by electron microscopy were cytolysis, dyskeratosis and acanthosis (179), but no feature specific to this group only was found. In spite of the common gene defect in these two subtypes (2, 64), none of the EBS Köbner families had a single member with the localized form of the disease, neither did any EBS Weber-Cockayne family contain any case with generalized EBS Köbner. The penetrance of D-EBS was high, with no instance of a generation passed over without manifest disease to be observed in any family.

Hyperhidrosis of the feet is a symptom which is often mentioned in connection with EBS. Left lumbal sympathectomia had been performed in one case (FEB 3) with fairly good results, but the operation had no effect on the blistering tendency (68). Another disease which showed a possible association with EBS was myopia magna. There were altogether four cases (one in FEB 7 and FEB 18 and two in FEB 3), the diopter values for which were between −6.5 and −16,25.

### *Dominant EB herpetiformis Dowling-Meara (II)*

In addition to six probands, the genealogical analysis revealed one further case who was still alive, the father of a proband (FEB 11). There was also a history of EB in three relatives of one proband (see appendix FEB 13). The occurrence of EBH in two generations in families FEB 11 and 13 confirms the dominant mode of inheritance.

The diagnosis of this subtype is based on an electron microscope finding which shows deformation of the typical converged tonofilament structures. These are seen as peculiar round bodies with well-defined boundaries in the lower keratinocytes (84, 85). Pearson was the first to use the term 'tonofilament clumping' to describe a similar phenomenon (73). In this study the ultrastructure of the lesions of six probands had this common specific feature (179), which formed the basis for the diagnosis. This feature was also found in healthy skin and in adulthood, after daily blistering of the skin had stopped.

Clinically, there are spontaneous herpetiformic eruptions, burning of the palms and soles with squamous hyperkeratosis, thick, deformed nails and post-traumatic blisters of the skin. The most seriously affected patient had enamel defects on the teeth, erosions on the mucous membranes and hair loss. Most blisters healed without scarring, but minimal scars had been left on the most severely affected sites. Besides periods of fever, sea-side baths in a warm climate, which were tried in one case, had a favourable effect. The symptoms of all the patients had become milder with age. Psychic stress appeared to cause a temporary worsening in one case (FEB 13).

*Dominant EB Bart*

The disease of one family (FEB 1) with father and daughter affected was classified as EB Bart. The father's mother had evidently had the same disease, which also suggests dominant inheritance. The main diagnostic criteria were blistering of the skin and congenital ulcerations with skin defects mainly on legs. In addition, spotted pigmentation and nails curved lengthwise were conspicuous features, as have also been described as being typical of D-EBS mottled (78).

There has been no previous ultrastructural description of the Bart syndrome. The ultrastructure of the father's skin proved to be peculiar (178), differing from that of the other types of EB, especially D-EBS mottled as presented by Gedde-Dahl et al (79). There was an intraepidermal cleavage through the basal cells, which were themselves filled with granular material. There were only a few small, rudimentary tonofilaments, and the desmosomes were normal but infrequent (180).

## Dermal forms

*Dominant EB dystrophica Cockayne-Touraine, dominant EB dystrophica Pasini*

D-EBD Cockayne-Touraine and D-EBD Pasini were found altogether in eight families with 55 affected members, of whom 41 were still alive. This suggests that the different clinical extents of these subtypes are manifestations of the same gene defect. D-EBD Pasini was found in three cases belonging to two families (FEB 31, 32), while the other 22 affected members of these two families, as well as the other six families, all represented D-EBD Cockayne-Touraine. One of the cases (FEB 38) was the only case without a family history. The family FEB 32 has been partly described earlier by Rehtijärvi et al (181). Two newborn infants, both belonging to FEB 32, had had aplasia cutis.

In most cases the diagnosis of D-EBD is easy to establish on the basis of family history, clinical features and the typical location of the scars with miliae and defective nail growth. The ultrastructure of the lesions showed dermal cleavage, but the number of anchoring fibrils was not specifically diagnostic, since it varied from a clear reduction to normal numbers (95, 96, 179).

*Recessive EB dystrophica*

R-EBD inversa and R-EBD Hallopeau-Siemens were seen in one family each (FEB 39, 40). Clinically, R-EBD Hallopeau-Siemens represented the most troublesome subtype of EB, with scar contractions and mutilation of the hands and feet to little more than skin bags, while in R-EBD inversa it was limited to syndactylia. Both types had strictures of the gastrointestinal tract and the patients were small in size and anaemic and had widespread destruction of the teeth.

Ultrastructural examination revealed a splitting of the dermal level and reduction of the anchoring fibrils.

*Acquired EB dystrophica (III)*

The acquired form of dystrophic EB was found in one female (FEB 27) when post-traumatic blisters appeared in connection with her third parturition (III). Direct immunofluorescence examination of a sample of healthy skin showed a linear deposition of IgG and complement in the basement membrane zone, but the indirect immunofluorescence findings were negative. Electron microscopy revealed blister formation to be taking place immediately beneath the basal lamina within a band-like amorphous but not amyloid zone. The number of anchoring fibrils in the lesions was reduced,as in earlier reports (120).

## Junctional forms

*Recessive EB atrophicans*

In each of three families two children with lethal R-EBA (FEB 2, 28, 29) had died before the age of one year. Unfortunately, no electron microscopy specimens were available. Besides these six lethal cases, there was one affected member alive in family FEB 29, a 20-year-old female, who was typed as having EBA mitis. The epidermis was normal microscopically and there were small subepidermal vacuoles at the dermo-epidermal junction. The ultrastructural cleavage was seen between the basal lamina and the intact basal cell plasma membrane. The attachment plaques were uneven, the hemidesmosomes were reduced in number and the anchoring filaments appeared rudimentary. There were no immunoglobulins at the basement membrane zone.

Volvulus intestini was detected at obduction in one of the lethal cases (FEB 29). The families FEB 28 and FEB 29 may have had a common gene source, as they were from the same part of the parish of Peräseinäjoki in southern Ostrobothnia, but no consanguinity could be demonstrated.

*Unclassified cases*

The classification of the probands in two families (FEB 24, 30) was problematic. In FEB 30 a 30-year-old woman had had post-traumatic blistering ever since childhood. The blisters healed leaving atrophic scars, and she also suffered from muscle dystrophy. Her brother, who had died in 1971 at the age of 35, had also had skin symptoms compatible with EB and muscle dystrophy.

The other case was a five-year-old girl (FEB 24), whose clinical features partly resembled those of R-EBA-mitis (FEB 29), but the family history supported recessive inheritance of the disease and the symptoms were slightly scarring blistering without seasonal changes, nail and tooth defects and mild anaemia.

Splitting was seen between the basal lamina and basal cells in electron microscopy in both cases, but the hemidesmosomes were normal. These cases suggest greater heterogeneity in junctional EB than has been allowed for hitherto (132, 133).

## Indirect immunofluorescence for the diagnosis of EB (IV)

Specific antibodies to types IV and V collagen and to proteoglycan proved useful for pinpointing the level of the cleavage, which helps in determining the type of EB concerned. In D-EBS Köbner (FEB 3, FEB 26) and D-EBS Weber-Cockayne (FEB 6) all these three immunofluorescent antibodies were located in the floor of the blister, while in both the dominant dermal EB (FEB 32, FEB 34) and the recessive inherited form (FEB 40) all three components were located in the roof of the blister, suggesting cleavage occurring beneath the basal lamina.

In D-EBH Dowling-Meara (FEB 22) the antibodies against types IV and V collagen were attached to the dermal floor, whereas the proteoglycan antibody was located on the roof of the blister. A characteristic feature was the microscopically visible looseness of the dermo-epidermal junction seen in the non-blistering areas, together with the tonofilament defect. Staining of the proteoglycan component produced a broken line, around which microblisters were seen, especially on the tips of dermal papillae.

The preliminary histological diagnosis for the proband of FEB 30 was junctional EB, but all the antibodies in indirect immunofluorescopy showed staining in the floor of the blister and the hemidesmosomes were found by electron microscopy to be normal. These findings together suggest the existence of a junctional type with normal hemidesmosomes, the cleavage occurring directly below the basal cells but above the proteoglycan level in the lamina lucida.

## Galactosylhydroxylysine glucosyltransferase (GGT) in one family with dominant EB simplex Köbner (FEB 3) (VI)

The mean serum GGT activity was significantly reduced compared with the control material in twelve members with D-EBS Köbner. Two members without clinical D-EBS Köbner also had low enzyme activities, but the other eight apparently healthy members studied had normal activities.

GGT activity was assayed in skin specimens from nine family members of FEB 3, of whom eight had the disease. The values showed a significant correlation with those found in the serum. Low GGT activity was also demonstrated in cultured skin fibroblasts from the same subjects, the lowest values being about 25 % of the control mean. In contrast with these findings, the assays of immunoreactive prolyl hydroxylase in serum and of prolyl hydroxylase, lysyl hydroxylase and hydroxylysyl galactosyltransferase activities in skin biopsy specimens were normal when compared with the control values.

Excretion of hydroxyproline in the urine was normal in all the cases assayed, indicating normal collagen turnover. Urinary secretion of glucosylgalactosyl hydroxylysine was low, and there was a significant correlation between this and the serum GGT values. Seven out of eight family members without EBS Köbner had normal urinary glucosylgalactosyl hydroxylysine excretion, the only abnormal values without EBS Köbner belonging to one member who had low GGT values in both serum and skin (VI). Even lower urinary glucosylgalactosyl hydroxylysine excretion could have been expected on the basis of his GGT values, however. Eight patients representing three other families with D-EBS (FEB 5, 7, 17) and two patients with R-EBD (FEB 39, 40) had normal serum GGT activities.

The results suggest that low GGT values are due to low enzyme activity in at least some tissues, and that this low enzyme activity may be associated with reduced excretion, and hence probably also synthesis, of glucosylgalactosyl hydroxylysine. GGT deficiency may be aetiologically related to EBS in some families, but other defects may be the cause in other cases.

### Collagen metabolism in two forms of recessive EB, dystrophica and atrophicans (V)

80,6 % of the total collagenase was in the active form in the proband of FEB 39 (R-EBD inversa), 38,5 % in that of FEB 40 (R-EBD Hallopeau-Siemens) and 62,3 % in that of FEB 29 (R-EBA mitis), while the proportion of active collagenase in the controls was only 5,4 %. The total collagenase values in cell culture media per cell after trypsin activation in cell lines from two cases of R-EBD and one case of R-EBA mitis showed no difference from those of the controls, although collagenase activity without trypsin activation was significantly higher in the cell culture media of fibroblast lines from all three of these patients compared with the normal material.

All the cell lines from the patients with R-EBD and R-EBA produced two to five times more collagen than the control fibroblast cultures. The increased activities of prolyl hydroxylase and lysyl hydroxylase also suggested increased collagen production per cell. The hydroxyproline/proline ratio was increased, implying a specific increase in collagen compared with non-collagen proteins. The intracellular sugar transferases were not elevated.

### Treatment trials

One patient with R-EBD inversa (FEB 39) showed a favourable response to treatment with phenytoin (V), her skin becoming healthier at the margins of the scars after one year's therapy.

The pilot study with oxychloroquine treatment in four D-EBD Köbner patients did not show any improvement, and analysis of the files revealed that no patient had shown any response to treatment with vitamin E, which had been used for many years. Treatment of one case of D-EBH Dowling-Meara (FEB 22) by transfer to a warm climate for two months had a transient favourable effect.

## DISCUSSION

Clinical and genealogical aspects: The term epidermolysis bullosa has been accepted as a common label for the heterogeneous group of 13–17 mechanobullous diseases (3, 182). The classification of these has been based up to now mainly on microscopic findings, but because of new aetiological and biochemical data this classification will evidently be changed.

Eleven subtypes of EB were found in Finland , in addition two cases in which the final classification was left open. Although Finland is a propitious area for certain rare genetic diseases (183), no clear areal concentration was found, neither did any of the subtypes represent a special Finnish disease inheritance. Conspicuous was the very much lower incidence of R-EBD in Finland compared with that in Norway. A retrospective study of this type cannot be expected to reach all the milder cases of EB, especially as far as dominant EBS Weber-Cockayne and EBD Cockayne-Touraine are concerned. During the period of 1971–1980 the most common subtype, D-EBS Köbner, was found in five newborn infants, which would mean one case per 120000 births. It is surprising, however, that EBH Dowling-Meara, a new variant of EB, was the fourth commonest subtype. Only the types with recessive inheritance, EB atrophicans gravis Herlitz and EB dystrophica, are so severe that all the patients will probably be reached. The two cases with R-EBD could not be traced back to any common gene source and no consanguinities were shown. On the other hand, two isolated gene sources were found for R-EBA, one in the Åland Islands and the other in Southern Ostrobothnia, where families FEB 28 and 29 could be traced back to the same village of Peräseinäjoki.

In some subtypes, especially D-EBD Cockayne-Touraine, the correct diagnosis is often reached on the basis of clinical indicators and genealogical analysis. The problematic cases are the new mutations without a family history of a time, when the first blisters appear but no typical scars have yet developed. The most significant indicators which can be used to differentiate between the subtypes are the age of onset, preferred sites of blisters, the appearance of scars, condition of the hair, teeth, and nails, and the influence of temperature on the blistering tendency (3, 62, 64, 65, 184).

Characteristic of the two types of R-EBD were physical underdevelopment and small body size, anaemia, strictures of the gastrointestinal tract and syndactyly Scars with milia served as symptoms of the dermal forms. Hair loss was seen in D-EB Bart, R-EBA mitis and in the most serious cases of D-EBH Dowling-Meara (II), and the hair was thin in both cases of R-EBD. The teeth had been destroyed in R-EBD Hallopeau-Siemens and R-EBD inversa, and also in R-EBA mitis and both the unclassified cases. Nail disturbances were seen in all the dermal types. Diagnosis of the dystrophic forms should not be based on nail deformities alone because post-traumatic disturbances in their growth are also seen in the subtypes of EBS. The rise in temperature caused a worsening of the subtypes of EBS in this study from May to August, a symptom which tends to become milder in adulthood. Worth noticing as well is the improvement in skin symptoms during fever periods in D-EBH Dowling-Meara (3). It seems that hyperhid-

rosis is a symptom often found in EB patients. Whether high temperature is a common factor behind both EBS and hyperhidrosis is a question which should be considered in greater detail in future. There are also certain diseases of symptoms not related to the skin in EB. The finding of volvulus intestini at obduction in one case of FEB 29 is consistent with the reports on an association between anomalies of the gastrointestinal tract and R-EBA (139, 140, 141, 142, 143). The weak association between D-EBS Köbner and myopia magna in four subjects in this material and the link between muscle dystrophy and EB (FEB 24) require more attention (3).

Morphological and immunological aspects: It is important to find a blister as fresh as possible for the biopsy (182). The purpose of the light microscope examination of biopsy samples is to rule out the bullous diseases with a specific histology, while the direct immunofluorescence method serves to rule out a wide range of bullous diseases (185, 186, 187). The same applies to routine laboratory investigations, which should be used to exclude porphyrins at least.

The ultrastructural entities of the main intraepidermal forms of EB (179), 1. cytolysis and acantholysis in D-EBS Köbner and Weber-Cockayne 2. tonofilament clumping in D-EBH Dowling-Meara and 3. hypoplastic tonofilaments corresponding to the EB Bart form recognized in this study, are all unknown as far as their biochemical background is concerned. The effect of warmth in D-EBS in causing the cytolysis suggests an enzymatic pathomechanism, while the hypoplastic tonofilaments in D-EB Bart and their clumping in D-EBH Dowling-Meara, even on symptomless skin, are probably expressions of genetic defects in cellular structural protein, the role of which has been discussed in general by Lazarides (188).

A common feature characteristic of all the dermal types of EB examined here was a reduction in anchoring fibrils, the number of which varied from normal to a noticeable lack in D-EBD Cockayne-Touraine and was significantly reduced in both A-EBD and R-EBD (III). These findings are similar to those reported earlier (95, 96, 104). No specific histological marker was found to differentiate between the dominant and recessive forms of dystrophic EB.

Hypoplastic hemidesmosomes, the indicators of EBA, were found only in one case (FEB 29). The two so far unclassified cases with junctional splitting had normal amounts of hemidesmosomes. These findings support the reports of Tidman and Eady (132, 133) of a widespread reduction in dermo-epidermal adhesion with normal hemidesmosomes and suggest the existence of greater heterogeneity in the junctional EB forms.

Immunohistochemical visualization of the components of the basement membrane (161) was suitable for pinpointing the level of blistering (IV). The intraepidermal and dermal forms were clearly distinguishable, the splitting being located either above or below all three stained antigens, collagen types IV and V and proteoglycan. In the only junctional case examined the antibodies behaved similarly to those in the intraepidermal type, all being located on the floor of the blister. Hence the splitting must have taken place above the proteoglycan antigen, which has been shown to be situated in a narrow zone at the border between the lamina lucida and basal lamina (see 189, 190). As the antibodies against types IV and V behaved similarly in all specimens, it would be more practical to choose the pemphigoid antigen instead of collagen type V, this being located immediately below the basal cells (40). In this way the zone of available antigens would cover a wider area and two of the antigens would be situated in different levels of the

lamina lucida, in which there are probably several heterogeneous splitting patterns. This is also supported by the surprising finding of looseness and microblisters in the lamina lucida in D-EBH Dowling-Meara (IV), when using heparan sulphate proteoglycan antiserum.

Treatment: Because of the unknown pathomechanism of most subtypes, treatment is often limited to the use of local antibacterial therapy. Oxychloroquine (178) had no effect in four EBS Köbner cases, nor had vitamin E (145, 146, 147), which was used to treat various subtypes of the disease. Corticosteroids (152) should be reserved for emergency situations and for the prevention of strictures. Phenytoin treatment is worth trying on the basis of the results of this study (V) as well as those of others (3), and it would be interesting to try retinoids, although, further details are needed about the risks involved, especially when used for children.

Genetic counselling (156) in the case of the dominant forms should be given by the primary dermatologist or paediatrician responsible for the treatment of the patient, whereas in the recessive types the counselling should be concentrated in the departments of medical genetics, which could develop and maintain an organization for antenatal diagnosis and prevention (157, 158, 159).

Biochemical aspects: Disturbances of the post-translational enzyme reactions involved in collagen biosynthesis are reflected in fragility and hyperelasticity of the skin (18, 19, 20, 21, 22, 23, 24, 25, 26). A significant association was found between D-EBS Köbner and GGT deficiency in family FEB 3 but no GGT deficiency could be demonstrated in three other families, which suggests that it is not a general aetiological factor in D-EBS Köbner. The splitting patterns, cytolysis, acantholysis and dyskeratosis, nevertheless involve such unspecific findings that they could be caused by several pathomechanisms. Epidermal cells in vitro adhere preferentially to basement membrane collagen (191), which is known to contain a high number of glycosyl units. The association of EBS Köbner with GGT deficiency could give additional support to this theory, since one of the roles of the glycosylation reactions is thought to be the attachment of collagen fibres to the surrounding tissue.

One argument against the aetiological significance of GGT deficiency in FEB 3 is the fact that two members (members 5, 21 in paper VI) had this enzyme deficiency without manifest D-EBS, the penetrance of which is known to be very strong. In spite of the reports of unaffected carriers of EBS (192), no family was found in this Finnish series in which EBS crossed a generation without penetrance. In the light of paper VI, however, the GGT deficiency may be involved in the pathogenesis of D-EBS Köbner in family FEB 3, nor can the possibility of a close gene linkage be excluded. Screening of GGT in the isolated population of the community from which FEB 3 originated would perhaps give interesting information on this subject. In addition to defects in collagen biosynthesis, reduced mechanical strength of the skin may result from a disturbance in collagen degradation. There are studies reporting on increased collagenase activity (112, 113) and increased concentrations or preferential translation of collagenase messenger RNA (114, 115), and these authors have also suggested the presence of structural aberrant collagenase in R-EBD. Bauer has suggested that increased synthesis of collagenase, reduced thermal stability and diminished affinity for $Ca^{+}$ serve as reliable in vitro markers for F-EBD (115).

The amounts of total collagenase found in the cases of R-EBD and R-EBA mitis had

not changed when compared with the controls, but the fraction present in the active form in the culture media was significantly higher. These results support earlier reports on the significance of collagenase in the pathomechanism of R-EBD, and the favourable response obtained to the administration of phenytoin in order to inhibit the increased collagenase activity (153, 154). The observation of an increased amount of active collagenase in R-EBA mitis necessitates further studies on the biochemical nature of collagenase. In earlier in vitro studies no changes had been demonstrated in collagen metabolism in this disease (113). On the other hand, examples are also found among the inborn connective tissue errors of one biochemical defect causing two separate diseases (26). There is nevertheless a possibility of two different genetic abnormalities occurring in these subtypes of R-EB; a defect in the regulation of collagenase and a different defect in structural proteins.

Present problems and prospects: The great number of subtypes of EB in the present taxonomy leads to a troublesome multitude of separate diseases. More exact information on their pathomechanisms is needed, especially to resolve the final position of rare marginal types such as EB Bart or EBS mottled. It may be speculated whether there are any particular reasons why the classification of some subtypes is based on their severity or clinical extent, e.g. R-EBD generalisata Hallopeau-Siemens, inversa and localisata or R-EBA gravis-Herlitz, mitis, inversa and localisata. The results of this study suggest that EBS Köbner and EBS Weber-Cockayne are genetically different diseases, while D-EBD Cockayne-Touraine and Pasini represent different clinical extents of the same gene defect.

The subtypes with a junctional splitting level form the most confusing group. The present heterogeneity of this group is greatly due to the structural importance of the hemidesmosomes, upon which the classification is based. The recent reports on junctional cases with normal hemidesmosomes (132, 133) may yet contribute to the revaluation of this group.

A-EBD has obtained a generally accepted position as a distinct entity, separated from the diseases of the pemphigoid group by the involvement of sub-basal lamina immunoreactants. The possible autoimmune pathomechanism will require further investigation, however.

No significant changes in the morphological classification of EB are to be expected in the next few years, but biochemical data on the pathomechanism of the different subtypes are increasing with the growth of available specific antibodies against different structural components (193). In addition, characterization of the structural components of the basement membrane responsible for skin stability or pathological fragility will give new clues to the inborn errors responsible for mechanobullous diseases.

# APPENDIX

*The Finnish EB families*

The EB families identified during this study are listed below in the order in which information was received on them. At least the probands of the families were all examined personally during the years 1980–1981, and the families are briefly described with special reference to cases with diagnostic problems or of general interest. The grouping of the families according to their subtypes of EB is recorded in Table II.

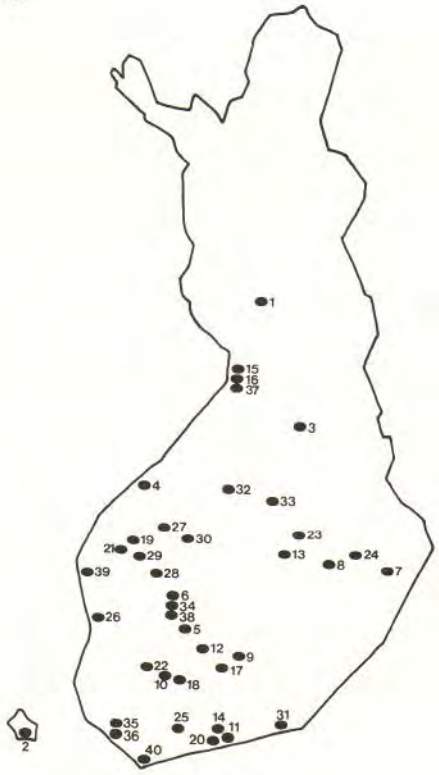



*Fig 2. Areal distribution of EB families according to origin of probands. The numbers correspond to the numbering used for the families later in this appendix.*

*FEB 1; D-EB Bart.* Female proband born 1978. Father had the same disease and possibly also his mother. Significant symptoms were congenital aplasia areas on the skin, mostly on the legs, (Fig. 3.) There they left insignificant scars after healing. Abnormal fragility and blistering followed a minimal trauma and her hair loosened easily by pulling. The father had been under hospital treatment for months as a child because of a problematic blistering skin disease, but the blistering had gradually lessened after six years of age. Later his skin had become atrophic and pigment-

spotted, the nails were curved lengthwise and he had total alopecia. Electron microscopy revealed an almost total deficiency in the formation of tonofilaments in the normal skin of the father. This family has been described earlier by Kero et al (180).

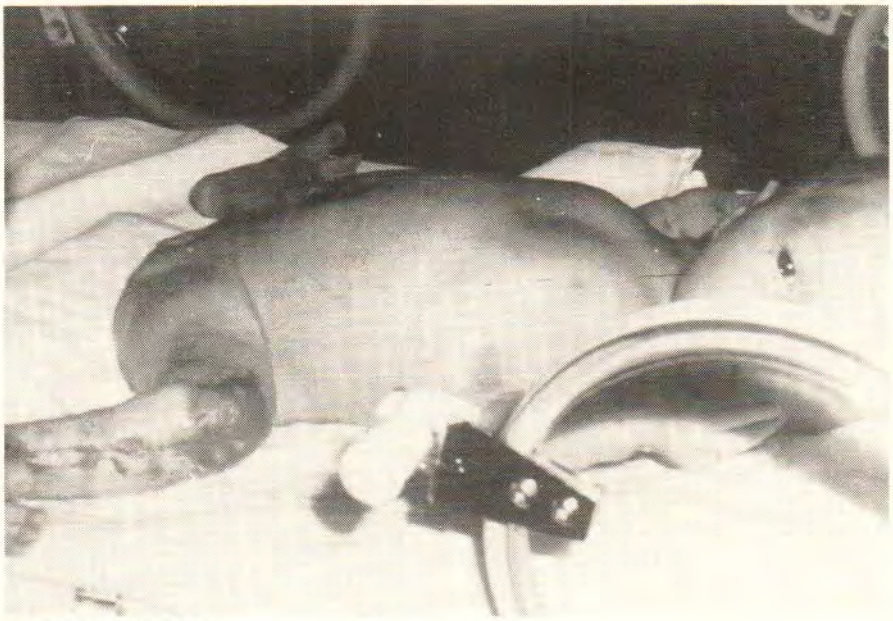


*Fig 3, FEB 1; D-EB Bart*

*FEB 2; R-EBA gravis Herlitz.* Two probands, male born 1968, died at the age of five months, and a male born 1980, died at the age of ten months. The second child of the family, a female born 1970, was healthy. The family was from an isolated area of the Åland Islands. No consanguinity was demonstrable between the maternal and paternal braches.

*FEB 3; D-EBS Köbner.* Female proband born 1940. A case report on this family was published by Sonck in 1948 (68). The pedigree is presented in detail in VI. A GGT enzyme deficiency was detected in members of this family. A specimen was studied by immunofluorescence in IV.

*FEB 4; D-EBS Köbner.* Male proband born 1965 (IV/I, Fig. 4). Six diseased members in four generations. Fig. 5 shows typical blisters on the palms.

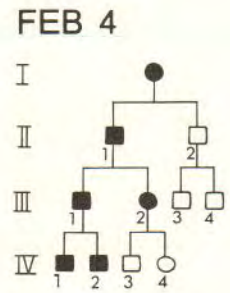



*Fig. 4*

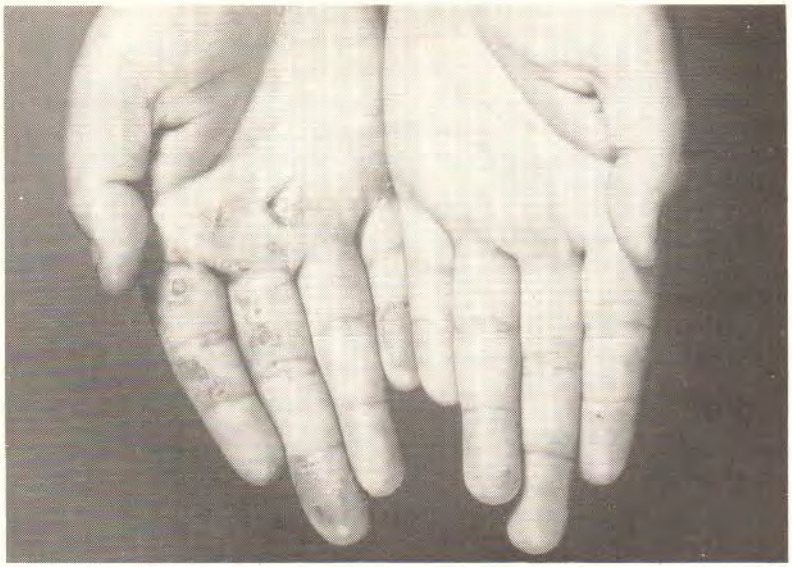


*Fig. 5, FEB 4; D-EBS Köbner*

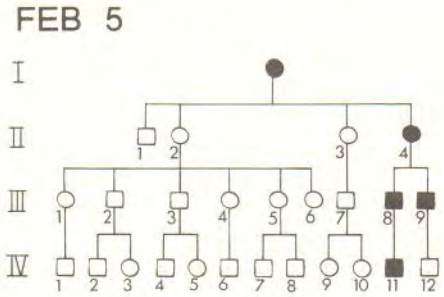



*Fig. 6*

*FEB 5; D-EBS Köbner.* Male proband born 1975 (IV/11, Fig. 6). Five diseased members in four generations.

*FEB 6; D-EBS Weber-Cockayne.* Female proband born 1922 (II/2, Fig 7). Her dead brother had an EB of the same type. No history of skin diseases in the parents now dead, although dominant inheritance would predict this. The splitting level was demonstrated immunohistochemically in IV.

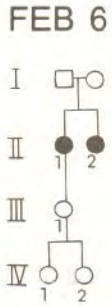



*Fig. 7*

*FEB 7; D-EBS Köbner.* Female proband born 1967 and her sister born in 1968. Their mother had the same type of EB.

*FEB 8; D-EBH Dowling-Meara.* Female proband born 1959. Youngest of five children, sporadic case. A large area of the skin was red and eroded after birth and general blistering continued to the age of eight years. Hyperkeratosis on the palms and soles started to develop at the age of one year. This case is described by Niemi et al (194).

*FEB 9; D-EBH Dowling-Meara.* Female proband born 1962, sporadic case. Blistering seen in infancy and childhood became milder at the age of 12. Marked palmar and plantar hyperkeratosis and hyperhidrosis were seen.

*FEB 10; D-EBS Köbner.* Female proband born 1933 (II/2, Fig. 8). Four cases in four generations.

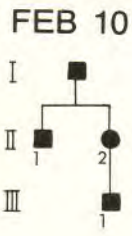



*Fig. 8*

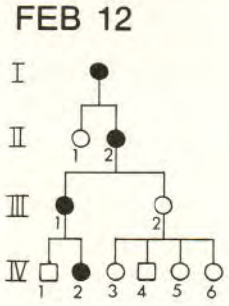



*Fig. 9*

*FEB 11; D-EBH Dowling-Meara.* Female proband born 1959. Disease was mild, blisters occurring occasionally on frictional areas. Her father, symptomless nowadays except for palmar hyperkeratosis, had had the same disease when young.

*FEB 12; D-EBS Köbner.* Male proband born 1940 (III/1, Fig. 9). Four cases in four generations.

FEB 13; D-EBH Dowling-Meara. Female proband born 1943. Her father suffered from EB, as also had her brother, who had died young and was reported as a case of lethal EB by Sonck in 1948 (68). The father's mother had had a similar blistering disease.

Blistering continued into adulthood in the proband and was worsened by menstruation and psychic stress, although she was symptomless during pregnancy. Fig. 10 shows the thick hyperkeratotic soles of the feet.

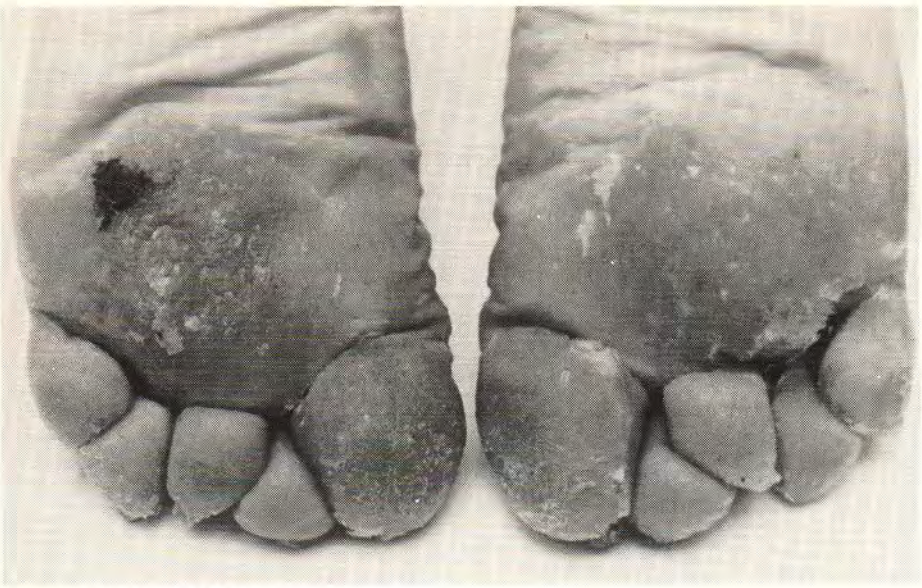


*Fig. 10, FEB 13; D-EBH Dowling-Meara*

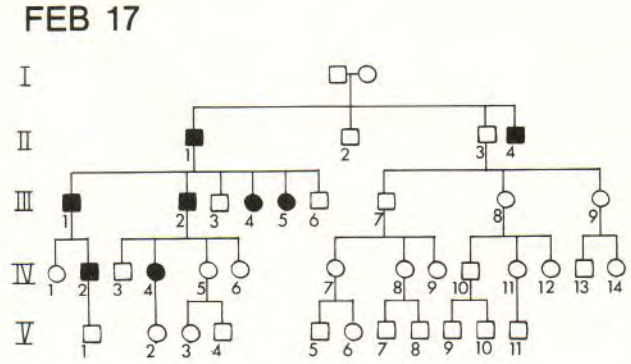



*Fig. 11*

**FEB 18**

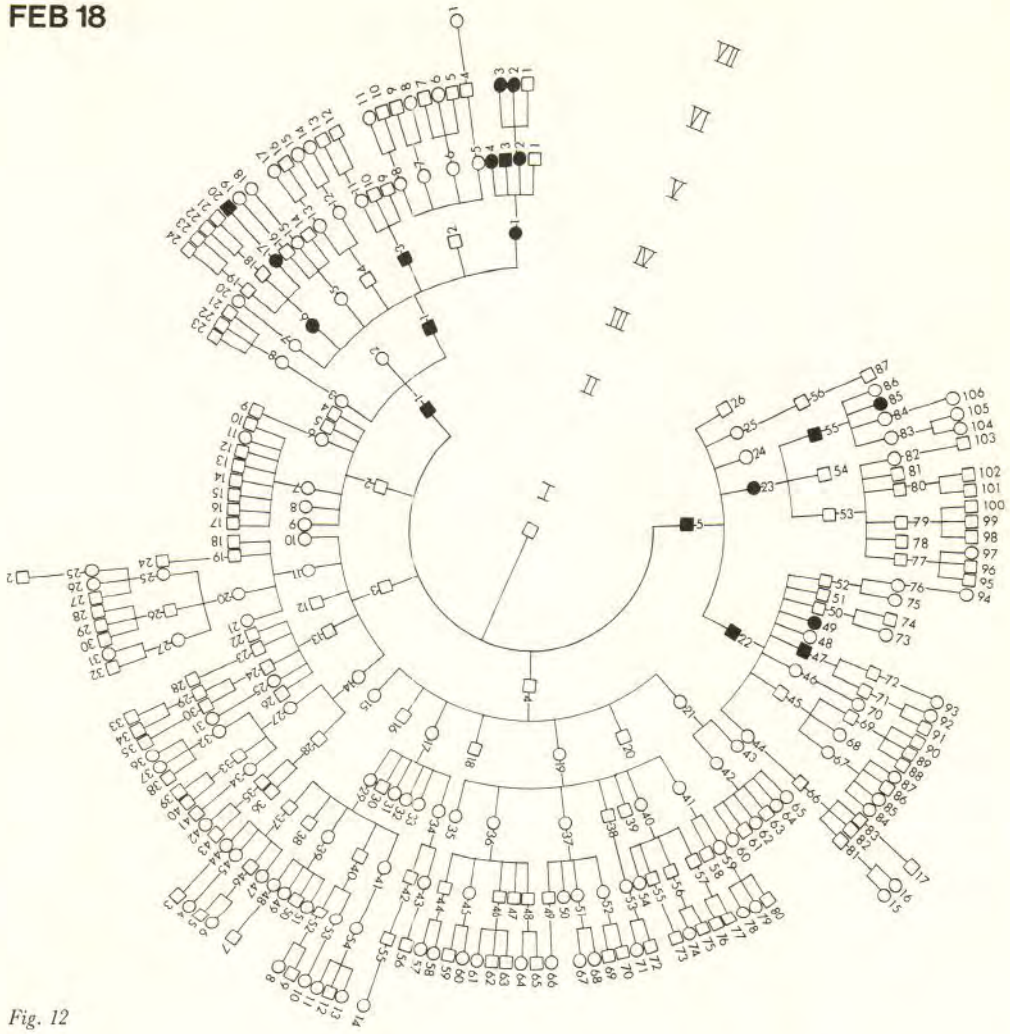


*Fig. 12*

*FEB 14; D-EBS Köbner.* Male proband born 1902, sporadic case. Died 1980 and was not seen personally.

*FEB 15; D-EBS Köbner.* Female proband born 1958. Mother had EB simplex as well. The present symptoms were few.

*FEB 16; D-EBS Köbner.* Proband born 1976, a sporadic female. Symptoms appeared at age 1,5 years. Worsened by heat, and was clearly noticed during a trip to the sunny south of Europe.

*FEB 17; D-EBS Weber-Cockayne.* Female proband born 1927 (III/4, Fig. 11). Eight affected cases in three generations.

*FEB 18; D-EBS Weber-Cockayne.* Female proband born 1952 (IV/85, Fig. 12). Nineteen affected cases in five generations.

*FEB 19; D-EBS Weber-Cockayne.* Female proband born 1972. Ten affected cases in four generations.

*FEB 20; D-EBS Weber-Cockayne.* A sporadic male proband born 1959, symptomless since the age of 16.

*FEB 21; D-EBS Weber-Cockayne.* A sporadic male proband born 1975. Typical mild disease.

*FEB 22; D-EBH Dowling-Meara.* Female proband born 1975, a sporadic case, reported in papers II and IV.
Fig. 13 shows herpetiformic blisters and Fig. 14 the faulty position of the left ankle caused by a burning pain on the soles of the feet. The patient moved about by crawling even at the age of seven. Seaside baths in summer time had a beneficial effect on the blistering.

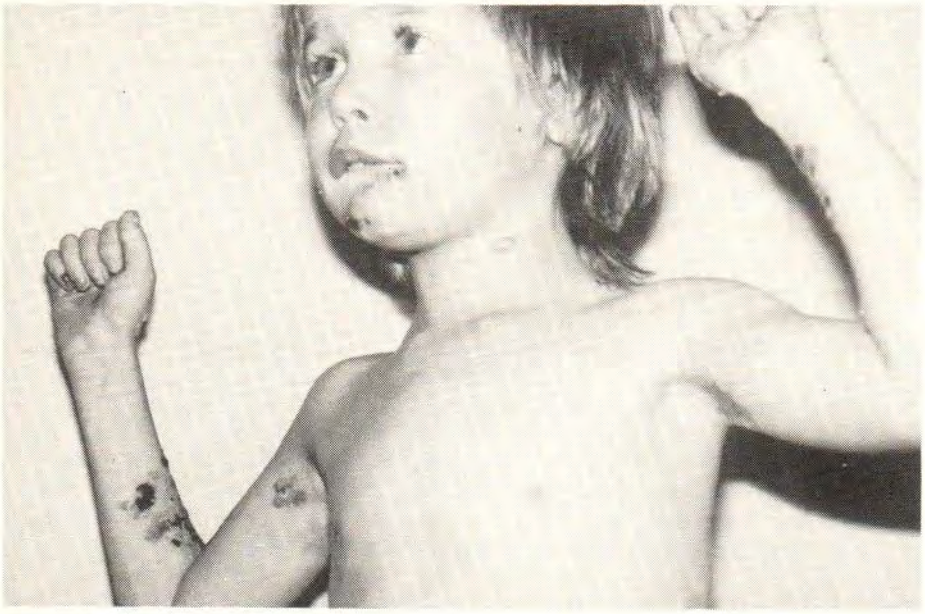


*Fig. 13, FEB 22; D-EBH Dowling-Meara*

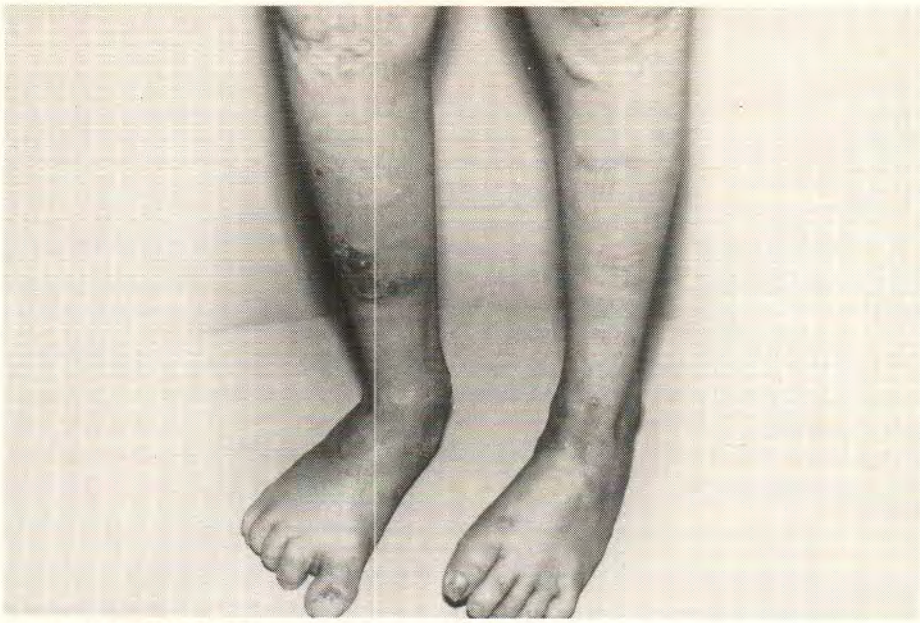


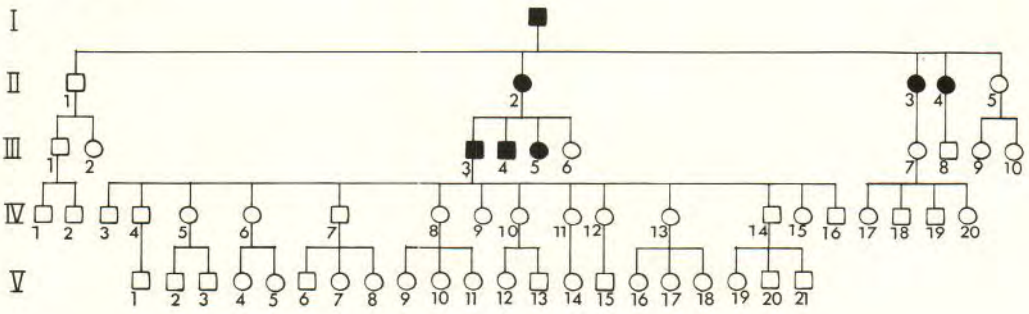
*Fig. 14, FEB 22; D-EBH Dowling-Meara*

*FEB 23; D-EBH Dowling-Meara.* Male proband born 1978, a sporadic case. Reported in paper II.

*FEB 24; Unclassified EB.* Female proband born 1947, the tenth in a family of twelve children. Besides EB, she also had muscle dystrophy, lactose intolerance and treated thyroid toxicosis. Her eldest brother, who had died at the age of 35, had also had EB and muscle dystrophy. The second and third children had died as infants in the 1930's. They had never been examined by a doctor, but according to the proband they may have had EB. Post-traumatic blisters could be seen everywhere, but the preferred sites were the feet, hands, elbows and shoulders. Atrophic scars were seen on the knuckles, but no miliae were present. The nails were deformed. Electron microscopy showed junctional blisters with normal hemidesmosomes.

*FEB 25; D-EBS Köbner.* Female proband born 1899 (II/4, Fig. 15). Seven cases in three generations. Of particular interest was the fact that none of the sixteen children of III/3 was affected.

**FEB 25**



*FEB 26; D-EBS Köbner.* Male proband born 1980, a sporadic case.

*FEB 27; A-EBD.* Female proband born 1923. The blistering of the disease started in connection with her third parturition and continued to the menopause. This case is described in detail in paper III.

*FEB 28; R-EBA gravis Herlitz.* There were two children with EB, a male (Fig. 16) and a female, who had both died of complications of EB in early infancy. No anomalies of the gastro-intestinal tract were found on obduction, however. A disturbance of the electrolytic balance was held to have been the immediate cause of death. These probands were not seen personally. The second child was unaffected. The parents had common ancestors five generations back and were from the same district as FEB 29.

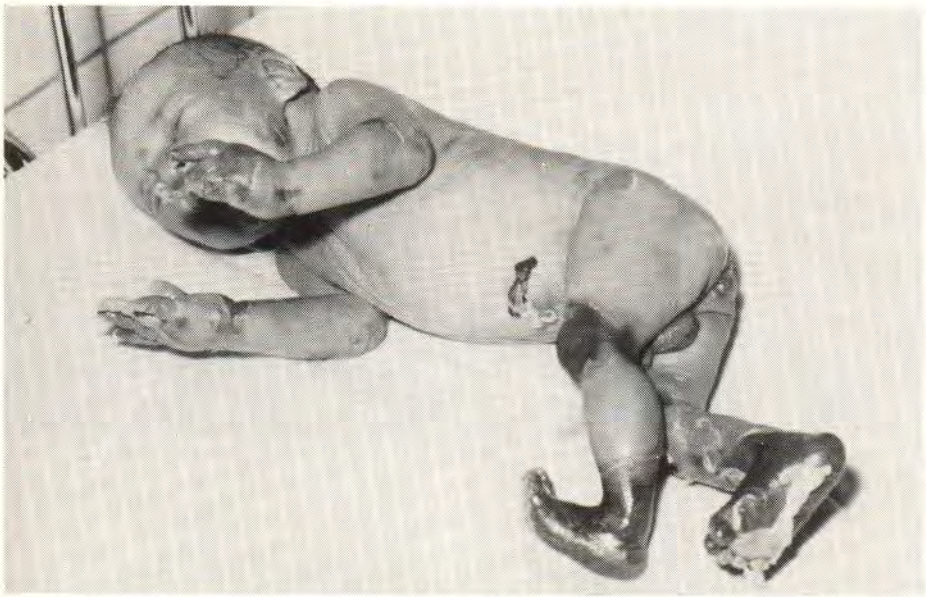


*Fig. 16, FEB 28; R-EBA gravis Herlitz*

*FEB 29; R-EBA mitis.* Female proband born 1962. The youngest of eight siblings. The five oldest were healthy, but the sixth, a female born 1955 with blistering EB, had died at the age of two years, with no obduction performed, and the seventh, a male born 1960, had died at the age of one month, with a finding of volvulus intestini on obduction. The clinical picture of the proband, with large blisters, anaemia, hoarseness, nail dystrophy and cicatricial alopecia, fitted well with EB atrophicans mitis, as did the ultrastructure of the skin, with hemidesmosomal hypoplasia and an increased amount of active collagenase (V).

The pedigrees of the parents did not show any consanguinity. The father's pedigree was traced back to the same village where the ancestors of FEB 28 were from, so that it seems probable that these two families had a common gene source.

*FEB 30; Unclassified EB.* Female proband born 1977, the youngest of five children in the family where blistering started in early infancy. Her brother, born 1963, had died at the age of one month, the immediate cause of death being thought to have been an infection. No intestinal anomalies were found on obduction. The evident recessive inheritance, destruction of the teeth

and nails, anaemia, alopecia and the lack of any dependence of blistering upon temperature were facts not typical of any known intraepidermal EB type (Fig. 17). The parents were from the same village, but no consanguinity could be demonstrated. Electron microscopy showed the cleavage to be taking place between the basal lamina and the plasma membrane of the basal cells. No clear hemidesmosome defect could be demonstrated. The immunofluorescence technique showed that the blister was situated above the proteoglycan zone in the lamina lucida, as in EB simplex (IV).

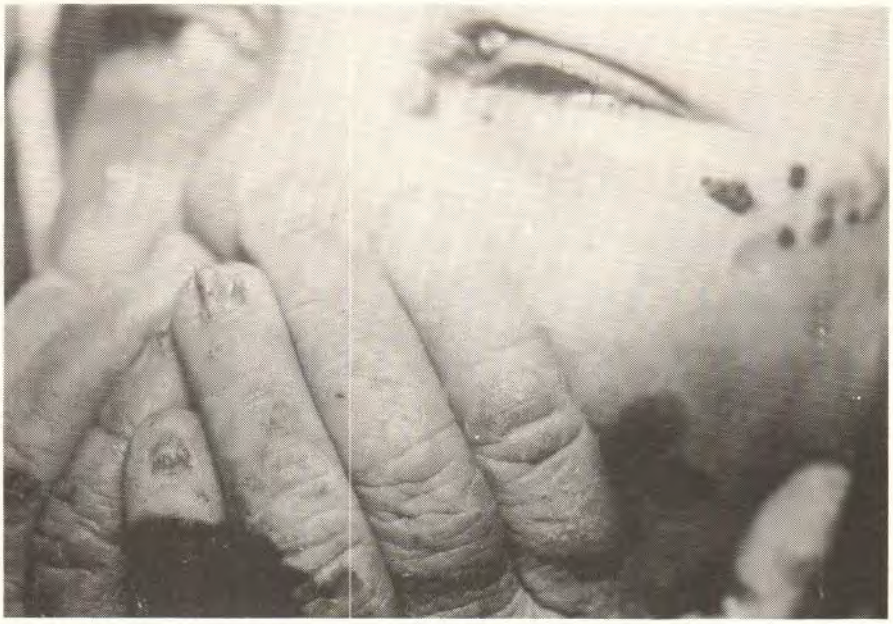


*Fig. 17, FEB 30; an Unclassified type of junctional EB.*

*FEB 31; D-EBD Pasini.* The proband was a female born 1945 (III/7, Fig. 18). There were five affected cases in four generations. (Fig. 18). Only the proband was examined personally. She clearly had the D-EBD Pasini, while the other members, with a more limited disease, were suggestive of D-EBD Cockayne-Touraine.

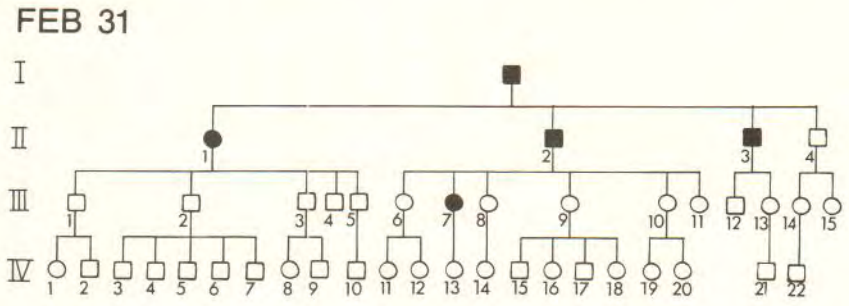



*Fig. 18*

*FEB 32; D-EBD Cockayne-Touraine/Pasini.* Female proband born 1947 (III/12, Fig. 19). Both generalized Pasini forms (II/1, III/12) and localized Cockayne-Touraine forms were found in this family. The latter cases included 15 patients who were still alive, nine of whom were examined personally. A child was born to III/13 after this study. Congenital aplasia cutis was shown in case

IV/11. A case report on this family has been published earlier (181). A specimen from proband was studied in IV.

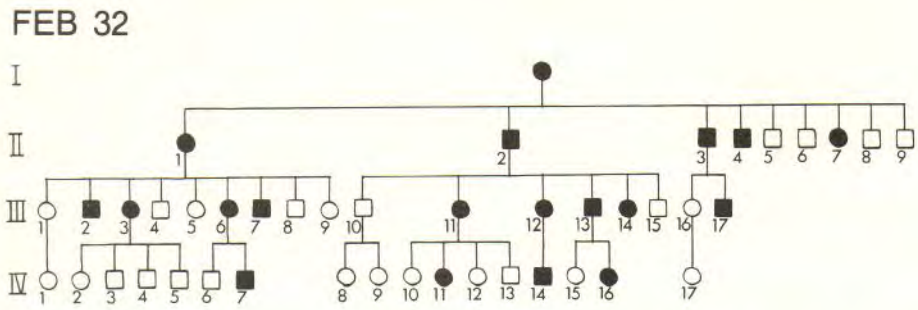



*Fig. 19*

*FEB 33; D-EBD Cockayne-Touraine.* Male proband born 1975 (III/25, Fig. 20). Nine cases were found in two generations.

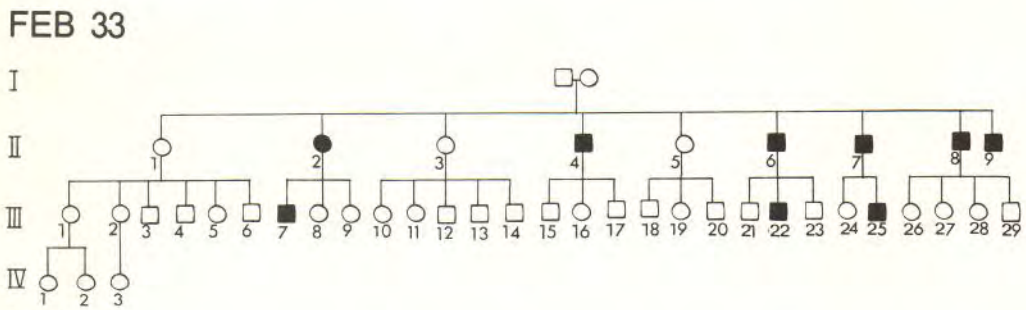



*Fig. 20*

*FEB 34; D-EBD Cockayne-Touraine.* Male proband born 1947. Four mild cases in three generations. A specimen was studied immuno-histochemically in IV.

*FEB 35; D-EBD Cockayne-Touraine.* Male proband (VI/1) born 1959 (Fig. 21). Ten members in seven generations had had EB. The proband had scars and contractures on the palms, an unusual location for such features. This was the result of considerable mechanical strain because of his job as an iron plate worker.

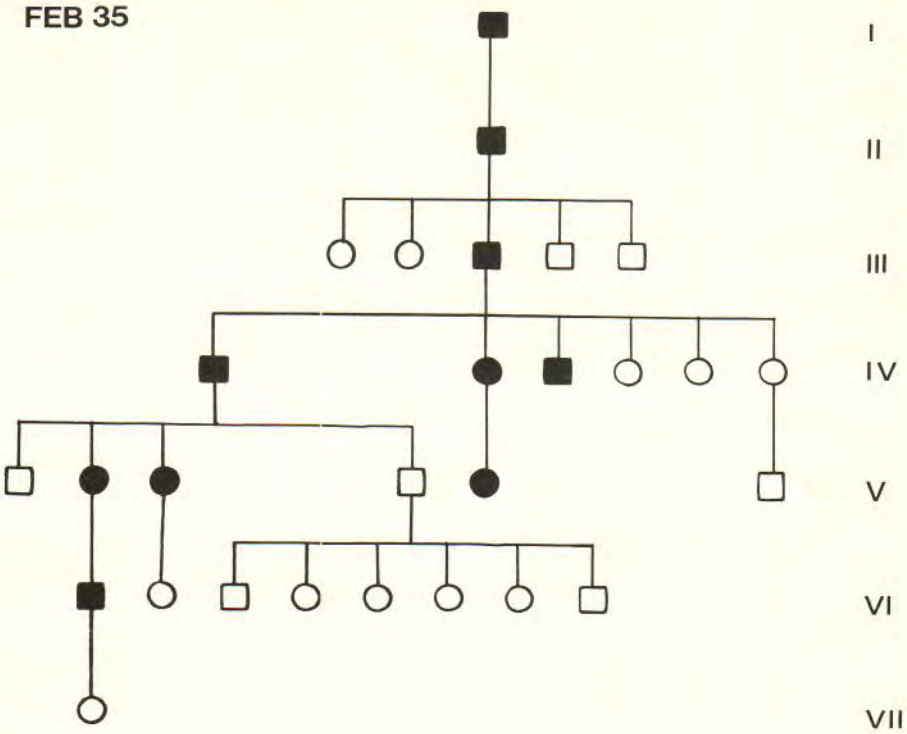



*Fig. 21*

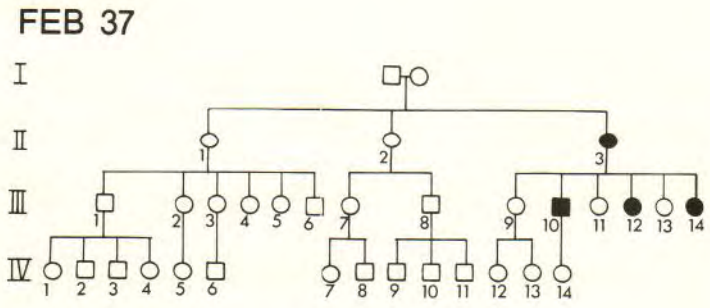



*Fig. 22*

*FEB 36; D-EBD Cockayne-Touraine.* Male proband born 1935. His father had also had the same disease. According to the proband his bullous eruptions required a strong tangential component of the blow to the skin.

*FEB 37; D-EBD Cockayne-Touraine.* Female proband (II/3) born 1920 (Fig. 22). Three of her six children were also typical cases of this disease.

*FEB 38; D-EBD Cockayne-Touraine.* Male proband born 1978, a sporadic case, who had a healthy twin brother. Their father was not known.

*FEB 39; R-EBD inversa.* Female proband born 1962, with one healthy brother. She had suffered since birth from susceptibility of the skin to mechanical traumas. Cleavage of the skin was most pronounced in a belt-like zone on the lower body and inguinal areas, but was also present on the

axillas and bends of the knees. The blisters healed leaving atrophic scars.(Fig 23)

Physically, the girl was retarded for her age. At the age of 11 she was 133 cm tall and weighed 24 kg. The mucous membranes of the mouth had always been broken, causing pharyngeal irritation and a hoarse cough. The teeth had decayed. There was syndactyly between the left II-III toes. She also had severe anaemia, which only partly responded to iron treatment. The amount of active collagenase was increased (V). The worst personal problem was ailing evacuation, which could take many hours because of pain. No consanguinity could be demonstrated between the parents.

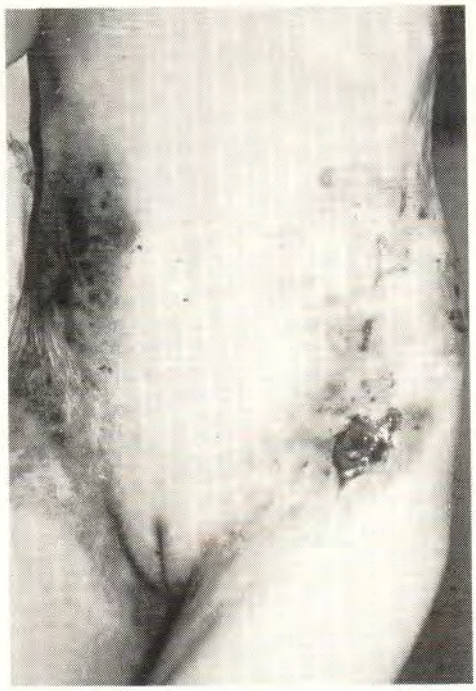
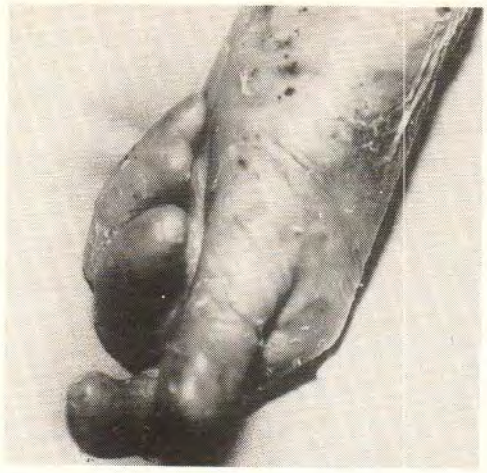


*Fig. 23, FEB 39; R-EBD inversa.*

*FEB 40; R-EBD Hallopeau-Siemens (mutilans).* Male proband born 1965, with one healthy brother. He had both histologically and clinically typical features as well as an immunohistology of dermal splitting (IV). The patient was small-sized and the amount of active collagenase was increased (V). He was anaemic and his teeth had decayed. The healing blisters left scars, causing joint contractures and oral and oesophageal strictures. The fingers and toes had fused together by pseudo-webbing of scar-tissue forming club-like bags(Figs. 24, 25).

Only the distal phalanges of the thumbs and index were unconstrained. In spite of this, the boy played the piano skilfully.



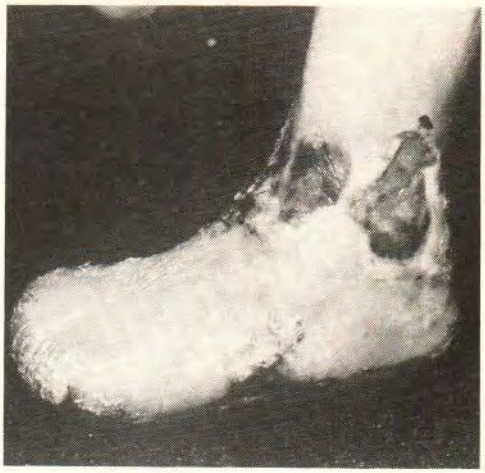


*Fig. 24 and 25, FEB 40; R-EBD Hallopeau-Siemens (mutilams)*

The author has been informed of three additional D-EBS Köbner families, one EBS Weber-Cockayne familyand two A-EBD families since 1980 which are not included in this survey.

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