EPIDERMOLYSIS BULLOSA IN SOUTH AFRICA

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C O N T E N T S

DECLARATION

This thesis is submitted for the degree of Doctor of Medicine of the University of Cape Town. The study was performed by the author under supervision of Professor Peter Beighton of the Department of Human Genetics, University of Cape Town

Signed

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August 1986

DEDICATION

To my husband, Rocky Gordon

ABSTRACT

A nationwide survey of Epidermolysis Bullosa in South Africa has been undertaken. Eighty-two affected persons in forty families have been investigated and the resultant findings are analysed and presented in this thesis. Current concepts of genotypic and phenotypic heterogeneity have been supported, while the invariability of clinical manifestations of EB in any particular kindred are confirmed.

Electron microscopic examination of the skin was performed with appropriate clinicopathological correlation. The ultrastructural changes in each subtype were consistent with those of other reported series. A new specific biopsy technique for the removal of optimal skin samples in EB has been developed.

A collagenase assay was established but this did not add any significant information to the investigation. Extensive linkage studies using histocompatibility antigens, red cell groups and enzymes as well as a molecular restriction fragment length polymorphism (L7) did not demonstrate positive linkage in any subtype of EB.

The management of EB remains problematic. However experience in this study suggests that the dilatation of strictures consequent upon oesophageal blistering in autosomal recessive EB Dystrophica is more successful and less hazardous than previously believed.

The skin lesions of EB had been mistakenly interpreted as child abuse in 3 children. The social implications of this chronic debilitating disorder are profound and special attention was paid to these aspects of EB.

Currently twenty-six of subtypes of EB have been delineated. A new unique South African subtype designated EB Dystrophica Generalisata Gravis is an autonomous entity, distinct from any subtype hitherto reported.

A classification of EB has been established in order to clarify the major and minor subdivisions of EB. In addition to a formal classification, a system of flow charts in the form of an algorithm has been devised to simplify the accurate diagnosis of any subtype of EB.

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ABBREVIATIONS USED IN THIS THESIS

EB	Epidermolysis Bullosa
EBS	Epidermolysis Bullosa Simplex
EBA	Epidermolysis Bullosa Atrophicans
DDEB	Autosomal Dominant Epidermolysis Bullosa Dystrophica
RDEB	Autosomal Recessive Epidermolysis Bullosa Dystrophica
EBSK	Epidermolysis Bullosa Simplex Koebner
EBSWE	Epidermolysis Bullosa Simplex Weber Cockayne
EBSO	Epidermolysis Bullosa Simplex Ogna
EBSHDM	Epidermolysis Bullosa Simplex Herpetiformis Dowling Meara
EBSMP	Epidermolysis Bullosa Simplex with Mottled Pigmentation
EBSN	Epidermolysis Bullosa Simplex Niemi
EBSL	Epidermolysis Bullosa Simplex (Lethal)
EBSMC	Epidermolysis Bullosa Simplex Mendes de Costa
EBAGG	Epidermolysis Bullosa Atrophicans Generalisata Gravis
EBAPA	Epidermolysis Bullosa Atrophicans with Pyloric Atresia
EBAI	Epidermolysis Bullosa Atrophicans Inversa
EBAGM	Epidermolysis Bullosa Atrophicans Generalisata Mitis
EBAL	Epidermolysis Bullosa Atrophicans Localisata
EBAM	Epidermolysis Bullosa Atrophicans with Muscular Atrophy
GABEB	Generalised Atrophic Benign Epidermolysis Bullosa
EBJD	Epidermolysis Bullosa Junctionalis, Disentis
EBDCT	Epidermolysis Bullosa Dystrophica Cockayne Touraine
EBDPA	Epidermolysis Bullosa Dystrophica Pasini (Albopapuloidia)
EBDP	Epidermolysis Bullosa Dystrophica Pretibial

EBDB	Epidermolysis Bullosa Dystrophica Bart
EBDI	Epidermolysis Bullosa Dystrophica Inversa
RDEBP	Epidermolysis Bullosa Dystrophica Progressiva
EBDHS	Epidermolysis Bullosa Dystrophica Hallopeau Siemens
EBDF	Epidermolysis Bullosa Dystrophica Fine
EBDGGW	Epidermolysis Bullosa Dystrophica Generalisata Gravis (Winship)

cm = centimetres g = micrograms

mm = millimetres ml = millilitres

GGT	Galactosyl hydroxylysyl glucosyl transferase
GPT	Glutanic - pyruvic transaminase
Glo-I	Glyoxalase-I
HLA	Histocompatibility Antigens
RFLP	Restriction Fragment Length Polymorphism
LOD	Logarithm of the odds
AD	Autosomal dominant
AR	Autosomal recessive
XL	XLinked
XLR	XLinked recessive
cM	centimorgans
CAMP	Cyclic adenosine monophosphate
CEA	Carcinoembryonic antigen

SECTION I

A BACKGROUND TO EPIDERMOLYSIS BULLOSA

CHAPTER 1 INTRODUCTION

1.1 INTRODUCTION TO EPIDERMOLYSIS BULLOSA

Epidermolysis Bullosa (EB) is a group of genetically determined disorders characterised by blistering of the skin and mucous membranes. These blisters may arise spontaneously or as a result of minor frictional trauma and this propensity has led to the use of the descriptive term "mechanobullous diseases" for these genodermatoses.

The different subtypes comprising EB are genetically distinct entities with specific clinical, histopathological and biochemical features. McKusick (1983) listed 15 different forms of EB in the 6th Edition of the catalogue of genetic disorders, Mendelian Inheritance in Man. By 1986, 26 subtypes had been delineated; these will be further outlined in the relevant chapters of this thesis.

Three major groups of EB are identified clinically according to the presence or absence of scarring and nail dystrophy. These are the **simplex** (non-scarring), **atrophic** (non-scarring with skin atrophy) and **dystrophic** (scarring) forms. These types are further categorised into minor subgroups according to the mode of inheritance and additional clinical concomitants, as well as the ultrastructural and biochemical features of the skin.

There is considerable variation in the clinical manifestations in the different subtypes of EB, which range in severity from the mild non-scarring through a spectrum of physically debilitating disorders to the lethal type. Involvement of the mucous membranes may lead to severe, even life-threatening complications.

Despite 100 years of investigation into EB, the basic defect has not been elucidated. However, the clinical, genetic and ultrastructural abnormalities have been determined and methods of prenatal diagnosis can now be employed in certain subtypes.

EB is a clinically significant genodermatosis and the continued investigation of all aspects of this disorder are important to the clinician, geneticist, basic scientist, and most notably, the affected individuals.

1.3 THESIS LAYOUT PLAN

This thesis comprises a nationwide study of 82 persons from 40 families with EB. The clinical, genetic, histopathological and epidemiological aspects gencdermatosis have been investigated and are presented in the appropriate sections. A detailed review of world literature will be discussed with relevance to this study.

The thesis consists of 15 chapters, divided into seven sections, covering the facets of EB which have been studied. The clinical findings in each kindred are presented according to subtype in Section III, Chapter 4. Patient data will be preceded by a short resumé of the form of EB discussed. Each report consists of a kindred description and pedigree, followed by illustrative clinical details of one family member with the characteristic phenotype. Data concerning other affected persons of the family will only be further elucidated where there is specific relevance to the discussion.

The results documented in Section III are analysed in Section IV, Chapters 6 to 10. Each of these chapters will begin with a review of literature pertinent to the subject, and the results of this study will then be discussed in relation to conventional concepts.

For the sake of clarity and ease of reading, certain information regarding presentation and layout will be repeated in the appropriate chapters. Similarly, where this is relevant cross-reference from chapter to chapter will be provided.

1.2 AIMS AND OBJECTIVES OF THIS STUDY

The occurance of EB in South Africa has not been well documented and the only prior report concerned the condition in African blacks in the Transvaal (Menter and Patz 1971). These authors stated that they had not encountered EB in the other ethnic groups of South Africa.

In view of the paucity of information relating to EB in this country, a nationwide investigation was conducted in order to document the disorder in South Africa. This study comprised investigation of the following facets of Epidermolysis Bullosa:

Clinical manifestations
Epidemiology
Genetics
Histopathology
Biochemistry
Management
Social implications
The delineation of a unique South African subtype of EB

In addition new proposals for the simplification of the classification of these genodermatoses will be suggested.

CHAPTER 2. HISTORICAL BACKGROUND

2.1 DEVELOPMENT OF THE NOMENCLATURE

'**Erblichen Pemphigus**' was the term employed in 1870 by von Hebra, who described a non-scarring, traumatically-induced blistering disease in a male patient who had been affected since early childhood. Three successive generations of the family had similar skin involvement. Twelve years later Goldscheider (1882) reported eight cases of '**Hereditare Neigung zur Blasenbilding**' in four generations of a family, which he believed to be a previously undescribed condition. Similar reports were made by Valentin (1885) who coined the name '**Hereditare Dermatitis Bullosa**'.

Koebner (1886) introduced the designation '**Epidermolysis Bullosa Hereditaria**' which has become the universally accepted name for this blistering genodermatosis.

Fox (1879) described the earliest dystrophic type of this disorder when he reported two siblings whose bullous lesions resulted in scarring and milia in association with nail dystrophy.

Fatal epidermolysis bullosa was first documented by Boeck (1878) - a non-syphilitic, non-infectious blistering disease, rapidly fatal, which he named '**Pemphigus neonatorum**'.

Confusion regarding nomenclature with this heterogeneous group of conditions arose in 1893 when Herzfeld stated that Kobner's nomenclature should be used only to describe the disorder when localised to the extremities. By the turn of the century, however, Koebner's Epidermolysis Bullosa Hereditaria (EBH) was accepted as the descriptive name for all the clinical manifestations of this condition.

The importance of distinction between the scarring and non-scarring forms of EBH was recognised at an early stage. Hallopeau (1898) differentiated between '**form bulleuse simple**' and '**form bulleuse et Dystrophique**'. At that time the distinction was not clear and it was not until 1921 that Siemens interpreted EBH in terms of Mendelian inheritance. He demonstrated an association between autosomal dominant inheritance and intra-epidermal blistering without scarring, and autosomal recessive inheritance with subepidermal blistering and scarring or nail dystrophies. This concept led to the introduction of the classification of these two types, on clinical and genetic grounds, as EB Simplex and EB Dystrophica. Siemens (1936) made the observation that EBS and EBD do not appear to occur in the same family.

Hoffman (1926) noted a form of dystrophic EB manifesting in an autosomal dominant pattern in a number of families. This concept was supported by Cockayne (1983) who studied 43 families with dominantly inherited EB Dystrophica. He recorded that their manifestations were of moderate severity, ranging somewhere between the dominant simplex and recessive dystrophic forms.

In 1928 a new type of EB was described by two independent workers, Pasini and Maschkilleison. This was a dystrophic form manifesting with albopapuloid lesions independent of the bullae. This variety was labelled as **EBD Albopapuloidea** and is now listed as an accepted subtype of the condition.

Boeck's fatal '**pemphigus neonatorum**' had meanwhile been reported again in the 1920's. Mautner (1922) and Kuse (1929) both named this condition '**Pemphigus hereditarius**', while Jenny (1927) described his patients as having a '**letal verlaufende form**' of EBH. Muller (1927, 1929) named this disorder EB Congenita. In 1933 Cockayne together with Siemens, agreed that this entity was a separate form of EB, but confusion persisted until 1935 when Herlitz introduced the term **EB letalis.** He differentiated this from EBD on grounds of lethal prognosis and healing of blisters without scarring. Herlitz also confirmed the autosomal recessive mode of inheritance from his study of eight cases in three families.

Even at this stage of the evolution of concepts concerning EB, heterogeneity was well recognised. In addition to the differentiation between scarring and non-scarring types, it was clear that further subtypes existed within these major divisions.

Readett (1961) described a family with an autosomal dominant pattern of transmission of EBS in whom the lesions were localised to the hands and feet. This condition acquired the descriptive designation of **Weber-Cockayne-type EB.** Davison in 1965 stated that this description applied only when the lesions of EB were confined to the feet.

Dowling and Meara (1954) described a non-scarring epidermolysis which presented in infancy and ameliorated by the seventh year of age. The phenotype strongly resembled dermatitis herpetiformis Duhring. This autosomal dominant form is now classified amongst the AD non-scarring variety as **EBS Herpetiformis Dowling Meara.**

2.1.1 Modern concepts

Bart and co-workers (1960) described yet another new variant of EB, in which bullous lesions were associated with congenital absence of the skin. The pattern of inheritance was autosomal dominant. This condition has been labelled as **Bart syndrome**, or as **EB Dystrophica Dominans (Bart)**.

Gedde Dahl (1971) presented his thesis based upon a study of EBH in Norway, in which he described three previously unrecorded types of EB:

EBS, Ogna type, which has been found only in one large Scandinavian family. This disorder differs from the other simplex forms by virtue of the distinctive association of skin bruising with the bulla formation.

EBD inversa, in which blistering and resultant dystrophy occur on the trunk, neck, thighs and legs without affecting the feet, hands, elbows or knees, as in the other dystrophic forms of EB.

EB Progressiva or **EBD Neurotrophica**, which had onset of bullae in late childhood, in association with a congenital and slowly progressive perceptive deafness. Subsequently, a family in the same population was noted to have the skin

changes but no hearing problems. Gedde Dahl suggested that these two syndromic features might be the consequence of gene defects at closely linked but separate loci.

Yet another form of EB was delineated in 1975 when Garcia and Perez described **pretibial EB** in two kindreds, a subtype which had been diagnosed earlier by Kuske (1946) and Portugal and Jacintho (1956).

Hashimoto (1976) reported an individual with EB Atrophicans who had survived into adulthood. Previously all persons with this subtype had died in infancy. This type, which he named **EB Junctionalis, Disentis type**, is possibly allelic with **EB Letalis.**

Fischer and Gedde Dahl (1979) reported a large family with autosomal dominant EBS in which ten family members with non-scarring EB presented with mottled changes of hyper- and hypo-pigmentation.

Hintner and Wolff (1982) studied eight patients with a third form of junctional EB, **generalised atrophic benign EB.** This is an autosomal recessive variety in which blisters heal without scarring but may result in skin atrophy. Unlike the infantile **EB Letalis**, the prognosis is good in this disorder.

The syndromic association of **Epidermolysis Bullosa Letalis** and pyloric atresia, reported by Bull (1980) was first recognised as a distinct entity by Korber (1977). Honig (1983) described an infant with EB letalis in whom pyloric obstruction occurred at the age of one month.

Niemi (1983) described yet another histological subtype of EBS in two patients. As yet the pattern of inheritance of this form is unknown.

Subtypes of EB which have been delineated more recently times will be discussed in Chapter 12, under the heading 'Classification'.

2.2 THE BASIC DEFECT

2.2.1 Historical views

A wide variety of theories have been put forward in an attempt to explain the basic defect in EB. Initially, at the turn of the century, it was suggested that a toxin within the body had the effect of diminishing the resistance of the skin. Various postulates of abnormalities of the nervous system and/or vasculature were made but could not be substantiated. An abnormality in elastin was long believed to be the underlying fault.

Engman and Mook (1906) suggested that the basic defect was an absence or underdevelopment of elastic fibres in the skin. This view was supported into the 1950's (Leoni, Dorn), but it is now widely accepted that there are no abnormalities in elastin in EB. Pearson (1962), studying the autosomal recessive type of EBD, (RDEB), advanced the hypothesis that there might be a collagen abnormality, or that the problem was caused by activity of a collagenase-like substance. Gedde Dahl (1966) studied haemostasis in EB following a claim by Tio (1963) that there might be abnormalities of the coagulation mechanism. However, he found no significant deviation from normal.

2.2.2 Biochemical defects

Despite extensive investigation, Fischer and Lodin (1966) could not demonstrate any specific biochemical or haematological abnormalities in EB. Rochman (1979) found raised serum plasma Carcinoembryonic antigen (CEA) in patients with RDEB. In the same year Bauer noted raised glycosaminoglycan accumulation in fibroblast cultures from patients with RDEB.

From the work of Bauer (1972, 1978), Eisen (1969, 1971), Stricklin (1978) and others, Pearson's earlier suggestion that a collagenase-like substance might be involved in the pathogenesis has been borne out and it is now well recognised that the rate of collagenase synthesis is increased in RDEB.

Savolainein (1981) described a deficiency of galactosyl hydroxylysyl glucosyl transferase, an enzyme which is involved in collagen synthesis, in a family with dominant EB Simplex.

The biochemistry of this genodermatosis is constantly under study in many centres but so far the basic defect has not been identified.

2.2.3 Ultrastructural defects

The clinical presentation of EB is complex and variable and it has long been accepted that histopathological description of EB and its subtypes is necessary for delineation and classification. It was recognised early that conventional light microscopy was inadequate for the differentiation of the various forms of EB, notably the junctional and dystrophic types. Since the advent of electron microscopy meaningful contributions have been made to histopathology, and Pearson (1961) commented that electron microscopy revealed distinctive pathological features in each form of the condition. At this time it was widely accepted that the level of cleavage with relation to the dermo-epidermal junction and resultant level of blister formation was the major diagnostic determinant in the various types of EB.

Pearson, in conjunction with Spargo (1961), recognised disintegration of the basal and suprabasilar cells. They attributed this feature to the release of a necrotising agent in the dermis and epidermis as a result of trauma, as well as an inherent weakness of cell structure.

Roberts and his co-workers (1966) suggested that the defect in the lethal recessive form was vesicular degeneration of the basal cells of the epidermis, which led to cleavage at the dermo-epidermal junction.

Arwell, Bergenholtz and Thilander (1968) added to the information concerning EB Letalis, where the level of cleavage is in the lamina lucida. They noted an structural abnormality in the hemidesmosomes, which was confirmed by Hashimoto (1976). In the benign junctional form of EB this lack of hemidesmosomes was also thought to play a part, although Tidman and Eady (1984) maintain that normal hemidesmosomes may be present in the regions of decreased dermo-epidermal adhesion.

Anton Lamprecht and Schnyder (1973) demonstrated that anchoring fibrils were missing in the junctional area beneath the basal lamina in dominant dystrophic EB, where other junctional structures were normal. The blistering in this type is subepidermal.

Briggaman and Wheeler (1975) observed abnormalities of the anchoring fibrils in RDEB. Hashimoto, Anton Lamprecht and Gedde Dahl in the same year reported that these anchoring fibrils did, however, differ in the autosomal dominant and autosomal recessive types. The present situation is confused as Tidman and Eady (1985) in a more recent study could not distinguish between these genetic entities by examination of anchoring fibrils.

In contrast to the other forms of the disorder, the basal layer with the basal cells remains relatively intact in EB Simplex apart from subnuclear splits. The dermo-epidermal junction is well developed with numerous hemidesmosomes, anchoring fibres and normal basal lamina.

The histopathological changes in the variants of EB will be described in detail in Chapter 10.

2.3 MANAGEMENT

The management of EB is difficult. Many and varied approaches have been attempted, but none have proved to be completely effective.

Oral and topical antibiotics and steroids, as well as Vitamin E have been used with varying degrees of efficacy. Plastic surgical repair has been attempted where lesions have impaired the patient's functional ability.

Human growth hormone replacement has failed to produce any beneficial effect in the management of RDEB. Most recently, pharmacological doses of phenytoin, an anti-epileptic agent, have been shown to inhibit collagenase activity and there have been reports of improvement in the skin lesions of infants with both RDEB and EBA treated in this way.

Current management will be further discussed in Section 6, Chapter 13.

2.4 EPIDERMOLYSIS BULLOSA ACQUISITA

A review of EB would be incomplete without mention of the acquired form of EB. This non genetic entity, Epidermolysis Bullosa Acquisita, is defined by strict diagnostic criteria (Roenigk, 1971):-

i) Bullae over joints of hands, feet, elbows and knees secondary to minor trauma.

ii) Atrophic scars, milia, nail dystrophies.

iii) Adult onset.

iv) Negative family history for EB.

v) Exclusion of other bullous disease (lichen planus, erythema multiforme and bullous drug eruption).

More recently additional criteria have been laid down by Woolley (1983) for the diagnosis of EB Acquisita.

Relative Criteria

Acral Distribution
Skin Fragility
Lack of inflammation
If inflammatory, predominant neurophils
Relative steroid resistance
Associated systemic disease

Specific Criteria

Separation site of blister is below the lamina densa

Circulating IgG antibodies directed against a sub-lamina densa component of normal skin

Immunoglobulin and complement deposits immediately below the lamina densa

Milia formation

Scar formation

Lack of family history for bullous diseases

Adult onset of disease.

EB Acquisita was first described by Fox (1897) and subsequently named by Kablitz (1904). The pathogenesis is not fully understood but associations have been noted with inflammatory bowel disease, especially Crohn's disease, tuberculosis, systemic lupus erythematosus, renal dialysis and high dose furosemide therapy.

Since this thesis is concerned with the genetic forms of EB, no further discussion on EB Acquisita will be included.

SECTION II

METHODOLOGY

CHAPTER 3 METHODOLOGY

CHAPTER 3 METHODOLOGY

3.1 COLLECTION OF DATA - PATIENT ASCERTAINMENT

3.2 ASSESSMENT OF PATIENTS

3.2.1 Clinical evaluation

3.2.2 Diagnostic criteria

3.3 SPECIAL INVESTIGATIONS

3.3.1 Histopathological investigation

3.3.2 Biochemical investigation

3.3.3 Linkage analysis

3.1 PATIENT ASCERTAINMENT

An attempt was made to identify all persons in South Africa with Epidermolysis Bullosa. The following data sources were employed:

Data sources

a) A computerised hospital record system for the academic hospitals in the Western Cape.

b) Record systems from other major hospitals throughout the country.

c) Departments of Human Genetics, Dermatology and Paediatrics at the University Medical Schools and teaching hospitals.

d) A circular requesting patient referral was sent to every dermatologist and paediatrician on the South African Medical Register.

e) A letter was published in the South African Medical Journal inviting the referral of patients.

f) A review article on EB appeared in the South African Medical Journal, in which the request for patient referral was repeated (Winship 1986).

(Copies of documents are included in the Appendix.)

3.2 ASSESSMENT OF PATIENTS

3.2.1 Clinical evaluation

All patients referred for the study were evaluated according to a specially designed protocol and the findings were recorded on a proforma, a copy of which is included in the Appendix.

A detailed history of each patient was recorded with special emphasis on pedigree data. Important facts which were established included age of onset, precipitating factors in the formation of blisters, and anatomical distribution of lesions. The occurrence of complications, most notably scarring, and the course or natural history of the skin lesions were documented. Details of the nature and effects of previous treatment were recorded.

Physical examination was performed using standard clinical procedures. Detailed appraisal of the skin and its appendages focused on the distribution and morphology of the lesions. The severity of EB in each patient was assessed according to the status of acute lesions as well as the presence and extent of scarring. Examination of all other systems was undertaken to exclude physical signed of disease, either unrelated or in association with EB. Clinical photography formed an important adjunct to clinical assessment.

3.2.2 Diagnostic criteria

The clinical evaluation of a patient is the most important factor in the diagnosis of EB.

The characteristic presentation of EB is the formation of bullous skin lesions as a result of minimal trauma to the skin. Similar changes may occur in other conditions, which must be excluded.

Onset in infancy is usual, though the milder, more localised types of EB may only develop after the affected person becomes ambulant. Indeed, the autosomal recessive dystrophic form, **EB progressiva** may present as late as adolescence.

Areas of absence or aplasia of skin are useful diagnostic indicators if associated with bullous formation, though differentiation between widespread aplasia cutis congenita and EB with congenital absence of skin may pose a diagnostic dilemma in the neonatal period.

The nails of the fingers and toes may be involved. The subungual blister formation, shedding of nails, and dystrophic nails are important clinical signs in the diagnosis of EB. In addition, bullae of the oral mucosa, nasopharynx, trachea, oesophagus and tongue serve as useful pointers.

Patients had to fulfill the established clinical criteria before being included in the present study. Diagnosis was confirmed against a "check list" of important positive and negative features:

i) Traumatically induced bullous formation; spontaneous blistering.

ii) Increased skin fragility as a concomitant to a blister formation.

iii) Repeated development of bullous lesions.

iv) Absence of an infective agent or systemic illness preceding onset.

v) Absence of intake of drugs or toxic agents.

vi) Absence of evidence of photosensitisation of skin as a cause for blistering.

vii) Clear serous or serosanguineous content of blister fluid, without evidence of infections.

viii) Exclusion, where necessary, of other conditions entering into the differential diagnosis of EB, notably aplasia cutis congenita, cutaneous porphyria, bullous pemphigoid.

ix) The presence of a positive family history for EB.

Further criteria were applied for differentiation into major and minor subgroups of EB:

i) The presence of scarring, milia or atrophy of the skin.

ii) Involvement of nails of fingers or toes, including shedding and dystrophy of nails.

iii) Bullae formation within mucous membranes.

iv) Involvement of the teeth.

v) Gastro-intestinal involvement.

vi) Bruising associated with lesions.

vii) Age of onset of EB.

viii) Distribution of lesions and predilection sites.

ix) Clinical severity.

x) Death in early infancy.

xi) Pattern of inheritance as established in a kindred.

xii) Advanced paternal age effect.

xiii) Parental consanguinity.

3.3 SPECIAL INVESTIGATIONS

Special investigations were carried out on patients with specific types of EB, as outlined below.

3.3.1 Histopathological investigation

Two elliptical skin samples were obtained from the patients examined in this way. An area of 'normal' skin ± 3 mm in diameter was removed from the medial aspect of the upper arm. The 'abnormal' biopsy consisted of the edge of a very fresh blister. If no such lesion was present, a blister was induced by rubbing the medial aspect of the upper arm for several minutes with a soft pencil eraser. The area of skin to be biopsied was anaesthetised using 2% lignocaine (without adrenalin). A 23 guage needle was then inserted into the required area of skin, and the sample portion lifted off with a scalpel. Forceps were not used because of the damage caused to the ultrastructure by their pinching forces. Punch biopsy is unsuitable in the diagnostic evaluation of EB because the torque action of the punch may cause separation of the epidermis, leading to potentially inaccurate results.

The skin sample was placed immediately in Karnovsky's fixative (see Appendix for formula) using a soft probe to remove it from the blade edge. It was then divided into sections one millimetre in width with a blade, working in a drop of fixative on a soft dental wax surface to avoid the stress which would be imposed on the skin by cutting on a hard surface. The specimens were then fixed, post-fixed, dehydrated, impregnated, and polymerised using standard methods employed in transmission electron microscopy. Details of the preparation of blocks are provided in the Appendix. Ultra-thin sections were cut and stained using urylacetate and lead citrate, using prior light microscopy for "survey section" to isolate the area of interest.

These sections were studied electromicroscopically and the features of 'normal' and 'blistered' skin were complared. Examination was undertaken using a Phillips 420 Transmission Electron Microscope.

3.3.3 Biochemical investigation

Fibroblasts were cultured from a sample of unaffected skin for the assay of collagenase. A portion of the 'normal' biopsy specimen obtained for histopathological examination was divided off prior to fixation, under sterile conditions. This investigation was undertaken in patients with autosomal recessive EB Dystrophica (RDEB).

Fibroblasts were cultured under standard sterile tissue culture conditions. Nutrient mixture F-10 Ham IX with 1-glutamine supplemented with 20% foetal calf serum was used during the initial stages until the primary culture had been trypsinised and plated onto large petri dishes. Thereafter the culture was maintained with Hams F10 supplemented with 10% foetal calf serum until the cultures were sufficiently confluent ($+$ - 1 000 000 cells) for the collagenase assays to be undertaken. Cystapen, streptomycin sulphate, tylosin and phenol red were also added to the F10 medium throughout. Cultures were initiated on one cm. square sterile coverslips and then passaged after $+$ - four weeks onto 85 mm. diameter plastic petri dishes.

Collagenous extracellular matrix (ECM) covered petri dishes were prepared for the assay of collagenolytic activity of these fibroblasts.

Rat arterial smooth muscle cells were cultured for 14 days in 35 mm. petri dishes in the presence of 50 µg/ml. of ascorbic acid which was added daily (Jones et al 1979). After 10 days of culture, cells were grown in the presence of 10 µg /ml (3/f) proline in addition to ascorbic acid. At the end of the culture period (14 days) the medium was removed and the cells washed three times with phosphate buffered saline (PBS). Addition of 25 mm NH_4OH to cell layers resulted in complete lysis of all cells and cell debris and intracellular material was removed by repeated washing and aspiration using sterile distilled water. Matrix layers were then treated with a sterile solution of elastase (50 µg./ml.) in 50 mm Tris HCl (pH 8.0) for 12 hours to remove all but the collagen component of the ECM (Jones et al 1979). After removal of elastase solutions and further repeated washings, ECM-coated were ready to be used for collagenolytic assay procedures (Jones and Scott-Burden 1979).

Confluent cultures were passaged by trypsinisation and used for the preparation of conditioned medium or were cultured directly upon the ECM-coated dishes. 100 ml aliquots of either medium were removed from the ECM-coated dishes and counted to assess release of degraded collagenous peptides. In all experiments, controls that consisted of eith fresh unconditioned medium or normal skin fibroblasts added to ECM-coated dishes, were employed.

3.3.3 Linkage analysis

Conventional Markers

Linkage analysis with conventional gene markers was undertaken on families with autosomal dominant inheritance of EB. The following systems were employed:

i) HLA typing

ii) Red cell enzymes

iii) Serum proteins

iv) Blood grouping

These tests were performed by staff of the Provincial Laboratory for Tissue Immunology, Cape Town, and were carried out using standard techniques.

Restriction Fragment Length Polymorphisms

Both the genes for human skin collagenase and for RDEB have been assigned to Chromosome 11 (Church 1983). L7, a polymorphic single copy genomic clone localised to 11q23 (Warnich 1985) was used in the linkage analysis of five families in which the proband had recessive dystrophic EB. L7 identifies a simple two allele polymorphism with a band at either 13,9 kb (A1) or 10,9 kb (A2) at a frequency of 0,24 for the minor allele A1.

Genomic DNA was isolated from whole blood of the probands in these families, their parents and siblings. Blot hybridisation analysis of this DNA was performed using the methods elaborated by van den Plas et al (1984).

SECTION III

CHAPTER 4 CLINICAL FINDINGS

CHAPTER 5 RESULTS OF SPECIAL INVESTIGATIONS

CHAPTER 4 CLINICAL FINDINGS

4.1 INTRODUCTION

4.2 EPIDERMOLYSIS BULLOSA SIMPLEX

4.2.1 EBS Koebner

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4.4.1 Autosomal Dominant EB Dystrophica

4.4.1.1 EBD Cockayne Touraine

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4.5 A SOUTH AFRICAN SUBTYPE: EBD GENERALISATA GRAVIS

4.6 UNCLASSIFIABLE EB

4.1 INTRODUCTION: THE AFFECTED FAMILIES

82 affected persons from 40 families were identified according to the protocol elaborated in Section 2 (Methodology). These individuals all fulfilled the clinical criteria for the diagnosis of EB and each entered into the study with fully informed consent. Permission was obtained for special investigation of their biological material, for clinical photography and for publication of data and photographs.

Case reports of the persons seen in this study are presented as family units. The family history and pedigree precede a short description of the medical history and clinical findings of a specific person who exemplified the characteristic features of EB in that kindred. Details are confined to a description of the skin, with the addition of any relevant systemic involvement. Mention of other family members is limited to specific relevant details.

Case reports are be presented according to the clinical subtype, and a brief resumé of each broad category of EB precedes details of affected persons with that subtype of the condition. This chapter includes purely clinical data. The results of special investigations will be presented in Chapter 5.

4.2 EB SIMPLEX

EB Simplex (EBS) is the non-scarring form of this genodermatosis and is distinct from the other types.

The onset of bullae formation is usually at birth or in early infancy. Nails are rarely involved but if subungual blistering results in the shedding of nails, normal regeneration ensues. The dentition is normal. Life expectancy is not decreased in EBS, although quality of life may be markedly impaired.

Inheritance in the simplex forms is usually autosomal dominant. Clinical diagnosis is confirmed by histopathological demonstration of intra-epidermal blister formation.

Several forms of EB Simplex have been recognised - their distinguishing clinical features are presented in Table 4-I. Eponyms are listed using standard nomenclature.

TABLE 4-I

SUBTYPE	AGE OF ONSET	IN-HERI-TANCE	PROG-NOSIS	DISTRIBUTION OF LESIONS	CLINICAL CONCOMITANTS
1. EB Simplex Koebner (EBSK)	Birth	AD	Good	Generalised	-
2. EBS Weber Cockayne (EBSWC)	Infancy	AD	Good	Hands & feet only	-
3. EBS with GGT deficiency	Birth	AD	Good	Generalised	-
4. EBS Ogna	Birth	AD	Good	Generalised	Onygryphosis of great toes. Bruising with blisters
5. EBS with mottled pigmentation	Birth	AD	Good	Mainly limbs	Speckled hyper-pigmentation
6. EBS Herpetiformis Dowling Meara	Birth	AD	Good	Herpetiformis grouping of lesions con,junctival involvement	EB ameliorates during fever
7. EBS Niemi	Birth	AD	Good	Generalised	-
8. EBS, lethal type	Birth	?AR	Lethal	Generalised	Death one year
9. EBS Mendes da Costa	Infancy	XLR	Good	Mainly extremi-ties	Pigmentation; microcephaly

* Glucosylgalactosyl hydroxylysine, an enzyme that catalyses glucosylation of galactosyl hydroxylysyl residues in collagen biosynthesis.

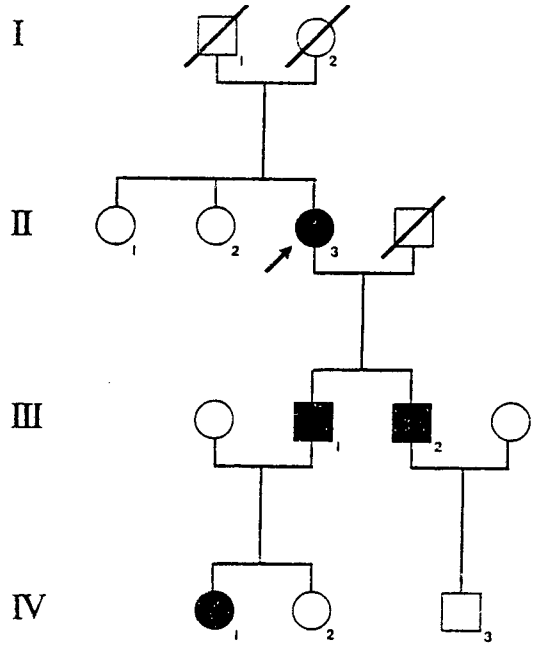
4.2.1 EBS Koebner

4.2.1.1 FAMILY 1

Family 1 consists of four affected persons in three generations of a South African family of British and German origin.

The proband was the second of three sisters; neither her parents nor her sisters were affected. Her father was 41 years of age at the time of her birth. Both sons of the proband and one granddaughter had EB.

Pedigree:



The proband, a 60 year old woman, experienced excessive blistering on her feet after she became ambulant at the age of one year. Blister formation resulted from any minor trauma or friction, or ill-fitting clothing or footwear. Healing, though slow, was complete and the lesions did not leave scars.

The course of the blistering was static until about 14 years of age. Thereafter there was slight improvement though propensity to form lesions has persisted into middle age. Bulla formation was markedly increased in the hot weather and a frequent complaint was lesions on the buttocks from long periods sitting on hard seats as a cricket spectator in the summer! She has had no systemic involvement nor have her nails been affected.

Treatment has been attempted with topical steroids and antibiotic ointments with no real effect.

Her skin was soft but of normal appearance with slight tanning on sun-exposed areas. There were no other pigmentary changes and no areas of scarring. She had two small blisters, one on the right great toe and one under the right breast. Both were attributed to friction caused by clothing.

The nails on both fingers and toes were quite normal and there was no subungal blistering. Examination of eyes, teeth and hair were within normal limits.

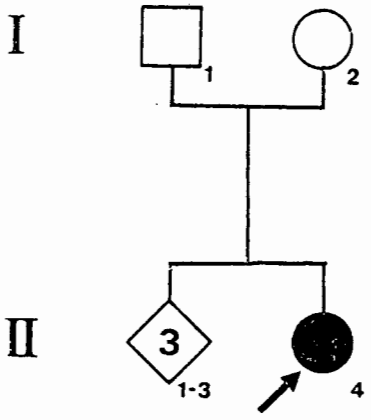
Subsequent examination of the other three affected persons in her family revealed identical findings, which were in keeping with a clinical diagnosis of Epidermolysis Bullosa Simplex.

Comment: The pedigree illustrates autosomal dominant inheritance. II-3 is the first affected person in the kindred and it is concluded that this is the consequence of new mutation of the gene. The advanced paternal age is compatible with the occurrence of new mutation. Despite the fact that onset was in late infancy and not at birth, the generalised distribution of non scarring bullae categorises this family to the subtype **EBS Koebner**.

4.2.1.2 FAMILY 2

The family were South Africans who were originally of European stock. There was no consanguinity and both parents were young.

Pedigree:



The proband was an eight month old infant female who presented with tiny areas of absent skin on the feet at the time of birth. From the second day, blistering of hands, feet, face and ears began, being induced by minor friction. The oral mucosa and tongue blistered, and areas within the mouth desquamated and were shed. Blisters formed underneath the nails of fingers and toes, which were lost. This blistering continued, with rapid healing without scarring and normal nails have regenerated.

Examination revealed the typical lesions of EB with involvement of face, hands, feet and groins. No scarring was present and nails were not dystrophic. A diagnosis of EBS **Koebner** was made.

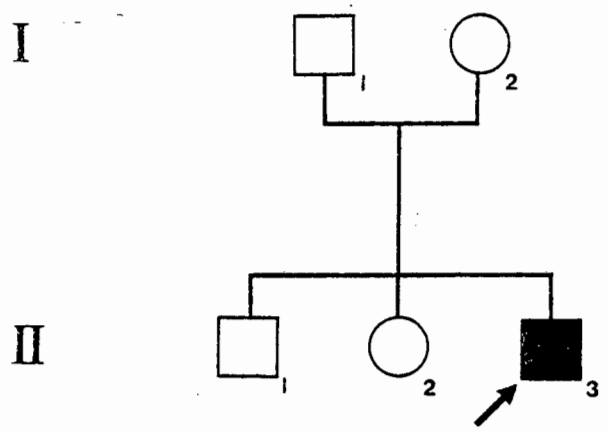
Comment: EBS is usually inherited as an autosomal dominant trait. In the absence of a family history of EB, this patient may manifest a new mutation of the gene, although there was no advanced paternal age. Autosomal recessive inheritance cannot be definitely excluded.

4.2.2 EBS Weber Cockayne

4.2.2.1 FAMILY 1

The family is South African of British and German stock. There is neither consanguinity nor advanced parental age.

Pedigree:



The proband was a 21 year old man who presented initially, at the age of one year with traumatically induced blistering. He has had bullous lesions predominantly on the hands, feet, knees and elbows. Very occasional blisters occur on the body with frictional trauma. No scarring resulted from blistering and there was no involvement of the nails or teeth.

He described a subjective worsening of his skin lesions when under emotional stress, and found that a highly refined diet caused a deterioration of symptoms. There had been some improvement in his condition since adolescence.

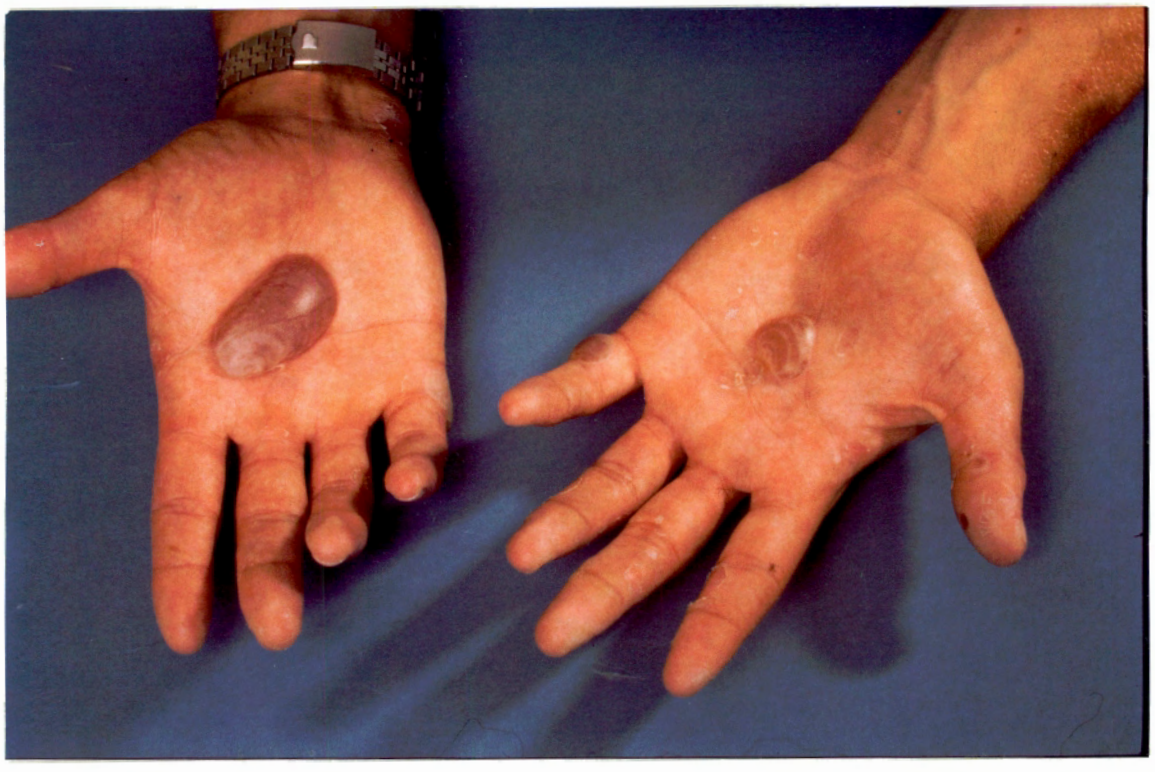


Figure 1. Large serous filled blister on the hand of a man with EB Simplex.

The only form of treatment which affected subjective relief was the topical application of ung. metallorum., which is no longer available.

Large serous-filled blisters were noted on both his hands and feet (Figure 1). There was neither scarring nor nail dystrophy and dentition was normal.

A diagnosis of EB Simplex of the **Weber Cockayne** subtype was made on the basis of the onset of lesions once he began walking, as well as the localised areas of involvement.

Comment: This form of EB is inherited as an autosomal dominant trait and the sporadic occurrence in this family is highly suggestive of new mutation of this gene.

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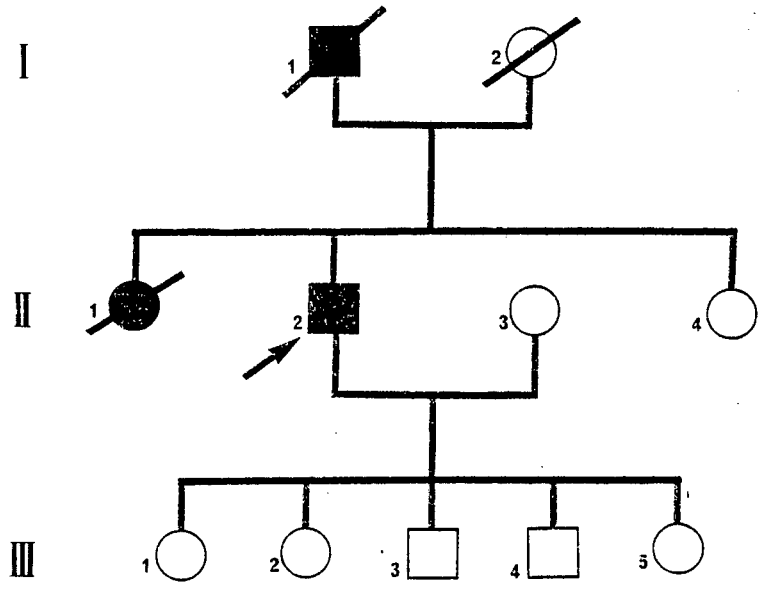
Comment: This form of EB is inherited as an autosomal dominant trait and the sporadic occurrence in this family is highly suggestive of new mutation of this gene.

4.2.2.2 FAMILY 2

This family are of Dutch stock, now residing in the Transvaal, South Africa.

Pedigree:



The proband was a 62 year old man. Blistering occurred soon after birth, and persisted throughout his youth. Lesions were confined to the arms and legs only, and healed well, without scarring. During adulthood his condition has improved markedly and in late middle age blistering was very infrequent and only in response to trauma.

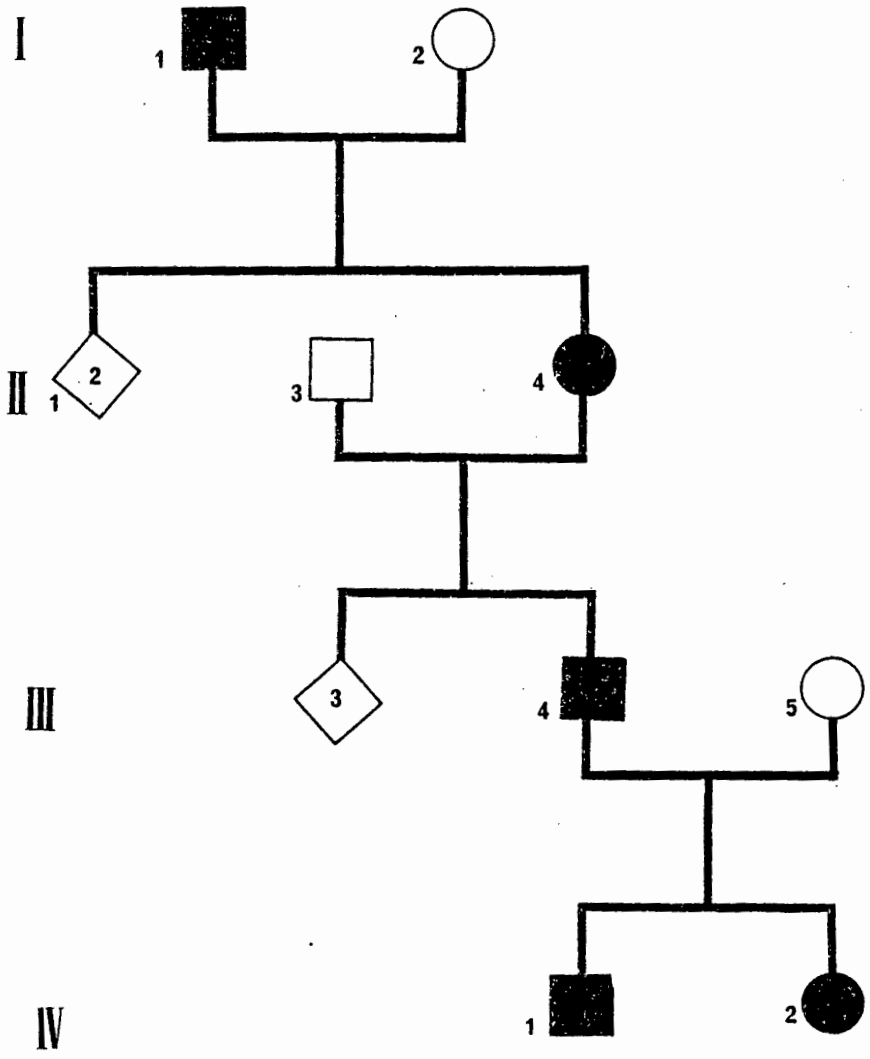
There was no significant nail involvement.

Comment: This man has the clinical features of **EB Simplex Weber Cockayne.** The pedigree data shows autosomal dominant inheritance. It is of interest that none of his five children were affected and it seems that the dominantly inherited gene has not been transmitted to the next generation.

4.2.2.3 FAMILY 3

This kindred are South Africans of British stock. There was no consanguinity and no advanced paternal age.

Pedigree:



The proband IV-1 was a 12 year old boy who presented with blistering of the skin of his feet at the age of 1 year, when he began walking and wearing shoes. Traumatic bullous lesions also occurred on the hands, but no other area of the body was affected.

The lesions which persisted since this time healed well without scarring. Neither the nails nor the mucous membranes were involved.

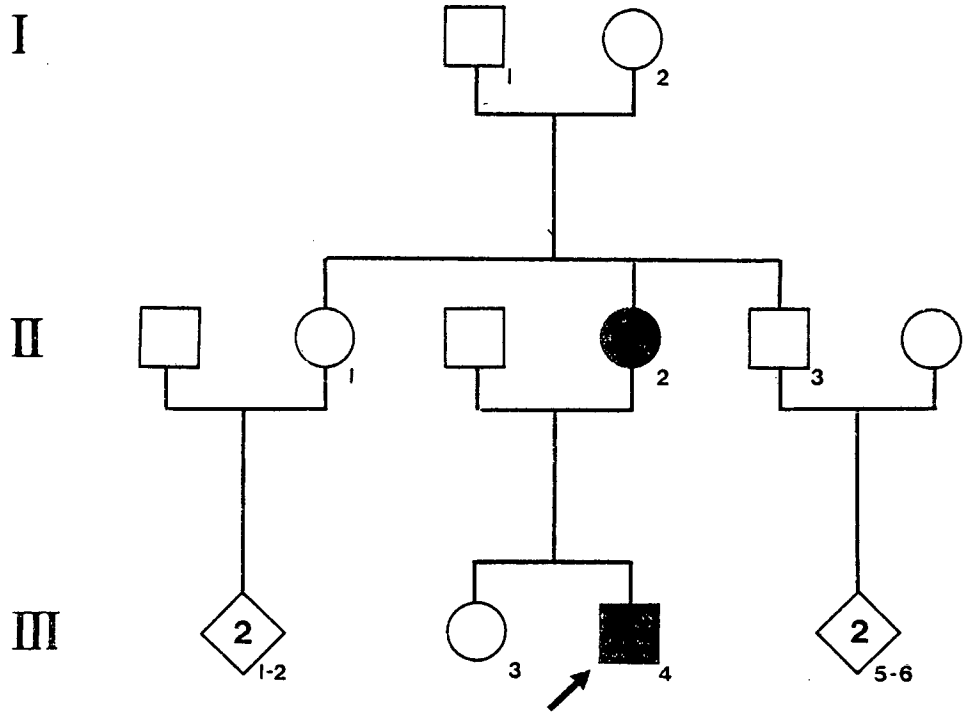
Identical lesions were noted in 4 generations of this kindred, who had non scarring EB localised to the distal extremities, consistent with the diagnosis of **EB Simplex Weber Cockayne.**

Comment: Autosomal dominant inheritance as seen in the pedigree is the accepted mode of transmission in EBS Weber Cockayne.

4.2.3 EBS Dowling Meara

This family consists of one affected person in 2 generations of a South African kindred of Afrikaner stock. II-2 is the only one of three siblings affected; neither of her parents has EB and it can therefore be assumed that she manifests a new mutation of the gene for EB.

Pedigree:



The proband was a seven year old boy (III-4) who had bilateral blistering of his thumbs at birth. Blister formation, especially with trauma as a precipitating factor, persisted through infancy to childhood, with some improvement latterly. Distribution of lesions was generalised, but hands and feet were most severely and most frequently affected. Bullous lesions were noted on the oral mucosa in infancy. The lesions

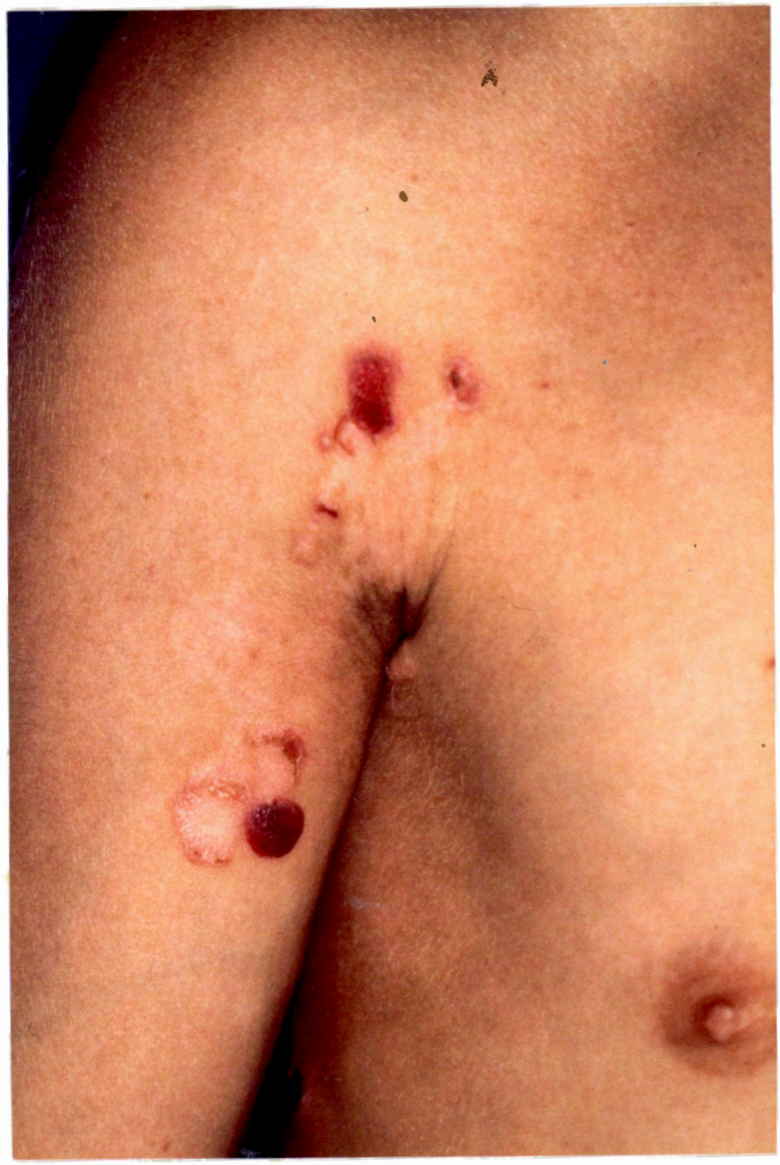


Figure 2. Herpetiform grouping of lesions in EBS Dowling Meara.

healed well, with slight depigmentation which was corrected on sun exposure. Subungal blistering occured with shedding of nails, which then regenerated normally. An exception to this was a single finger nail which was thickened and ridged.

Treatment with various topical steroids, antibiotics and zinc preparations had little or no effect on the course of this EB.

He was a healthy young boy who, apart from EB, had no notable physical signs. Small blisters, most of which were complicated by impetigo, were present on the face, shoulders, arms, legs, hands and feet. The lesions were grouped in the typical herpetiformis pattern as described in **EBS Dowling Meara** (Figure 2). Small, slightly depigmented areas occurred in a similar distribution but there was no scar formation. Apart from one mildly thickened fingernail, no nail dystrophy was evident.

His mother (II-2) was similarly affected in childhood. Spontaneous improvement ensued at the end of the second decade, and bullous lesions during adulthood was very occasional.

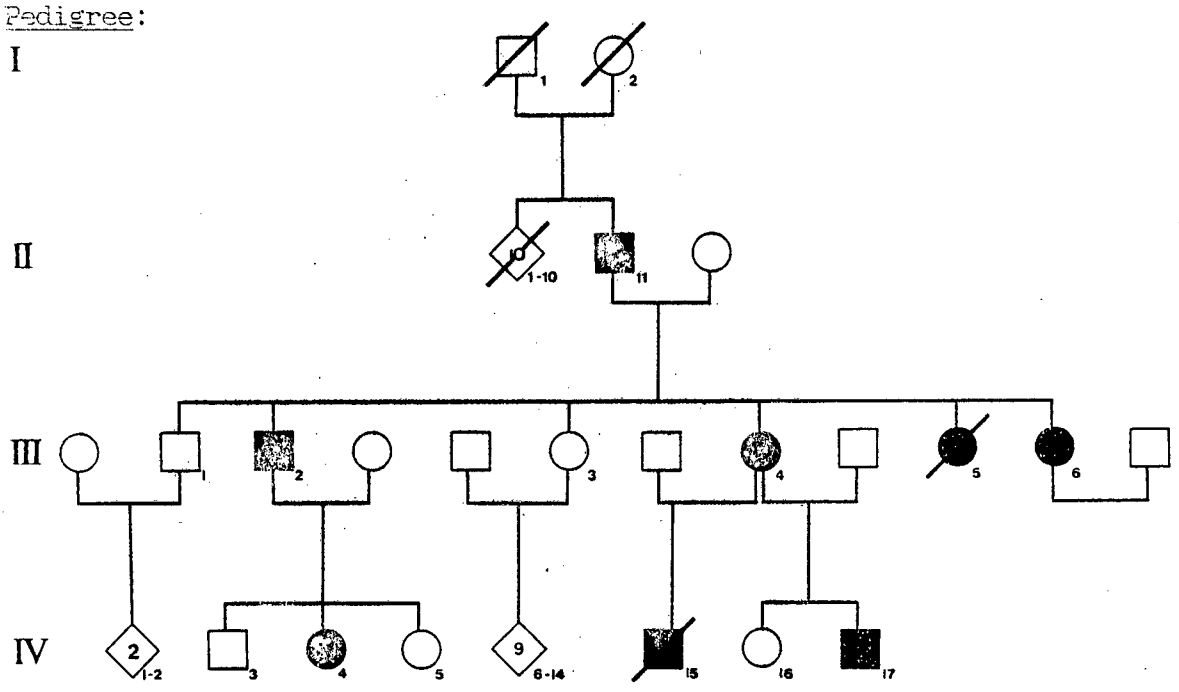
Comment: The clinical features in this family are the classical manifestations of **EBS Dowling Meara**. The pedigree confirms autosomal dominant inheritance. Since neither of the parents of II-2 have EB, it is evident that she has a new

mutation of the gene responsible.

4.2.4 EBS with mottled pigmentation

This pedigree illustrates eight affected persons in four generations of a large kindred of mixed British, European, Malay stock.

Pedigree:



IV-17, the propositus in this family, was born by normal vaginal delivery following an uncomplicated full term pregnancy. Because the foetus had an even chance of inheriting EB, delivery was in a maternity hospital with intensive care facilities. No blistering or skin loss was noted at birth, but during the course of the first day, bullae formation began. Initially the lesions occurred on

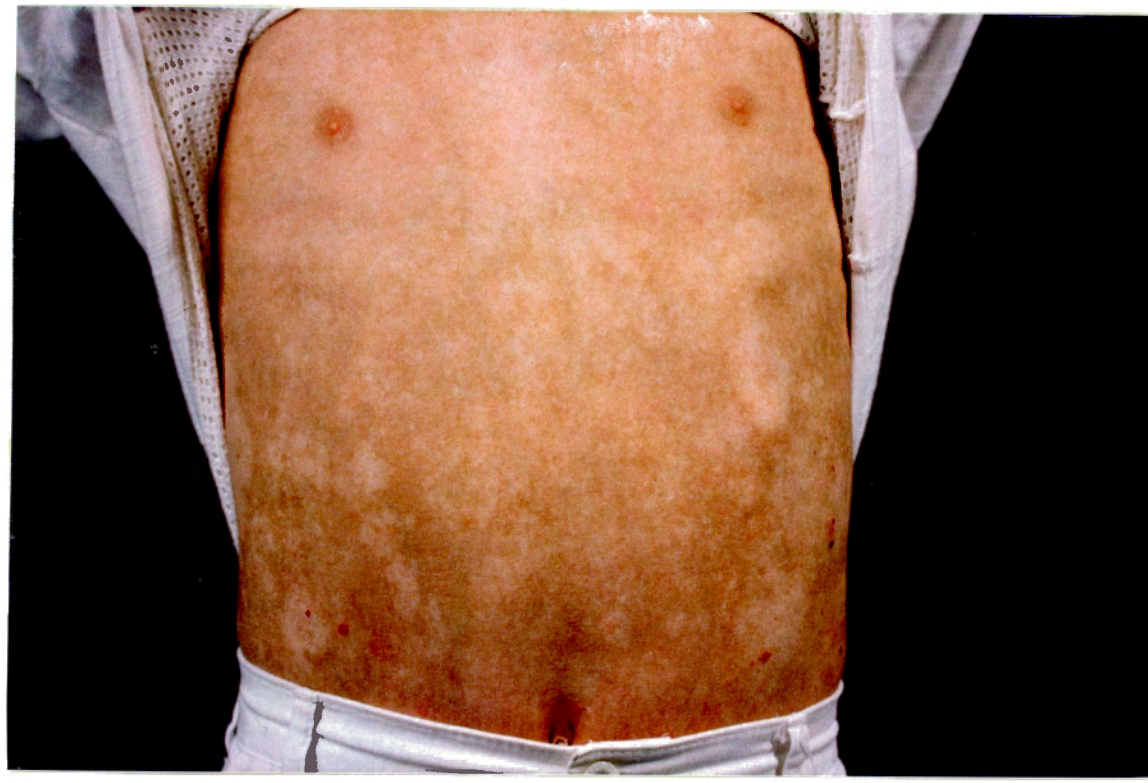


Figure 3. Hyper- and hypopigmentation of skin in EBS with mottled pigmentation.

the buttocks, followed by involvement of skin throughout the body. Blistering occurred in the oral mucosa and when breast feeding was attempted, the infant's oral mucosa was stripped, necessitating tube feeding was then instituted.

During infancy and early childhood blistering was moderately severe. Lesions were exacerbated by impetigo, and his problems were compounded by poor social circumstances.

His condition improved during later childhood. Bullous lesions were frequent and healed without scarring, although pigmentary disturbances resulted from blisters.

Treatment with various combinations of oral and topical antibiotics, steroids and emollients had no significant effect on the course of his EB.

Blisters, at various stages were evident ranging from fresh fluid-filled to impetigenous scabs. There was a generalised distribution of lesions. Although no scarring existed, there were areas of hyper- and hypopigmentation consequent upon recent blistering, (Figure 3).

The nails of his fingers and toes were unaffected. His teeth and eyes were normal.

A clinical diagnosis of **EB Simplex, with mottled pigmentation**, was made.

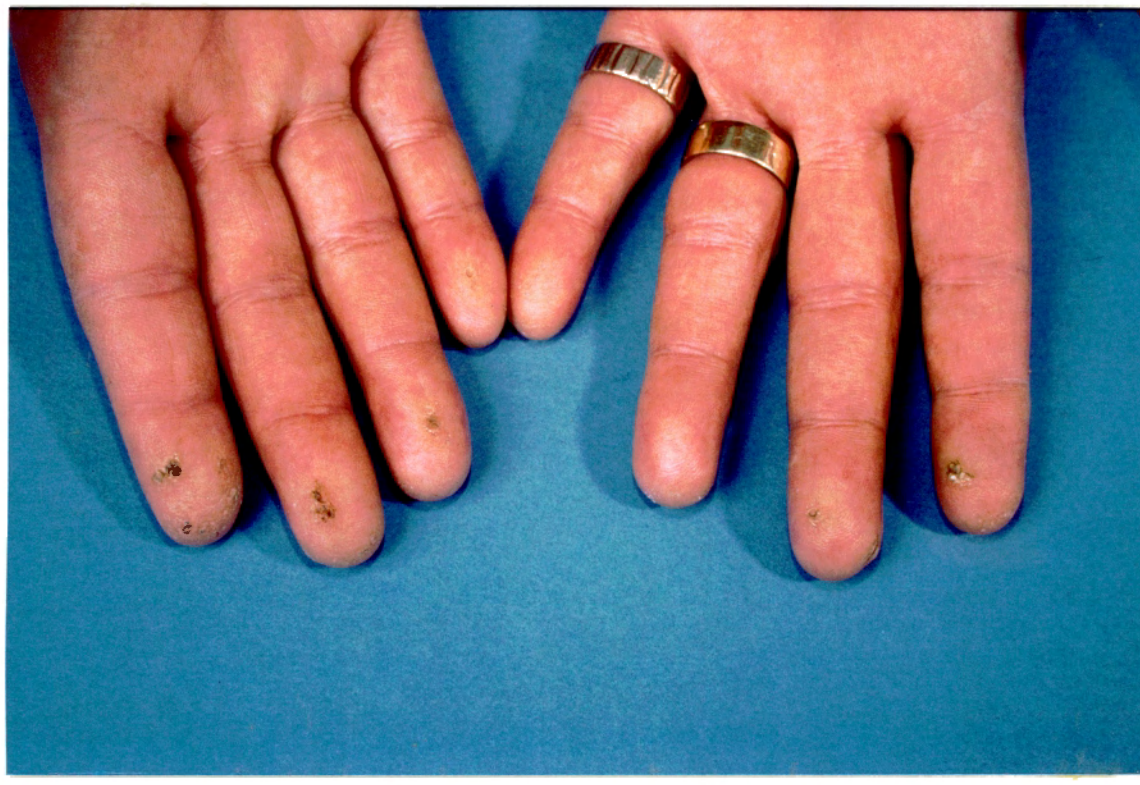


Figure 4. Pitted hyperkeratotic fingertips of an adult with EBS with mottled pigmentation.

Each of the other affected family members gave a similar history and showed clinical features of Epidermolysis Bullosa Simplex. The palms and soles were markedly hyperkeratotic. Two adults had pitting of the finger tips (Figure 4). All family members had areas of mottled pigmentary change. Of note is the fact that during her three pregnancies, III-3 had no deterioration of her condition.

None of the family members were severely affected and all have shown a degree of improvement after the second decade. IV-7 died during infancy as a result of septicaemia following systemic corticosteioid therapy.

Comment: This family manifest a mild form of EBS with generalised distribution of lesions and pigmentary changes, compatible with the diagnosis of **EBS with mottled pigmentation.** The focal hyperkeratoses on the finger tips are significant; similar observations were reported by Fischer and Gedde-Dahl (1979) in a family with EBS with mottled pigmentation. The pedigree shows autosomal dominant inheritance with complete penetrance, and apart from the infant who died of complications of the lesions rather than EB per se, phenotypic expression within the family is constant.

4.3 EPIDERMOLYSIS BULLOSA ATROPHICANS

EB Atrophicans or Junctional EB is a non-scarring form of EB. Repeated bullous formation results in atrophy of the skin, hence the name 'atrophicans'.

Blistering begins in the prenatal period, and bullae may be present at the time of delivery. The nails are not dystrophic, nor is there subungal blistering, although clubbing of fingernails is a recognised association. The dentition is abnormal, with punctated hypoplastic dental enamel. The inheritance of EBA of all subtypes is autosomal recessive. Histopathological examination reveals a cleavage plane for blister formation within the basal lamina, or so-called junctional blister formation. It is for this reason that EBA is widely named Junctional EB.

Prognosis for life in these forms is usually poor, and death within the first few months of life ensues. The classical EB **Atrophicans Generalisata Gravis** is also known as **EB Letalis.** As shown in Table 4-II, there are rare non-lethal form of EB Atrophicans, which manifest as widespread bullous lesions which result in skin atrophy without scarring.

The different subtypes of EBA are listed in Table 4-II.

TABLE 4-II SUBTYPES OF EPIDERMOLYSIS BULLOSA ATROPHICANS

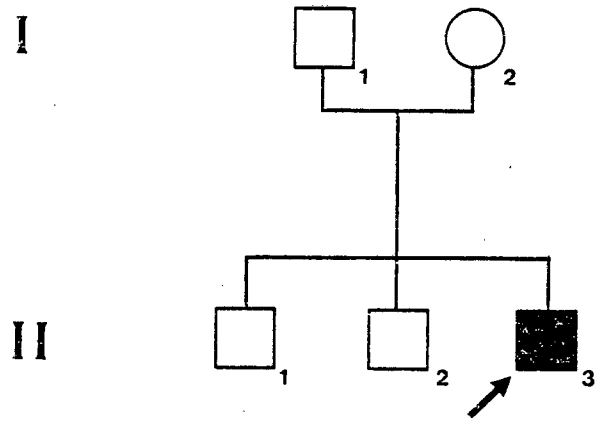
	Subtype	Age of Onset	Scarring	Nails	Teeth	Inheritance	Prognosis	Distribution of Lesions	Clinical Concomitants
1.	EBA Generalisata	Intra-uterine	Atrophy rare	Clubbing	Defective dental enamel	AR	Poor	Hands and feet spared	Laryngeal involvement
2.	EB Letalis with pyloric atresia	Intra-uterine	Atrophy rare	-	Defective dental enamel	AR	Poor	Generalised	Pyloric atresia
3.	EBA Generalisata Mitis	Birth	Skin atrophy	Clubbing	Punctated enamel	AR	Good	Generalised, hands & feet not spared	-
4.	EBA Inversa	Birth	Skin atrophy	-	Dental hypoplasia	AR	Females improve after menarche	Groins, genitals, axillae, trunk, shins	Albostriate skin changes
5.	EBA Localisata	± 6 years	Atrophy	Dystrophic	Enamel hypoplasia	AR	Good	Shins and soles	Private syndrome
6.	Generalised atrophic benign EB	Birth	Atrophy	-	Carious	AR	Good	Generalised	Scalp alopecia shagreen naevocytic naevi
7.	EBA = muscular atrophy	Birth	Atrophy	-	-	AR	Good	Generalised	-
8.	EB Junctionalis disentis type	Birth	Atrophy	Atrophic	-	AR	Good	Minimal on hands and feet	Absence of body hair. Naevoid palmar hyperkeratosis

4.3.1 EBA Generalisata Gravis (Herlitz Syndrome)

4.3.1.1 FAMILY 1

The kindred is a non-consanguineous family of mixed ancestry who resided in a rural part of the Northern Cape. There was no family history of EB or any other inherited disorder.

Pedigree:



The proband was a male infant who was noted at birth to have absence of large areas of the skin. At the age of 12 days, he was referred to a major centre for investigation.

There were generalised skin lesions, both bullae and well-demarcated areas of absent skin (Fig 5).

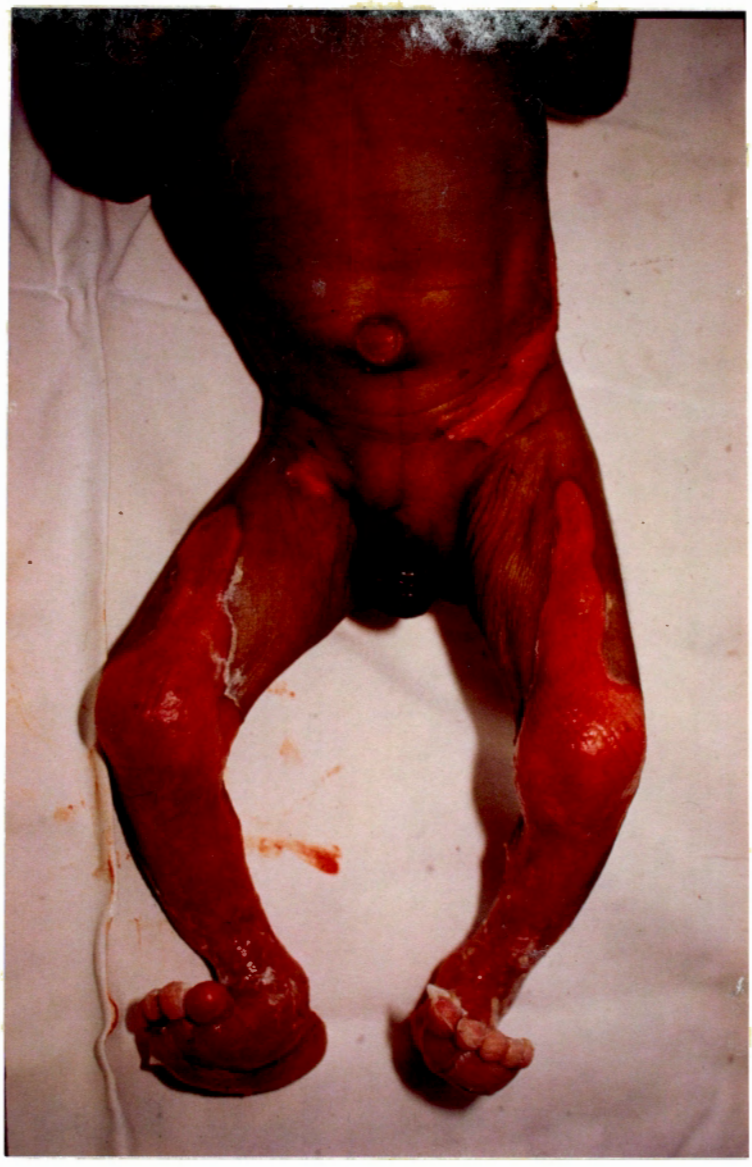


Figure 5. Absence of skin in areas of deroofed bullae in EBA Generalisata Gravis.

His nails had been shed from his fingers and toes. Blistering and ulceration of the oral mucosa were secondarily infected with Candida albicans.

A clinical diagnosis of EB was made. Topical treatment was instituted, with initial good effect. However, the underlying problem was complicated by a staphylococcal septicaemia and despite appropriate management the infant died at the age of 28 days.

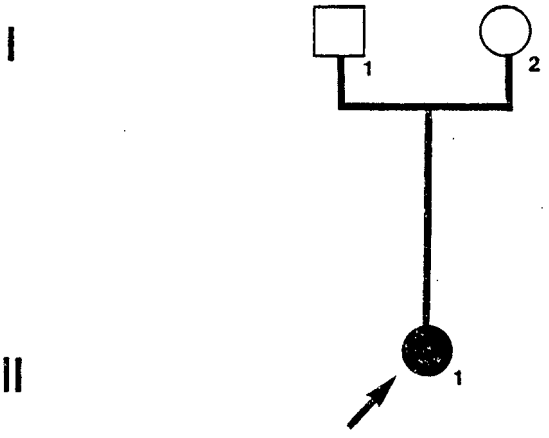
An autopsy was performed to assess the degree of systemic involvement. There was a large area of mucosal denudation on the tongue. The oro- and nasopharynx had numerous mucocutaneous lesions with superimposed infection. The vocal cords were pale, as was the trachea which appeared to have pinpoint areas of ulceration. The mucosa of the oesophagus had numerous areas of streaky ulceration. Further examination revealed signs compatible with severe bronchopneumonia and septicaemia.

Comment: The features of this patient are consistent with the diagnosis of **EBA Generalisata Gravis (EB Letalis)**. The pedigree does not confirm, but is compatible with autosomal recessive inheritance.

4.3.1.2 FAMILY 2

This family of mixed ancestry are farm labourers in a rural area in Namaqualand. There is no consanguinity and no knowledge of familial disorders.

Pedigree:



The proband, an infant girl, manifested widespread bullae at the time of birth. Several of these bullae ruptured within hours of delivery, leaving denuded areas of absent skin.

At the age of 17 days, the baby was referred in from a rural health centre to a tertiary care hospital.

Bullae were present throughout the skin in varying stages of development and there were areas of absence of skin as a consequence of deroofed blisters. The nails of the fingers and toes were hypoplastic.

A clinical diagnosis of EBA was confirmed histologically. The child was treated symptomatically and despite persistent blistering, she made moderately good progress. After a three-and-a-half month period in this neonatal unit, the infant was returned to the peripheral hospital for continued management.

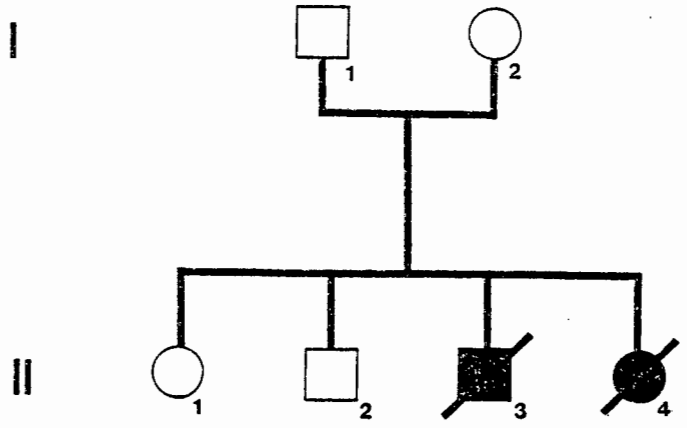
The infant died aged five months following a septicaemic illness.

Comment: The report illustrates **EBA Generalisata Gravis** with probable AR inheritance which was rapidly fatal, despite meticulous medical care.

4.3.1.3 FAMILY 3

The family were African negroes of the Xhosa tribal group and resided in a rural area in the Ciskei. No consanguinity was known.

Pedigree:



The proband, 11-4, was born with an area of skin absent on the right leg and the dorsum of the right foot. Blistering of the hands began soon after birth and hypoplastic nails were shed. The formation of bullous lesions spread to involve the peri-umbilical area, buttocks, head and limbs. There was desquamation of the buccal and nasal cavities.

Topical treatment together with Phenytoin led to an improvement in her condition. Staphylococcus aureus infection of the skin lesions complicated the condition of the infant, but response to Cloxacillin and Gentamycin was adequate. On day 38, the infant had a sudden deterioration and died, following septicaemia and resultant hypothermia.

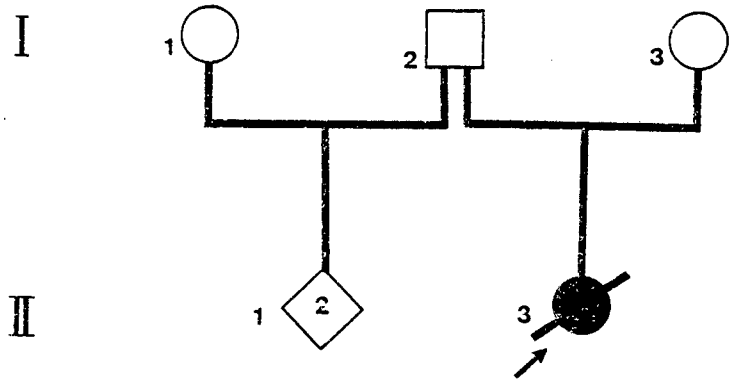
II-3 was an elder sibling of the proband who had died at the age of one month, following a similar course.

Comment: The diagnosis of **EB Atrophicans Generalisata Gravis** was made. No confirmatory histopathological studies were available. The pedigree data is consistent with autosomal recessive inheritance.

4.3.1.4 FAMILY 4

The proband was of South African Asiatic stock. Both parents were members of the Transvaal Moslem community but they were unrelated.

Pedigree:



The proband was an infant female who presented at the time of birth with widespread areas of bullous skin eruptions, initially peri-umbilical lesions. In addition, her oral mucosa was fragile and blistered and bled during feeding. The areas of blistering healed slowly without scarring. All finger and toenails, which were present at birth, were shed. A diagnosis of EBA was made.

She did not recover sufficiently to be discharged from hospital. She died at the age of three months.

Comment: It is noteworthy that **EBA Generalisata Gravis** occurs in persons of Asian stock. The sporadic presentation of EB is compatible with autosomal recessive inheritance.

4.3.1.5 FAMILY 5

The mother of the proband had had a mid-trimester abortion, at 22 weeks gestation, for unexplained causes. She was closely monitored in her second pregnancy, with serial ultrasonographic examination. At 16 weeks gestation, ultrasound scan demonstarated numerous bilocular lesions attached to the posterior aspect of the head and a large cystic lesion extending from lumbar region to thorax and axillae. A subsequent scan one week later demonstrated the accumulation of fluid in the thoracic and abdominal cavities of the foetus. A clinical differential diagnosis of cystic hygroma, hydrops foetalis or Turner syndrome was made. On the basis of these findings and with parental consent, the pregnancy was terminated at 17 weeks gestation by Prostaglandin induction.

The foetus delivered was a female of 17-18 weeks' gestation. Her limbs were oedematous and there was slight asymmetry, the right side larger than the left. There were numerous superficial cystic bullous skin lesions filled with clear fluid in the submandibular area, over the occiput of the skull and bilateral axillo-thoracic cysts. In addition, there was a large cyst of the abdominal wall which was filled with blood-stained fluid and the right kidney was large and cystic.

The multiple bullae were consistent with the macroscopic features of Epidermolysis Bullosa. Confirmatory histological examination, as well as chromosome analysis were undertaken and, unlike previous case reports which have dealt only with clinical findings, it is pertinent to detail these results in this subsection.

The epidermis was hypoplastic in comparison with foetuses of similar gestational age. Sections were made through various sites of the skin, and these demonstrated primitive mesenchymal tissue with hair follicles. There were multiple junctional bullae in early and late stages of development. Degeneration of the basal cell layer with marked vacuolation was evident, with areas where the basal cells had become separated from the epidermis.

Sections from macroscopically normal skin showed similar changes to those seen in the bullae. There was no inflammatory infiltrate nor vascular proliferation.

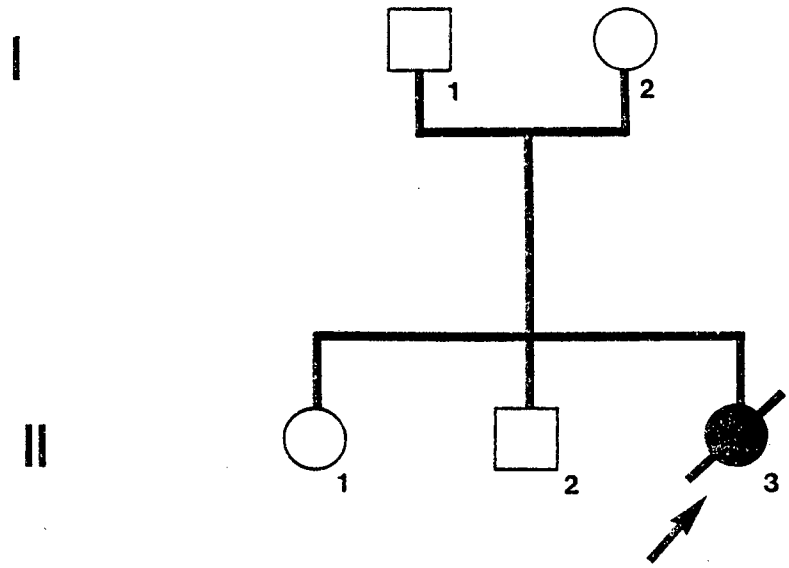
Histology of the kidney was compatible with a renal cystic dysplasia. Chromosomal analysis yielded the karyotype 45XO, or Turner syndrome.

Comment: The clinical features, confirmed by histopathological and cytogenetic analysis, were those of **Epidermolysis Bullosa Atrophicans** with co-existing Turner syndrome. This unusual association has not previously been documented.

4.3.1.6 FAMILY 6

The kindred were South African negroes of Xhosa stock, from the Transkei.

Pedigree:



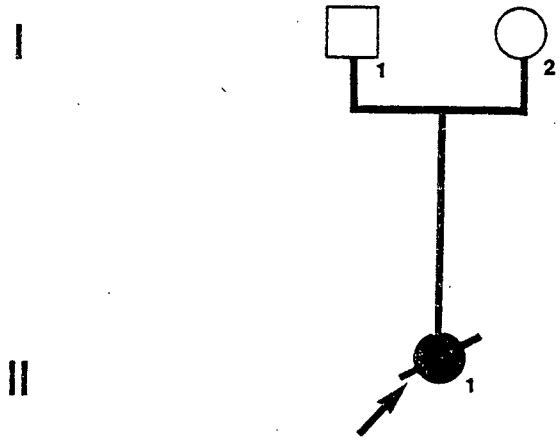
The proband was an infant female who presented with the classical bullous skin lesions of EB Atrophicans. Despite attempted management with topical and oral preparations of vitamins and antibiotics, the infant died several days after birth. A clinical diagnosis of **EBA Generalisata Gravis** was made, and histopathological studies were undertaken.

Comment: This lethal form of EBA has manifested in a Xhosa family, with probable autosomal recessive inheritance.

4.3.1.7 FAMILY 7

An infant was born to unrelated White South African parents.

Pedigree:



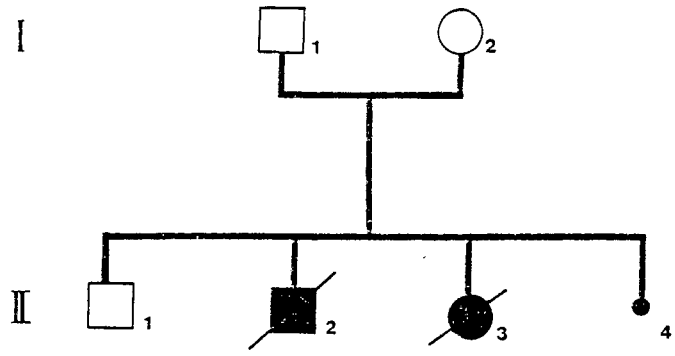
Bullous lesions appeared at the time of birth and a relentless course of blistering of skin and mucous membranes ensued. Despite appropriate management, the infant lived for only 14 days. A diagnosis of the lethal variant of EBA was made.

Comment: This infant was the only patient with **EBA Generalisata Gravis** in the White population group.

4.3.1.8 FAMILY 8

This family of mixed ancestry who lived in the Western Cape were non-consanguineous.

Pedigree:



The mother, 1-II, presented to a genetic clinic for counselling. Two of her four children had died in early infancy as a result of autosomal recessive **EBA Generalisata Gravis** and she was fourteen weeks pregnant at that time.

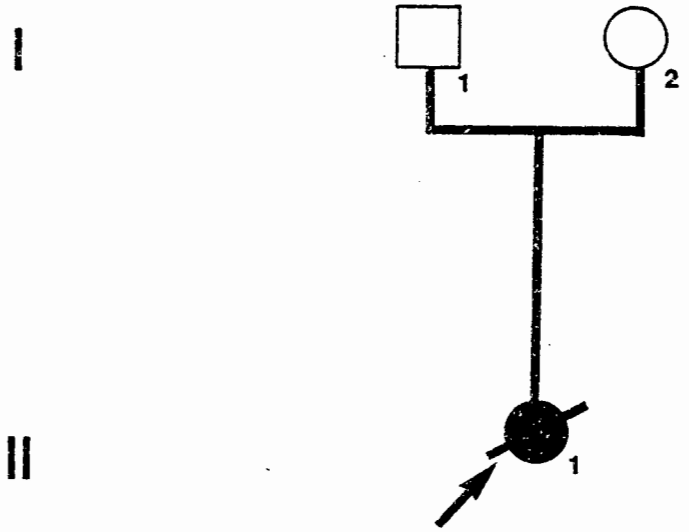
The patient was counselled by a member of the team as to the 1:4 recurrence risk of lethal EBA, and termination of pregnancy was requested. This was duly performed. No follow-up investigation of the abortus was done, and it is unknown whether the foetus was affected.

Comment: This family demonstrates **EB Atrophicans Generalisata Gravis**, with typical autosomal recessive inheritance.

4.3.1.9 FAMILY 9

This family are African Negroes from the Transvaal. There was no consanguinity and the proband was the first-born child of teenage parents.

Pedigree:



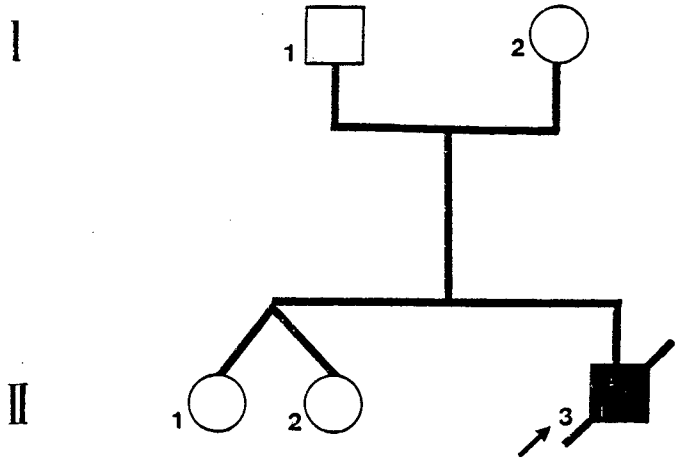
The infant presented at the time of birth with the typical lesions of EB involving mainly the limbs and the peri-umbilical area. Sub- and peri-ungual blistering resulted in shedding of the nails. The infant had a stormy course and died aged nine days.

Comment: This report illustrates a typical example of **EBA Generalisata Gravis.**

4.3.1.10 FAMILY 10

This family were of Xhosa stock, and non-consanguineous.

Pedigree:



Blistering began on the fingers of this infant several days after birth, and thereafter mild trunk involvement was noted. Initially, a few small lesions formed in his mouth, but suckling was unaffected and weight gain was good.

Bullae continued to occur in a widespread distribution, Deroofing of blisters resulted in large raw areas where skin was lost. Nails were hypoplastic and subungal blistering occurred.

At the age of six weeks he had a marked deterioration with very severe oral mucosal involvement. The attendant paediatrician inserted a nasogastric tube to facilitate feeding as the pain induced by sucking resulted in minimal effort from the boy, who failed to gain weight. At the age of

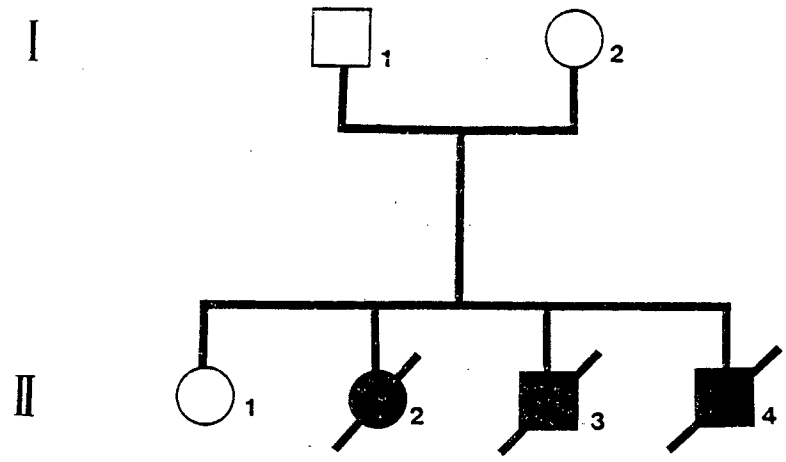
nine weeks, respiratory failure consequent upon pneumonia caused his death.

Comment: Although sporadic, this presentation of the lethal form of EBA is probably due to autosomal recessive inheritance.

4.3.1.11 FAMILY 11

This family of mixed ancestry lived in the Western Cape. There was no consanguinity.

Pedigree:



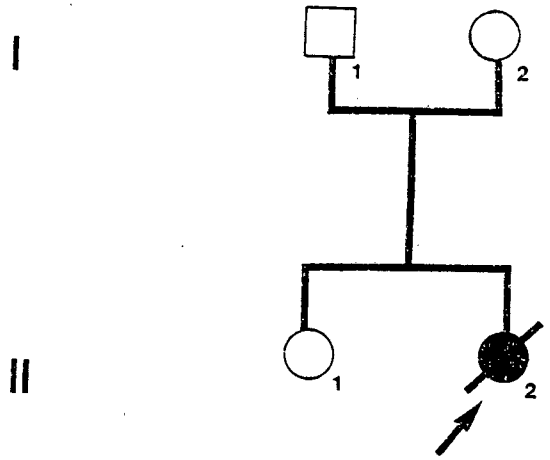
The proband was II-4, who was noted to have blistering of the skin at the time of birth. The infant's mother immediately recognised the lesions as EB and the child was referred for special care. He had the typical skin manifestations of EB with some involvement of the oral mucosa and he died at the age of 14 days.

Comment: Autosomal recessive inheritance of **EBA Generalisata Gravis** is demonstrated in this kindred.

4.3.1.12 FAMILY 12

This family were South African Blacks of the Xhosa and Sotho tribal groups. Both parents were young and there was no consanguinity.

Pedigree:



The infant presented 2 days after birth with periumbilical blistering. Lesions soon appeared on the buttocks, and then in a widespread distribution. The infants clinical condition deteriorated and she died at the age of two weeks.

Comment: This case illustrates probable autosomal recessive inheritance of **EB Atrophicans Generalisata Gravis.**

4.3.2 EBA with pyloric atresia

A South African Black infant was born in a rural area of the Southern Transvaal with the characteristic manifestations of EB. She was transferred to a major hospital where the diagnosis of EBA was confirmed. In addition, the infant was found to have pyloric atresia.

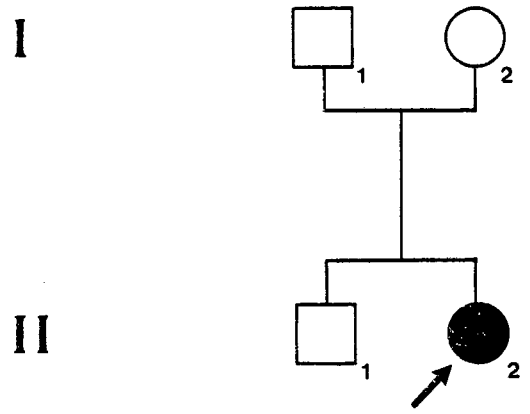
Shortly after admission, she died. No pathological or post-mortem studies were done.

Comment: This patient illustrates the well-documented relationship between lethal EBA and pyloric atresia. No other infants with this association were encountered in this study.

4.3.3 Non-lethal EB Atrophicans Generalisata Gravis

This kindred of British stock was non-consanguineous and had no family history of EB. There was no advanced paternal age.

Pedigree:



The proband was a 10 year old girl. Her earliest sign of EB was circumferential blistering of a single finger which was evident at the time of birth. Bullous lesions of the skin induced by minor trauma soon followed with involvement of face, hands and feet. During infancy the scalp was affected, with delayed growth of hair although latterly hair growth was normal. The most severe manifestations were facial, which caused a consistent mask-like pattern of blistering and scab formation.

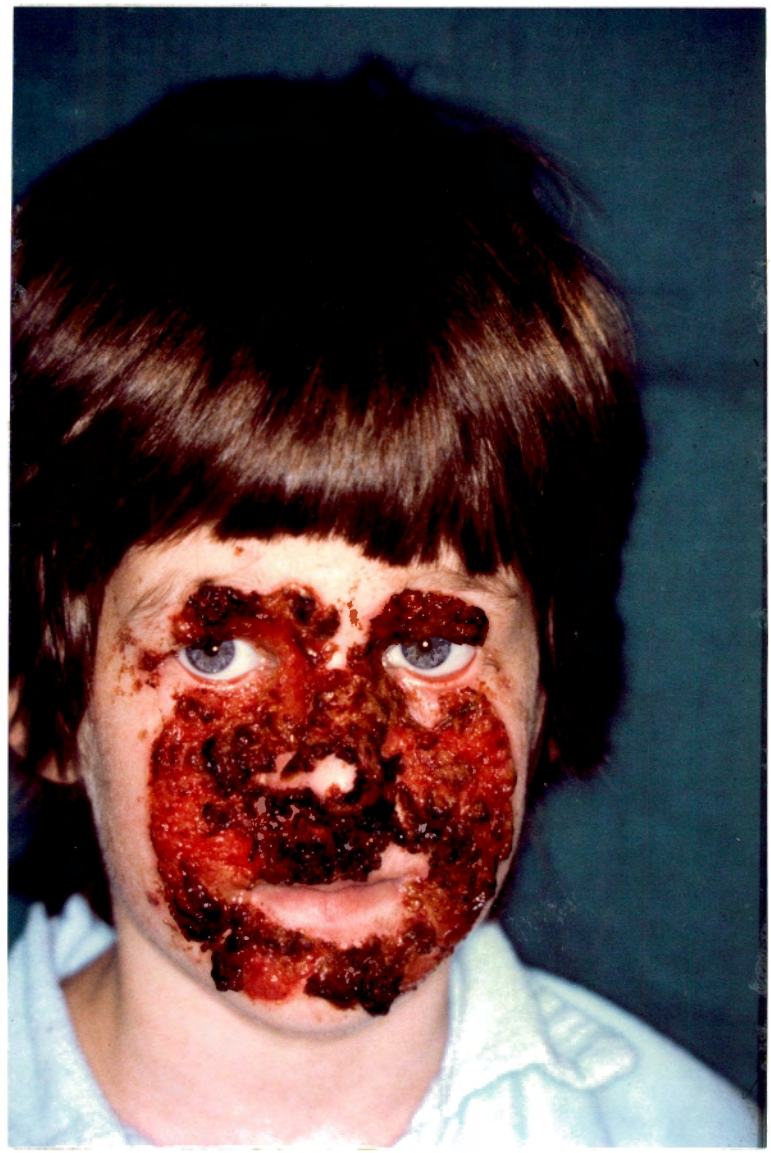


Figure 6. The "mask" of lesions in the child with EBA Generalisata Gravis (non lethal).

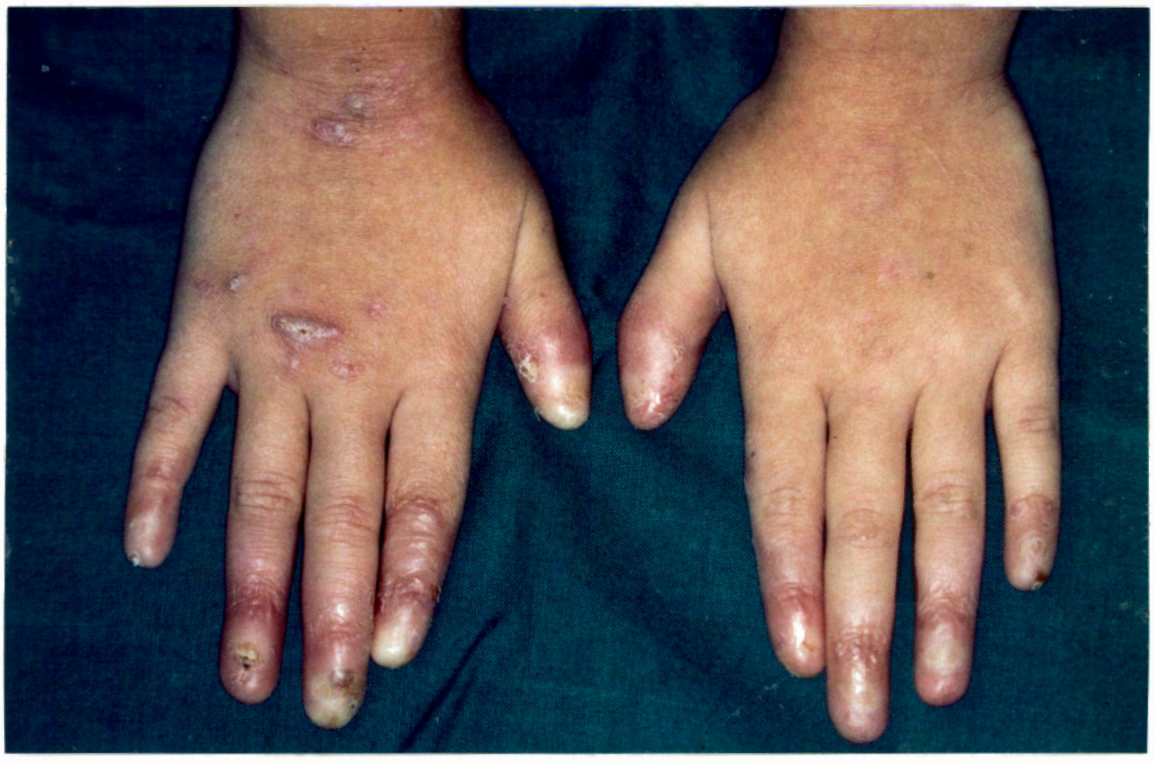


Figure 7. Absent fingernails and atrophic skin in the child with non lethal EBA Generalisata Gravis.

Nails of fingers and toes were shed, without regeneration. Teeth, though overtly normal, had a tendency to caries. Blistering of the eyelids occurred and no eyelashes developed in the lower lids. There was no corneal involvement. Exhaustive attempts at treatment were unsuccessful.

The most striking feature on examination was the involvement of the face. A confluent mask of fresh serous blisters, haemorrhagic blisters, and scab formation covered most of her face, surrounding her eyes, nose and mouth and extended onto upper eyelids (Fig 6).

Although this "mask" fluctuated in severity, she was never free from involvement of this area. The healed skin on the hands, feet, arms and legs appeared thin and atrophic. No nails were present on the fingers or the toes (Fig 7). Her teeth were overtly normal.

Comment: The distribution of lesions predominantly on the face is fairly characteristic of **EBA Generalisata Gravis**, or **Herlitz Syndrome**. Histopathological confirmation of her clinical subtype is discussed more fully in Section 5.1.2. This type of EBA is rapidly fatal, in infancy, and an explanation for her survival is needed. Two possibilities exist: in view of the marked heterogeneity in EB, she may be a

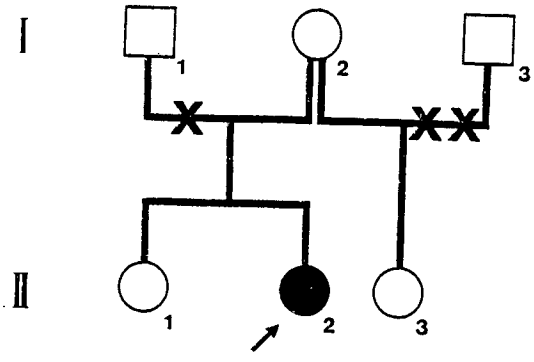
genetic compound. Alternatively, she may be a manifestation of yet another subtype of EBA. The Disentis type of EBA described in 1976 by Hashimoto should also be considered in the differential diagnosis of her EB, although she is not typical of this subgroup.

The parents of this child were visited on several occasions by members of the Department of Social Welfare, who were informed of suspected non-accidental injury, or child abuse. This erroneous diagnosis of non-accidental injury in children with EB has emerged as a very important issue, and will be discussed more fully in Section VII.

4.3.4 EBA Generalisata Mitis

This kindred was of English and Afrikaner stock. There was no notable family history, and no consanguineous marriages had occurred. Both parents of the proband were in their early twenties at the time of conception.

Pedigree:



The proband was a five year old girl who presented at birth with widespread lesions of EB. She has had a stormy course with severe blistering sparing no area of the body. Bullae resulted from minimal trauma and occurred spontaneously. Her nails were dystrophic and severe involvement of the scalp led to very sparse hair growth. In addition, teeth were very carious despite meticulous dental hygiene. Treatment was palliative, and served only to prevent secondary infection of the lesions. The condition of this child was consistently

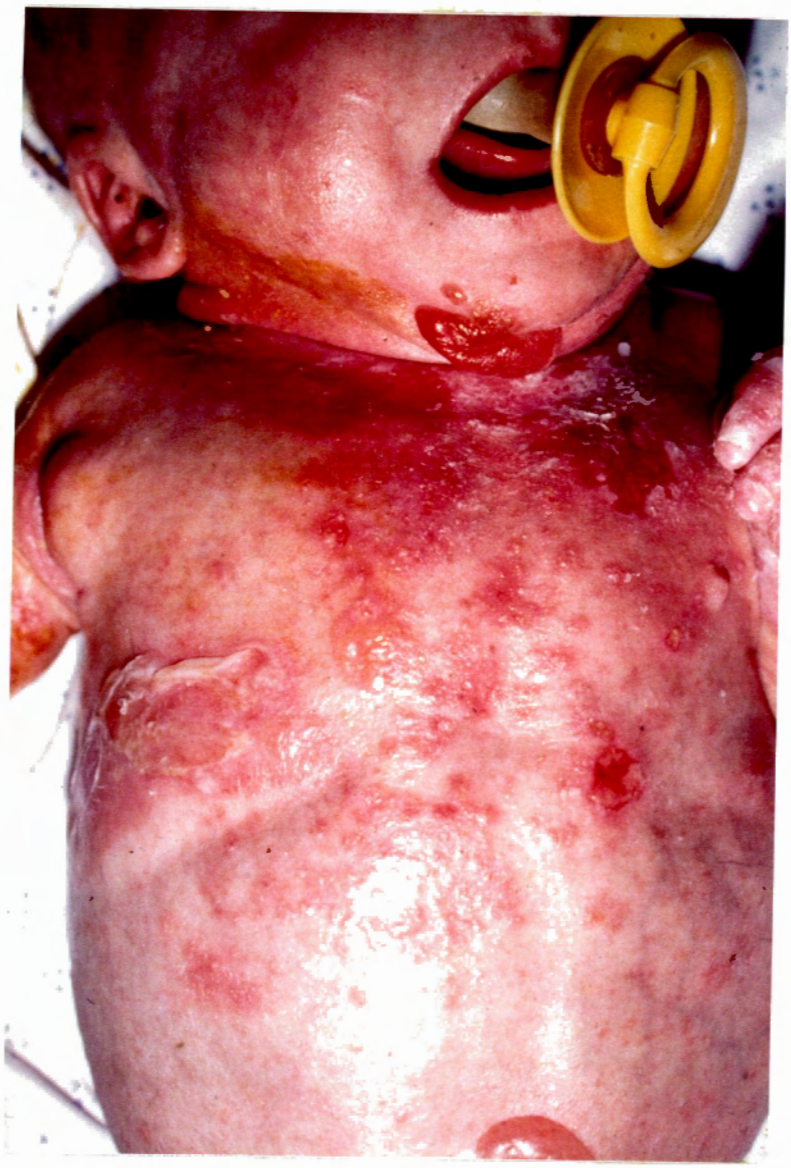


Figure 8. Widespread bullous lesions in a child with EBA Mitis.

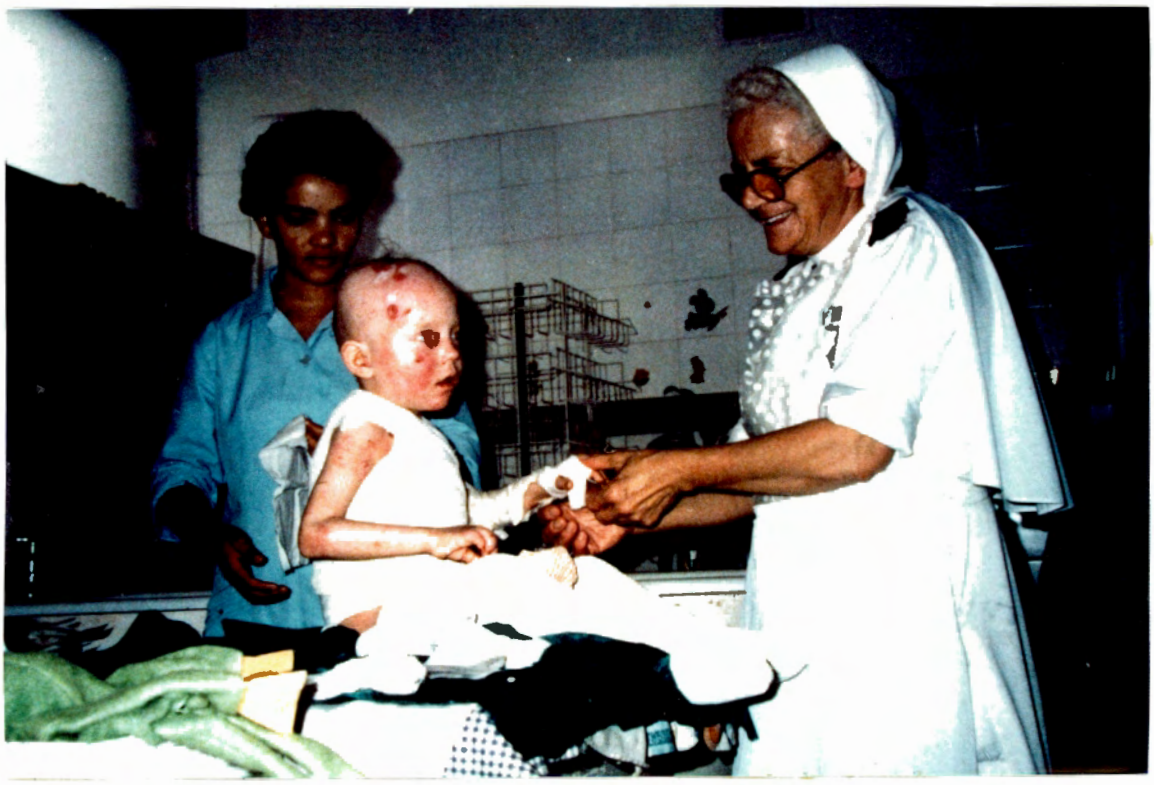


Figure 9. Alopecia in EBA Mitis - an institutionalised child requiring daily dressings.

severe and following repeated admissions to hospital in infancy and very early childhood, she is now resident in an institution which provided full nursing care.

There was a generalised distribution of the typical bullae of EB. Her hands and feet were most remarkable, but there was no area of the skin was spared. (Fig 8).

Finger and toe nails were absent. Teeth were extremely carious and required extraction. There was severe blister formation of the scalp and the hair was very sparse and fine, with large areas of alopecia. (Fig 9). There was no scarring although the skin was atrophic with areas of hyper-pigmentation.

Comment: This girl manifests a non-lethal form of EB Atrophicans. The areas of alopecia especially on the scalp and the defective dentition are highly suggestive of **EBA Generalisata Mitis.** There are no other concomitants such as shagreen patches or naevocytic naevi to support the diagnosis of the other non-lethal EBA subtypes. The pedigree data is compatible with autosomal recessive inheritance.

4.4 EPIDERMOLYSIS BULLOSA DYSTROPHICA

The term Epidermolysis Bullosa Dystrophica (EBD) encompasses all those forms of EB where scarring and milia result from blister formation. Nail dystrophies are an integral part of the dystrophic subgroup.

EBD may be inherited in an autosomal dominant or recessive pattern. The scarring forms of EB will therefore be divided for purposes of this thesis into Autosomal Dominant Dystrophic Epidermolysis Bullosa (DDEB) and Autosomal Recessive Dystrophic Epidermolysis Bullosa (RDEB). Within the two broad groups, each can be subcategorised into to various subtypes as will be elaborated in subsequent sections. For ease of discussion, and since X-linked inheritance is not in question here, the word 'autosomal' may be omitted, although implied.

4.4.1 Autosomal Dominant EB Dystrophica (DDEB)

Atrophy of the skin, scarring and milia result from bullae formation. Milia are discrete white subepidermal cysts which are seen within the scar tissue. Onset of skin lesions in the different types of DDEB is variable and range from birth through adolescence to the third decade. The nails are dystrophic and if shed are seldom regained. Involvement of the dentition is unusual.

Prognosis for life in this form of EB is good although there may be a marked impairment in the quality of life.

Histopathological studies confirm intradermal blister formation with alteration of structure and quantity of anchoring fibrils.

Certain clinical concomitants are significantly associated with the different forms of DDEB, the most serious of which is the development of malignant change within scar tissue. These clinical variations are outlined in Table 4-III.

TABLE 4-III

SUBTYPES OF AUTOSOMAL DOMINANT EPIDERMOLYSIS BULLOSA DYSTROPHICA

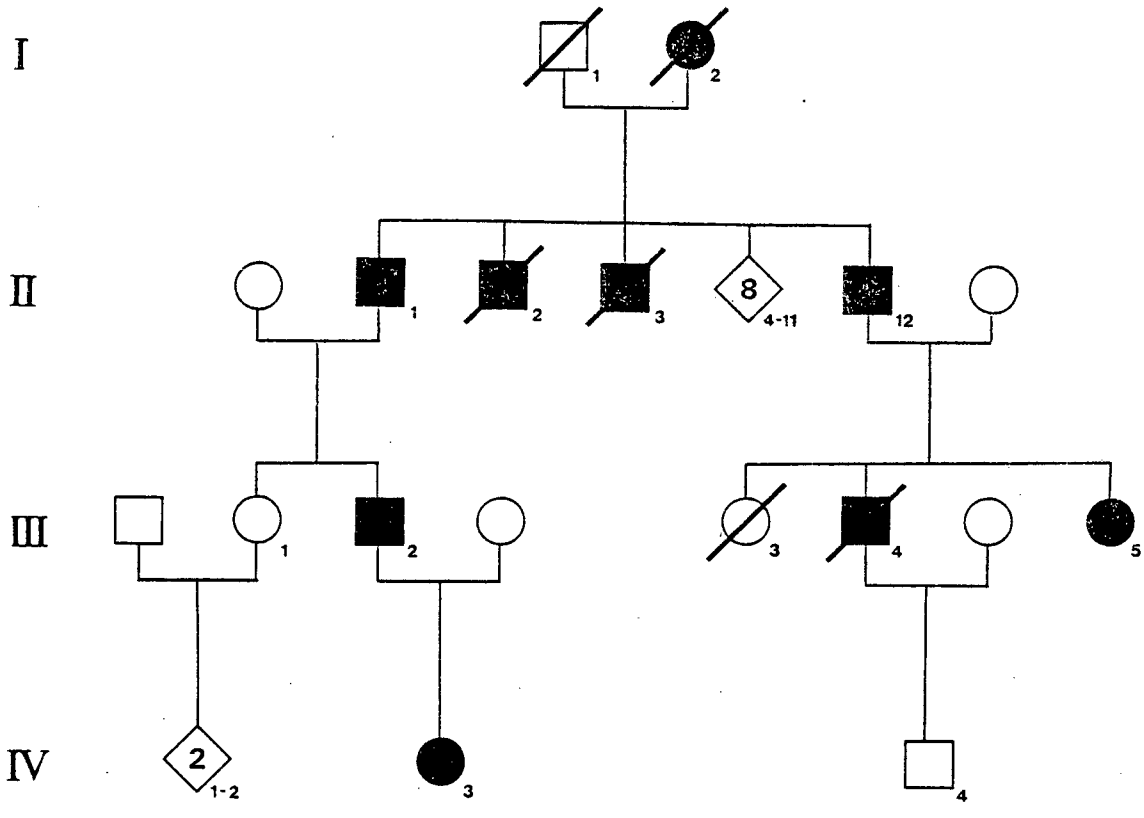
	Subtype	Age of Onset	Distribution of Lesions	Clinical Concomitants
1.	EB Dystrophica Cockayne Touraine	0-5 years	Dorsum of extremities	Malignant change in scars
2.	EBD Pasini	Neonatal	Mainly limbs; oral mucosa	Albopapuloid patches present in adolescence
3.	EBD Pretibial	11-24 years	Pretibial	Occasional albopapular lesions
4.	EBD Bart	Intra-uterine	Generalised: corneal	Congenital absence of skin and skin ulceration.

4.4.1.1 EB Dystrophica Cockayne Touraine

4.4.1.1.1 FAMILY 1

This South African family of British descent have nine affected members in four generations as seen in the pedigree below.

Pedigree:



The proband, III-5, was a 22 year old female who presented had blistering of the skin and absence of her toenails at birth. Her skin was fragile and blister formation resulted from any minor trauma. Lesions were localised to the lower legs, forearms, feet and hands. There was no involvement of face or oral mucosa, nor trunk. Subjectively, she found that blistering was more severe in the hot summer months.

Healing was slow and resulted in scarring. The nails of her fingers were shed periodically, and on regenerating were small and thickened. Her toenails did not develop. Her teeth were unaffected. There was a marked improvement in her condition after puberty. No treatment altered the course of her EB.

Unrelated to her skin lesions, she had congenital micrognathia and underwent a successful reconstruction of the jaw in her late teens.

Typical scarring and milia consequent upon bullae were present on the skin distal to her elbows and knees. (Fig 10).

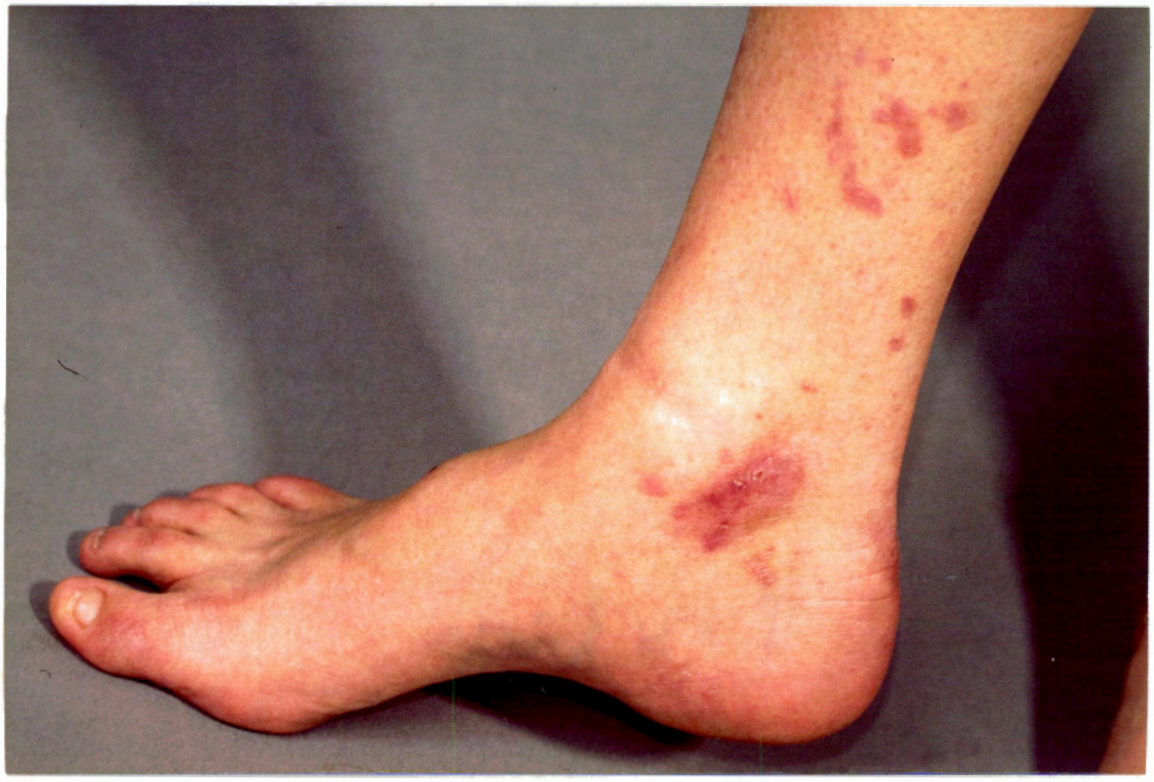


Figure 10. Scarring consequent upon blistering in DDEB Cockayne Touraine.

No fresh bullae were present. Her finger nails were markedly dystrophic and her toenails were absent.

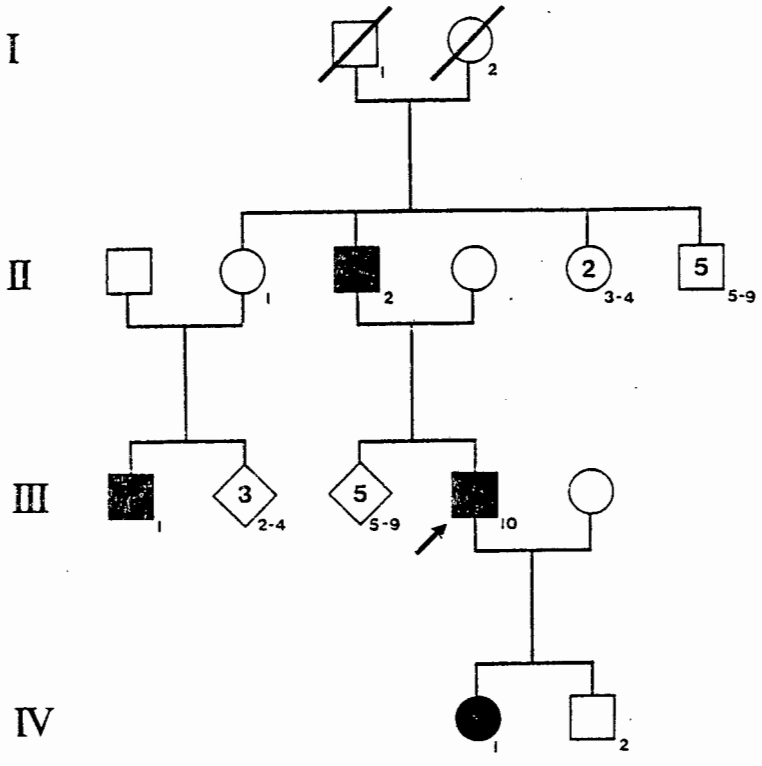
Her family history and the localised distribution of her lesions were consistent with **DDEB Cockayne Touraine.**

Comment: This kindred manifested the autosomal dominantly inherited **EBD Cockayne Touraine,** where blistering is localised to the distal part of the extremities, particularly the dorsal surfaces. All affected members had identical manifestations of EB and those who were deceased had died of unrelated causes.

4.4.1.1.2 FAMILY 2

This family of mixed ancestry resides in the Western Cape and Transvaal.

Pedigree:



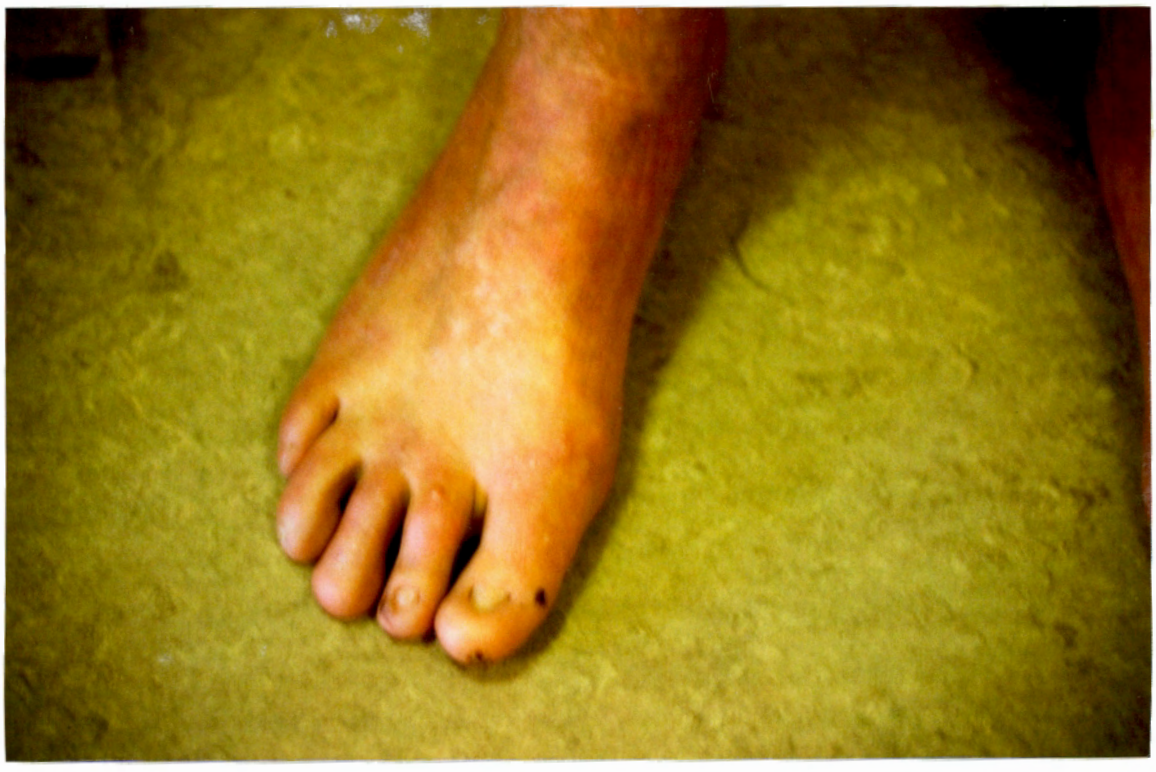


Figure 11. Nail dystrophy in DDEB Cockayne Touraine.

The proband was III-10, a thirty year old man. Since birth his skin was fragile with a tendency to blister on minor trauma. The distribution of lesions was primarily on the distal extremities. The bullae healed but resulted in scarring. Although his fingernails were unaffected, his toenails were abnormal. There was no change in his condition with age, nor worsening of symptoms in the summer.

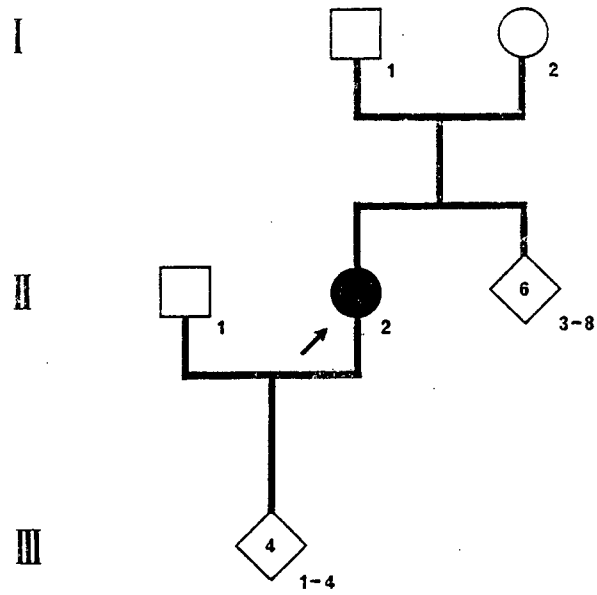
At the time of examination there were no bullae. There was marked scarring of the skin of the distal extremities and the nails of his toes were dystrophic (Fig 11).

Comment: This data is consistent with autosomal dominantly inherited **Epidermolysis Bullosa Dystrophica Cockayne Touraine.** There was discrepancy in the data regarding inheritance in this family; III-1, who had EB, is the son of II-1 who is reputed to have no dermatological problems. II-1 was not available for investigation. This may be a demonstration of incomplete penetrance of the gene for EBD Cockayne Touraine, although without objective proof, no such conclusion may be drawn. All other affected family members had manifestations of EB identical to the proband.

4.4.1.1.3 FAMILY 3

The family were of South African Black stock. There was no known consanguinity.

Pedigree:



The proband was a 45 year old woman who manifested traumatically induced blistering of the skin since birth. Lesions were confined to the distal extremities, and healing resulted in scarring and milia in these areas. Nails, teeth and hair were unaffected.

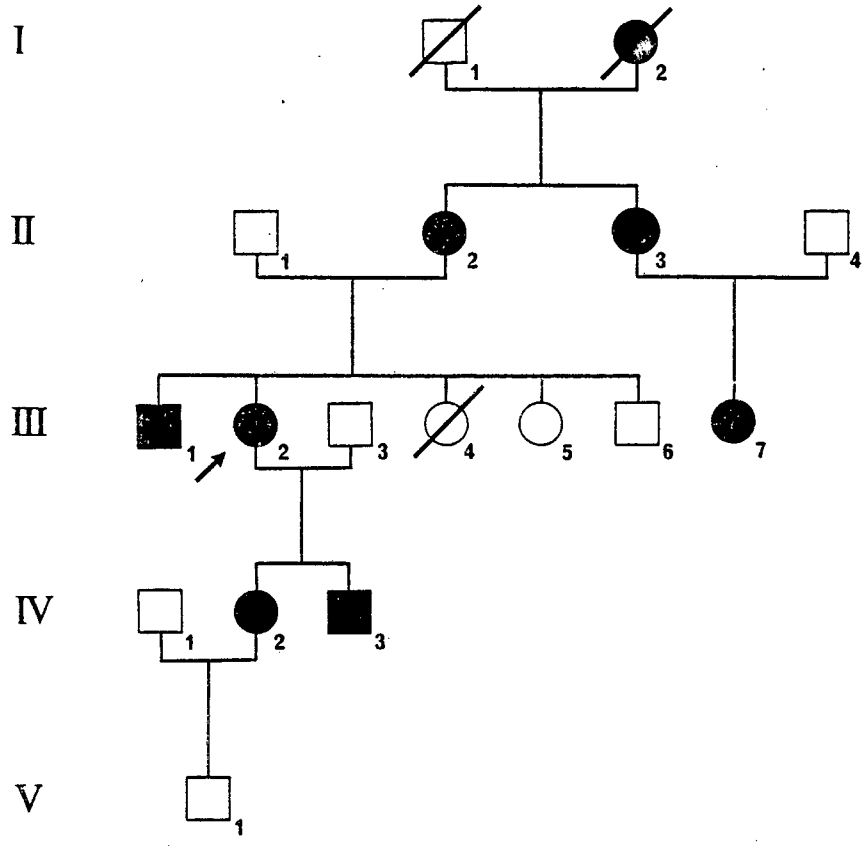
She had shown some improvement with advancing age, but her occupation in domestic service predisposed her to a moderate amount of trauma, and active blistering occurred frequently. She had bullous lesions on the hands and feet, but marked scarring over the knees, elbows, shins, hands and feet. Her nails were unaffected.

Comment: Her clinical findings were compatible with the diagnosis of **EBD Cockayne Touraine**. The pedigree may be consistent with an new mutation for this autosomal dominant condition. It is of interest that none of the proband's children are affected.

4.4.1.2 EBD Pasini

The kindred was a large Sotho family residing in a rural farming community in the north-eastern Transvaal. Members of four generations were examined, of whom 7 persons had EB. There was no consanguinity in any of the marriages shown in the pedigree.

Pedigree:



The proband was III-2, a 38 year old woman. Blistering of her skin began on the second day of life with a specific predilection for the limbs, particularly over the pressure points. Lesions healed with scarring. There was no involvement of mucosal surfaces, teeth, nor eyes. Finger and toe nails were lost with trauma and were thickened and ridged when regained.

The condition of her skin gradually improved after the second decade. Her six pregnancies did not affect the condition of her skin. There was no seasonal variation in her blistering tendency.

Skin lesions were most marked on the extremities, worsening distally and over pressure points. Fresh and established blisters were present, an area of ichthyotic skin formed a transitional zone between blistered and affected skin and normal skin on the thighs. The face was spared.

The most striking feature was the nature of her scarring. Discrete opalescent white areas were present on her arms and legs, where repeated bullous lesions had led to pigmentary alteration (Fig 12). The nails of her fingers and toes were dystrophic.

There were confluent areas of papules, approximately four millimetres in diameter on the sebaceous parts of the body.

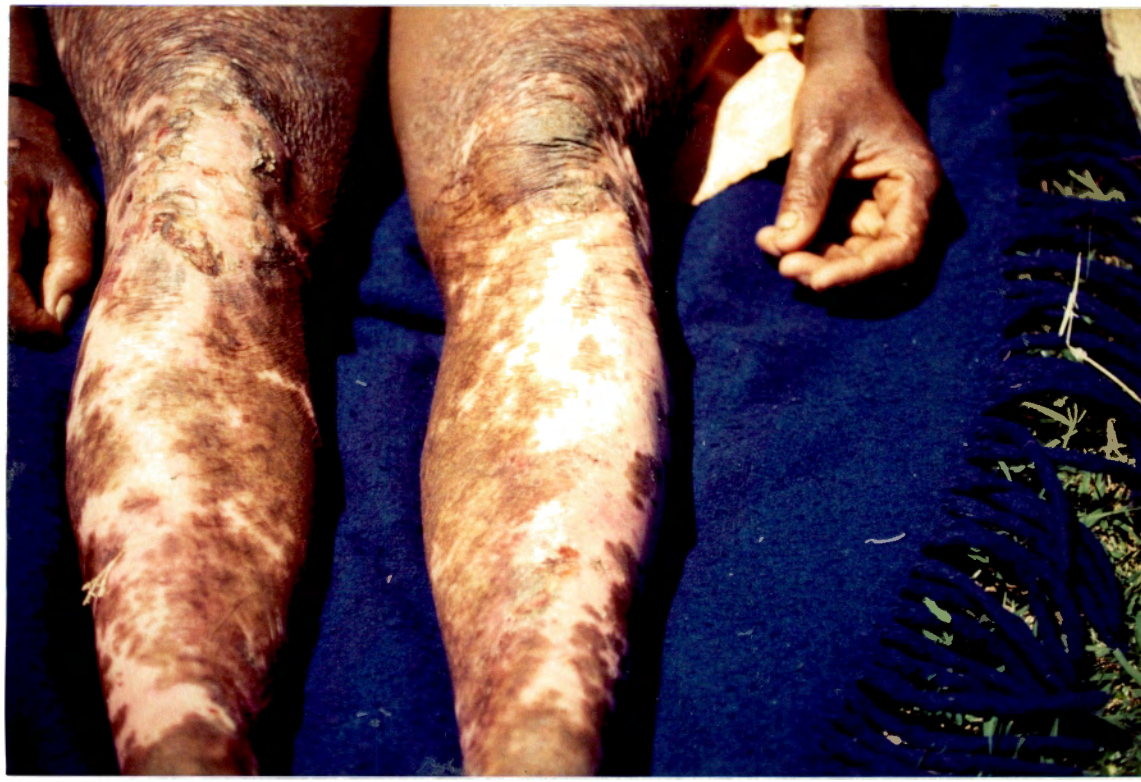


Figure 12. Depigmented scars on the legs of a woman with DDEB Pasini.

The patient was unable to recall the duration of these lesions. She was edentulous - complete extraction had been performed for replacement with cosmetically preferred dentures.

No medical attention had been sought and consequently no treatment had been attempted. None of the family members regarded their EB as significant.

A clinical diagnosis of **Epidermolysis Bullosa Dystrophica Pasini** was made on clinical grounds. Results of special investigations will be elaborated in the relevant sections.

Comment: The papules in the sebaceous areas bore striking resemblance to the so-called Pasini papules, although they were not depigmented, as are the classical albopapuloid lesions. The **Pasini** type is not well documented in dark skinned persons and these papules may be the manifestation of Pasini papules in a pigmented skin.

Autosomal dominant inheritance of **EBD Albopapuloidea (Pasini)** is demonstrated with complete penetrance. The presence of this condition in several generations is suggestive of an old and fairly stable gene.

4.4.2 Autosomal Recessive EB Dystrophica (RDEB)

The clinical features of RDEB are similar to those of DDEB; with skin atrophy, scarring and milia are a consequence of bullae and the nails are dystrophic. The phenotype and complications are, however, more severe. The onset of lesions is usually at birth or infancy, although the late onset of **EBD Progressiva** in adolescence is recognised. Mucous membranes are involved and ocular abnormalities may be seen. In addition, there may be hypoplasia of the dental enamel.

Life expectancy is not diminished. However, the more severe types of RDEB, with widespread blistering, complicated by scarring, syndactyly and contractures, may be mutilating.

The ultrastructural changes are identical in RDEB and DDEB; bullae are subepidermal and the anchoring fibrils are abnormal in structure and diminished in size and number. The latter may be identified in skin without acute blisters.

The various forms of RDEB are compared clinically in Table 4-IV.

TABLE 4-IV

SUBTYPES OF AUTOSOMAL RECESSIVE EPIDERMOLYSIS BULLOSA DYSTROPHICA

	Subtype	Age of Onset	Teeth	Prognosis	Distribution of lesions	Clinical Concomitants
1.	EBD Inversa	Birth	-	Good	Perianal, perivulvar	Anal and oesophageal strictures; sideropoenic anaemia
2.	EBD Progressiva	Late childhood/ adolescence	-	Good	Hands, feet, knees, elbows; oral mucosa	Progressive perceptive deafness
3.	EBD Hallopeau Siemens	Birth/ infancy	Hypoplastic enamel	Good	Generalised oesophageal and corneal	Syndactyly, contractures
4.	EBD Fine	Birth	-	Good	Proximal arms and legs	Centipetal progression of symmetrical blisters
5.	EBD Generalisata Gravis (Winship)	Birth	Hypoplastic	Good	Generalised	Alopecia, short stature, contractures

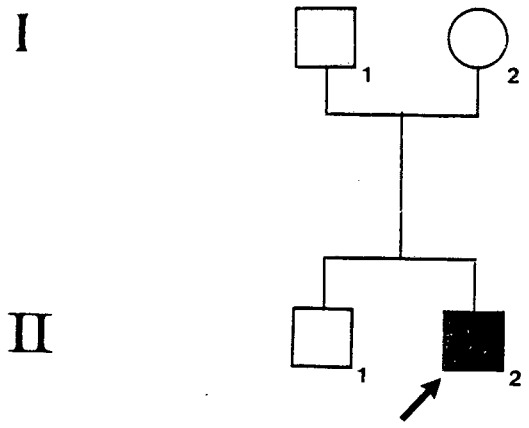
4.4.2.1 EBD Hallopeau Siemens

4.4.2.1.1 EBD Hallopeau Siemens - General

4.4.2.1.1.1 FAMILY 1

This kindred were of mixed ancestry and there was no known consanguinity. There was no positive family history.

Pedigree:



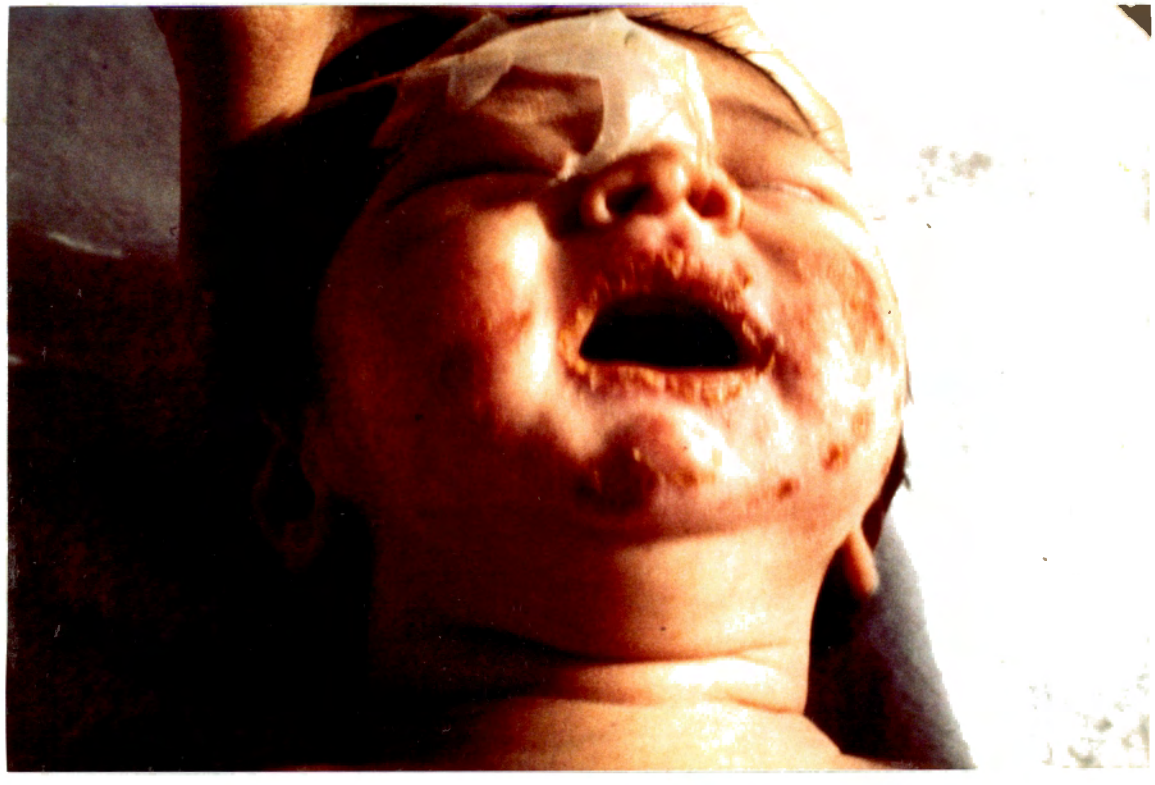


Figure 13. Lesions on the face of an infant with RDEB Hallopeau Siemens (General).

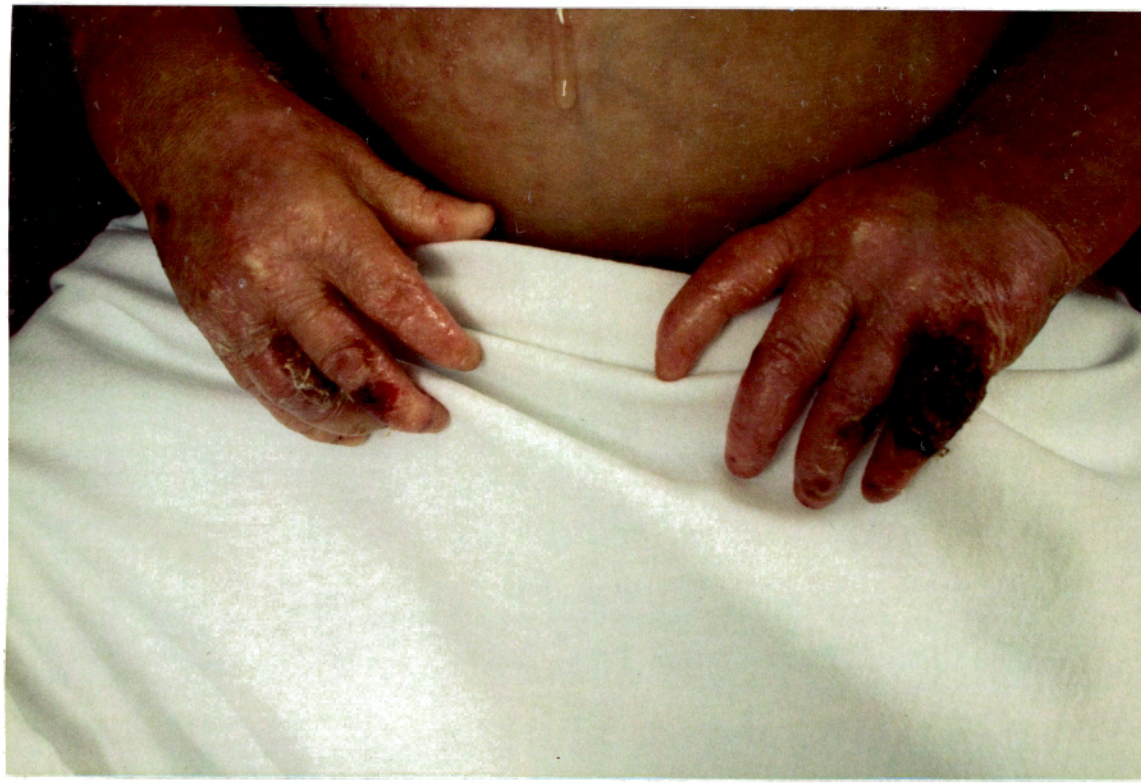


Figure 14. Dystrophic fingernails and bullous lesions in a boy with RDEB Hallopeau Siemens (General).

The proband was a 14 month old boy who presented at the time of delivery with blisters on both feet. Soon after birth, widespread bullae appeared, induced by very minor trauma to the skin. During feeding, the oral mucosa blistered and was almost totally stripped from the mouth, necessitating nasogastric tube feeding (Fig 13). In addition, his nails were shed periodically, with regeneration of dystrophic nails. Treatment with vitamins and zinc ointment had served to palliate his condition, but even symptomatic management was largely ineffective.

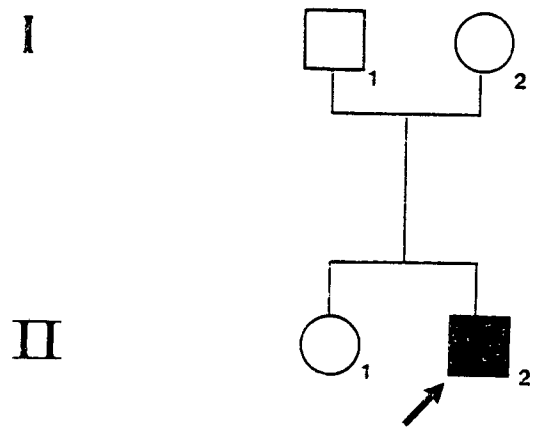
Bullous lesions and scars were distributed throughout the body. Lesions seen on the genetalia and in the mouth, in the neonatal period healed and were not present at subsequent examinations. His nails were shed due to subungual blistering, and regrowth had yielded dystrophic nails on fingers and toes (Fig 14).

Comment: This infant has **EBD Hallopeau Siemens - General.** The parents were frequently accused of child abuse, and a report had been made to the police regarding skin lesions which resembled cigarette burns. EB mimicking child abuse is discussed in a separate section of this thesis.

4.4.2.1.1.2 FAMILY 2

This family of mixed ancestry had no known history of any inherited diseases. No consanguineous marriages had occurred and the parents of the proband were both young.

Pedigree:



The proband was an 18 month old boy who presented with areas of denuded skin on both heels, at the time of birth. Widespread blistering of the skin and mucous membranes ensued, and subungual blistering resulted in the loss of his nails. His condition was better in cool weather and showed a marked deterioration in the very hot summer months. Initial treatment with Phenytoin in a neonatal unit was thought to be successful by the attending paediatrician, although no objective proof of its efficacy was evident.

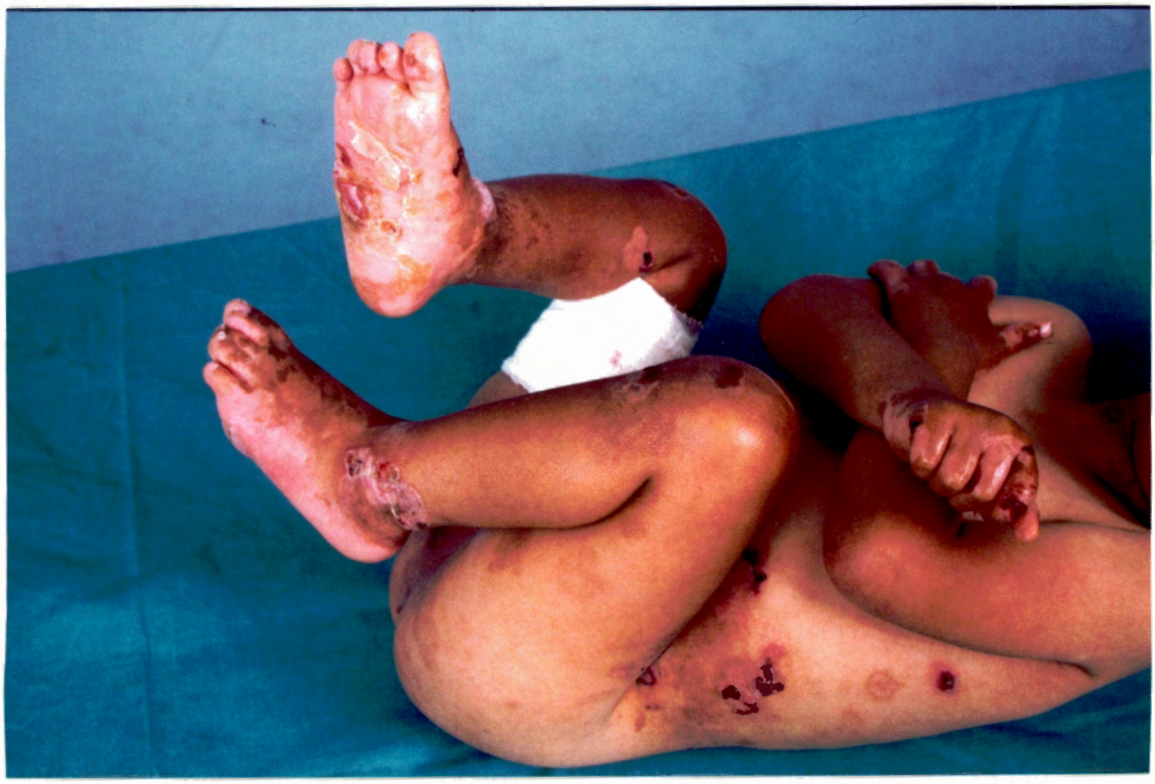


Figure 15. Generalised distribution of lesions in a boy with RDEB Hallopeau Siemens (General).

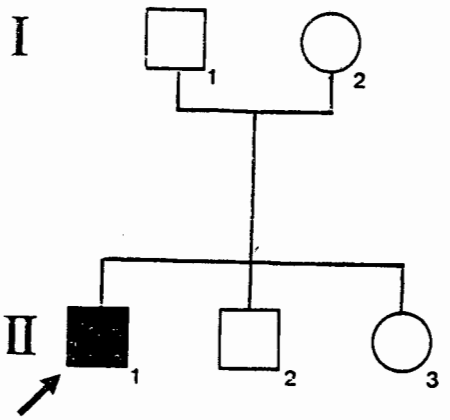
Examination revealed fresh blisters and scarring in a generalised distribution. (Fig 15). Several nails were dystrophic. Those teeth which had erupted appeared normal.

Comment: Cinical assessment together with genetic information were consistent with the diagnosis of **RDEB Hallopeau Siemens (General).**

4.4.2.1.1.3 FAMILY 3

The kindred were of English and Irish stock. There was neither consanguinity nor a family history of any inherited disorders.

Pedigree:



The proband was a 23 year old male who was referred for investigation by a gastro-surgeon, to whom he had complained of dysphagia. In addition he had traumatically induced bullous skin lesions which had persisted from infancy into adulthood. The lesions were in a generalised distribution and resulted in scarring. Subungual blistering had led to the shedding of all his nails which had not regenerated.
Since his early teens, there had been some improvement in the condition of his skin, although treatment in childhood had had limited success. Unlike many persons with EB, who suffer more

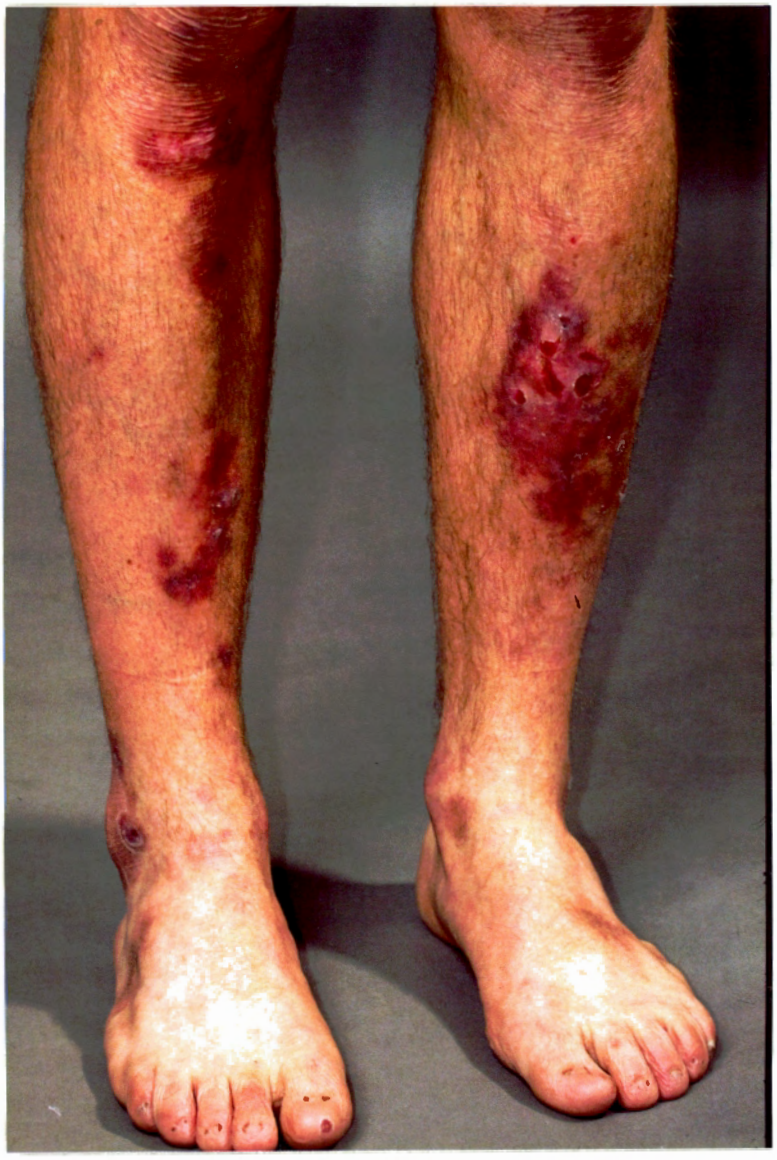


Figure 16. Scarring and nail dystrophy in an adult iwht RDEB Hallopeau Siemens (General).

blistering in the summer, he felt that his condition deteriorated in extremely cold weather.

Blistering of the tongue, palate and oral mucosa had continued into adulthood, and worsening dysphagia with a marked restriction of swallowing was his most troublesome symptom. Food would "stick" at the cervical region and, on some occasions, he could neither swallow nor regurgitate the bolus. There was no associated respiratory compromise.

There was widespread scarring of his skin with a few fresh serous bullae. Those nails which had regenerated were markedly dystrophic (Fig 16). His teeth were unaffected.

Blisters on the palate and oral mucosa suggested that his oesophageal involvement was still active, and that new lesions as well as strictures may have contribute to his dysphagia. Oesophagoscopy had previously been performed and had revealed numerous bullae on the entire length of the oesophagus mucosa. A barium swallow was performed and this revealed two obstructions in the cervical region with the features of oesophageal webs. The first obstruction, which was opposite the fifth cervical vertebra, was not markedly narrow. Three centimetres below this, and opposite the seventh cervical vertebra, was an extremely narrow web-like structure (Fig 17, 18). Between the 2 lesions was marked pseudodiverticulum formation. The nature of this lesion related to the history obtained, when he was unable to requrgitate the obstructing

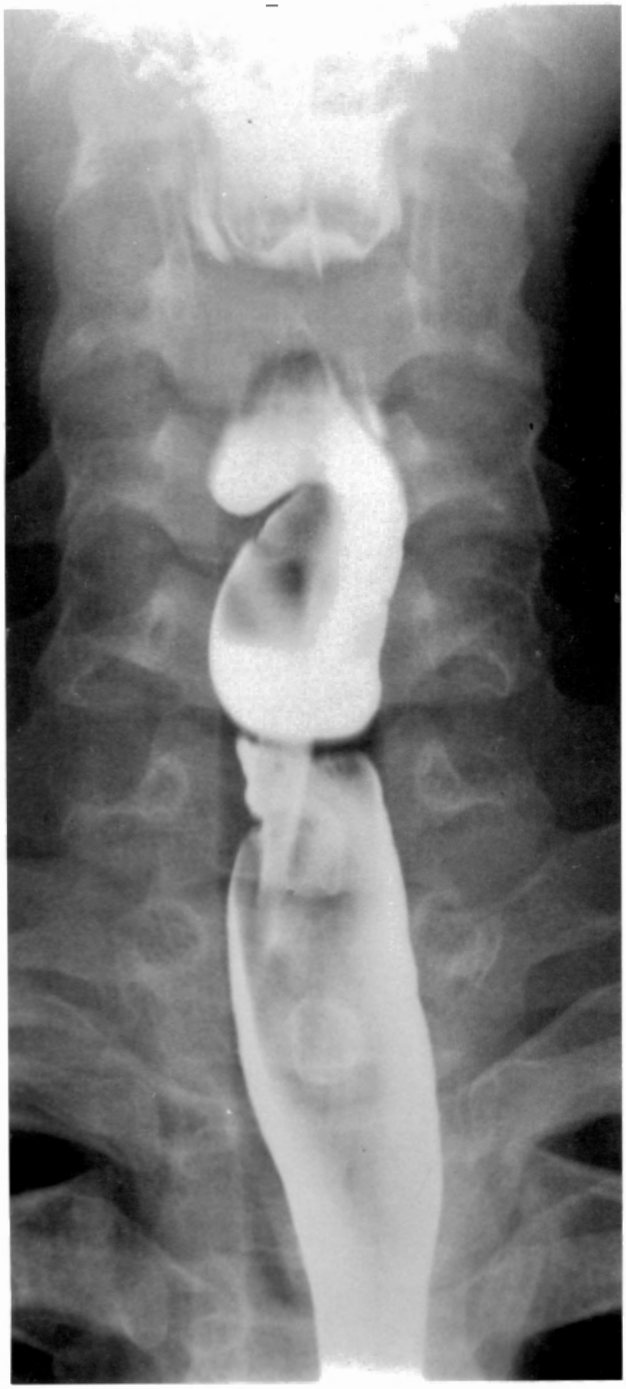


Figure 17. PA view of Barium swallow showing strictures are C5 and C7

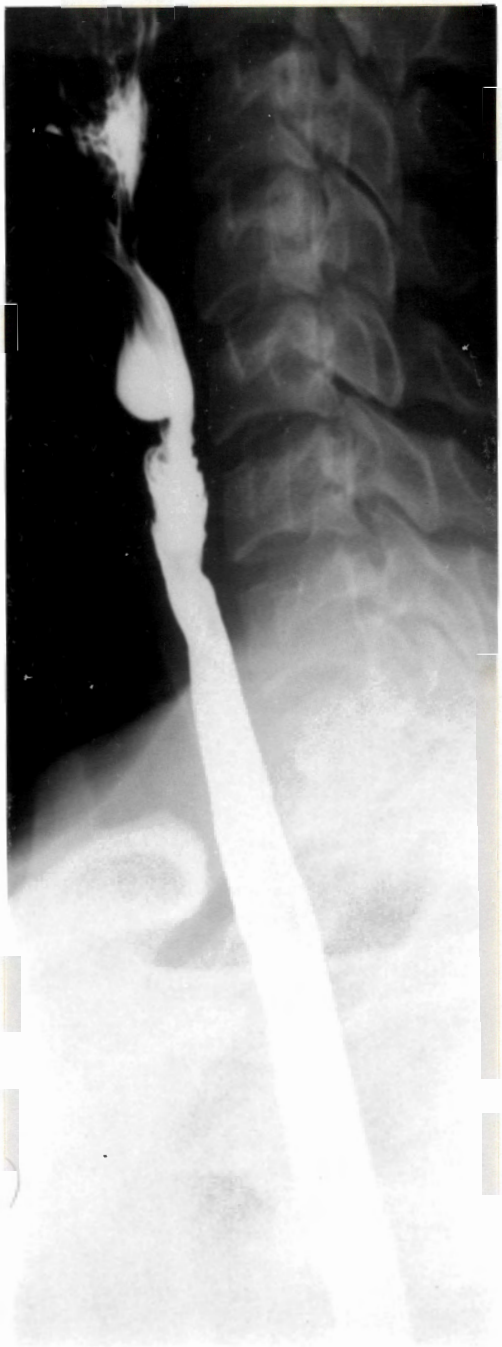


Figure 18. Lateral view of these oesophageal strictures

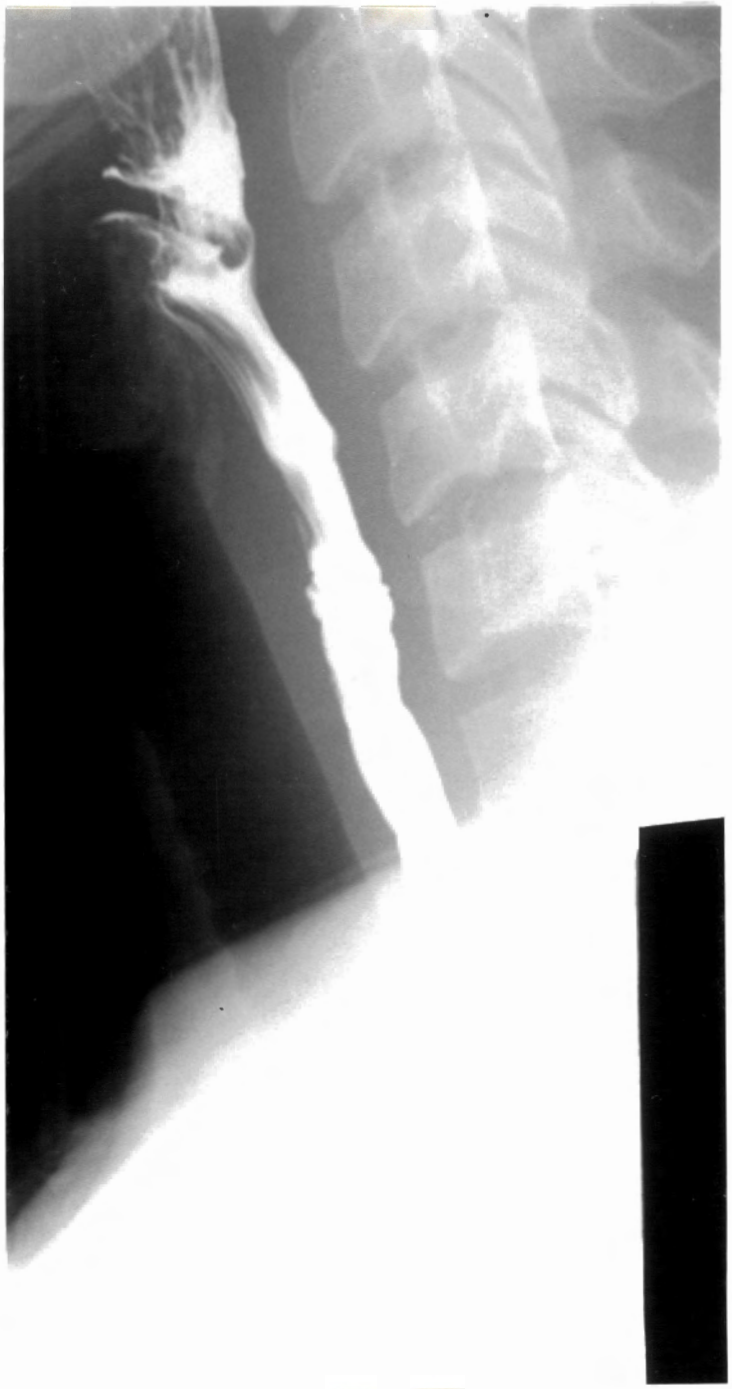


Figure 19. Barium study after dilatation demonstrating a marked reduction in the strictures

bolus; food may lodge between the two strictures thus explaining his symptoms. Oesophagoscopy was impossible due to the severely narrowed stricture.

Dilatation of the stricture was performed by balloon endoscopy. Under pethidium and valium sedation, a guide wire was passed into the stomach. An 8mm balloon was positioned accross the webs simultaneously and inflated for 30 seconds. This did not produce discomfort and the procedure was repeated with a 12mm balloon. At a second visit 5 days later, a Celestin dilator (Neoplex) was passed to size 36 French (12mm) with no discomfort although resistance was noted. Two days later the bigger Celestin dilator (French 34) was passed through 3/4 of its length. A repeat barium study following the dilatation showed a marked improvement of both webs (Fig 19). In the hope of preventing further cicatigation, visanic (sucralfate) 1g, dissolved in water was sipped 4 times daily for 2 weeks.

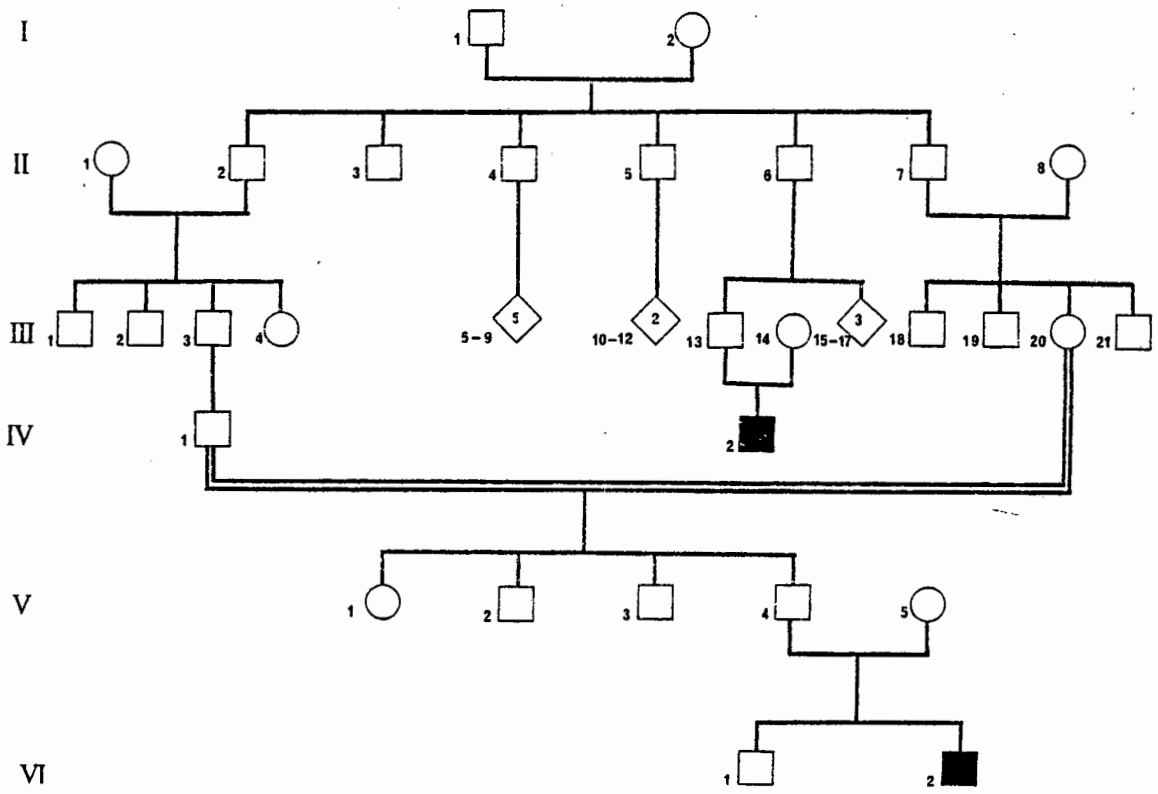
There was considerable symptomatic relief with this procedure and follow up correspondence 1 year later confirmed that he had had no recurrence of his dysphagia.

Comment: This association of severe oesophageal and buccal mucosal involvement is an unusual though well-documented complication of RDEB. Oesophageal strictures are known to occur as a complication of **EBD Inversa** - by virtue of the distribution of lesions, this subtype can be excluded clinically, and the diagnosis of **EBD Hallopeau-Siemens (general)** is favoured. Other relevant special investigations and management will be discussed in the subsequent chapters of this section. In view of his oesophageal strictures and the successful management of these lesions, Chapter 7 is devoted to the involvement of the oesophagus in EB.

4.4.2.1.1.4 FAMILY 4

This kindred was of Afrikaner stock from the Northern Transvaal. Although the proband's parents were not relatives, there had been intermarriage in the family, and his paternal grandparents were first degree relatives.

Pedigree:



The proband was a 15 year old boy who presented with blistering of the skin at the age of two months. The typical bullous lesions of EB were induced by trauma in any area of the body but especially the feet and legs, hands and ears. There had been involvement of the oral mucosa and palate, with recurrent blockage of salivary ducts in the mouth.

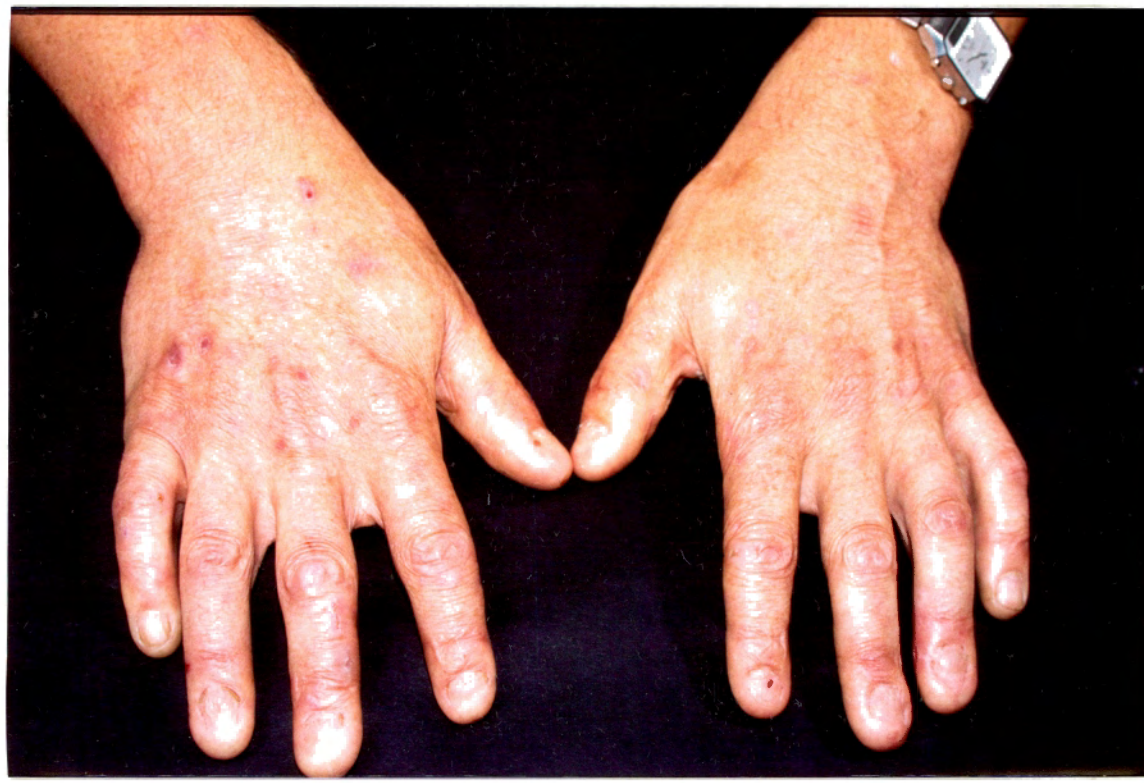


Figure 20. RDEB Hallppeau Siemens (general) nail dystrophy

Scarring had resulted from acute lesions, and in this respect there had been some sparing of the face, where minimal scarring had occurred. After repeated shedding, the dystrophic nails of his fingers and toes did not regenerate. His teeth and eyes were not involved.

He had the typical bullae and scars of EB Dystrophica. The distribution of lesions was generalised although involvement of the skin was most marked on the distal extremities and ears. All finger and toe nails had been shed (Fig 20). Small blisters and scars were seen on his palate. His teeth appeared normal and healthy.

IV-2 had the identical manifestations of EB Dystrophica.

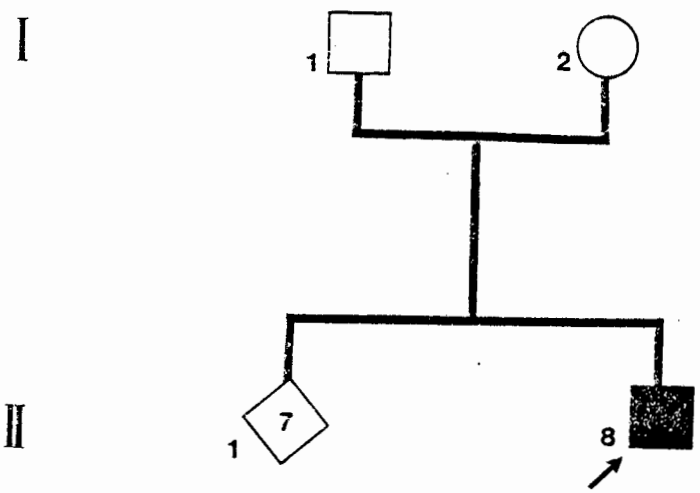
Comment: The pedigree data suggested autosomal recessive inheritance. Family members were from a small community and consanguineous marriage had occurred. The clinical and genetic data were compatible with the diagnosis of **RDEB Hallopeau Siemens (general).**

4.4.2.1.2 EBD Hallopeau Siemens - Mutilans

4.4.2.1.2.1 FAMILY 1

The family shown in the pedigree below were of Madeiran Portuguese stock. No consanguinity was known.

Pedigree:



The proband was a 14 year old boy who was diagnosed soon after birth as having EB, when typical bullous lesions developed over the entire body, induced by trivial trauma. The scalp was not spared and the oral mucosa was stripped during feeding. Widespread scarring resulted from his blisters.

The nails of his fingers and toes were shed as a result of subungual blistering, and did not regenerate. His teeth became carious in his early childhood, necessitating almost total extraction. In addition, he suffered from intermittent dysphagia, and this, together with inadequate dentition, allowed him to eat only a puréed diet.

Since the age of 12 years he noted some improvement in his condition. Nevertheless, he continued to blister on any frictional trauma and his skin, which was fragile, split open when subjected to minimal stress. No attempts at treatment had been successful.

This boy had marked short stature and was well below the third percentile for height, using standard charts. Fresh serous blisters, to raw, deroofed and desloughed sores, and crusted lesions in varying degrees of the healing process were evident on his skin. No area of the body was spared and blisters were present on the scalp.

His hands and feet were most severely affected. Scarring and fibrosis had led to flexion contractures with resultant syndactyly and ultimately mitten-like deformities of both hands and feet. No nails were present on the fingers or the toes. Similar complications of scarring peri-orally prevented full opening of his mouth. His four remaining permanent teeth were carious, and the enamel appeared to be hypoplastic. A clinical diagnosis of **EBD Hallopeau Siemens** was made.

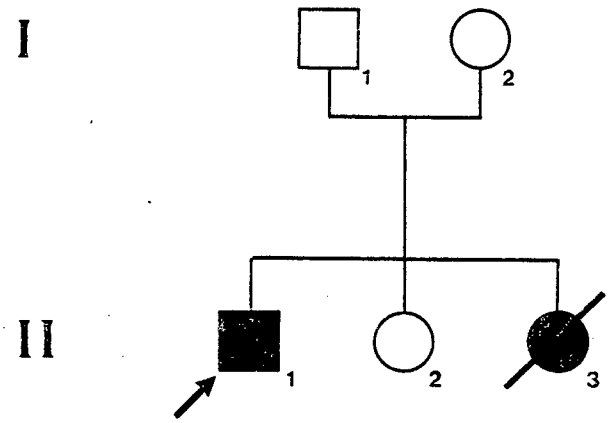
Despite symptoms of possible gastro-intestinal manifestations of EB, the patient and his family refused any further investigation of his condition.

Comment: This boy was the most severely affected person encountered in the study. His physical signs confirmed validity of the suffix 'mutilans' applied to certain types of **EBD Hallopeau Siemens.** The fact that the parents of the proband were from a small inbred community is in accordance with the autosomal recessive pattern of inheritance recognised in this subtype.

4.4.2.1.2.2 FAMILY 2

The family was of British and British/South African stock. The father of the proband was adopted in infancy, and no details of his family history were known. He emigrated to South Africa as an adult, and no consanguinity was suspected.

Pedigree:



The proband was a 22 year old man who had bullous skin lesions at the time of delivery. A wide distribution of traumatic and spontaneous blisters of the skin and mucous membranes ensued. These lesions healed with scaring and resultant deformities of his hands and feet.

His rudimentary nails were shed in early infancy and did not regrow. Dental involvement was severe. His primary teeth were poorly developed and eruption of his hypoplastic permanent teeth was delayed. Imperfect enamel had necessitated the crowning of his teeth. In addition, this young man had symptoms of dysphagia, dyspepsia and constipation, and

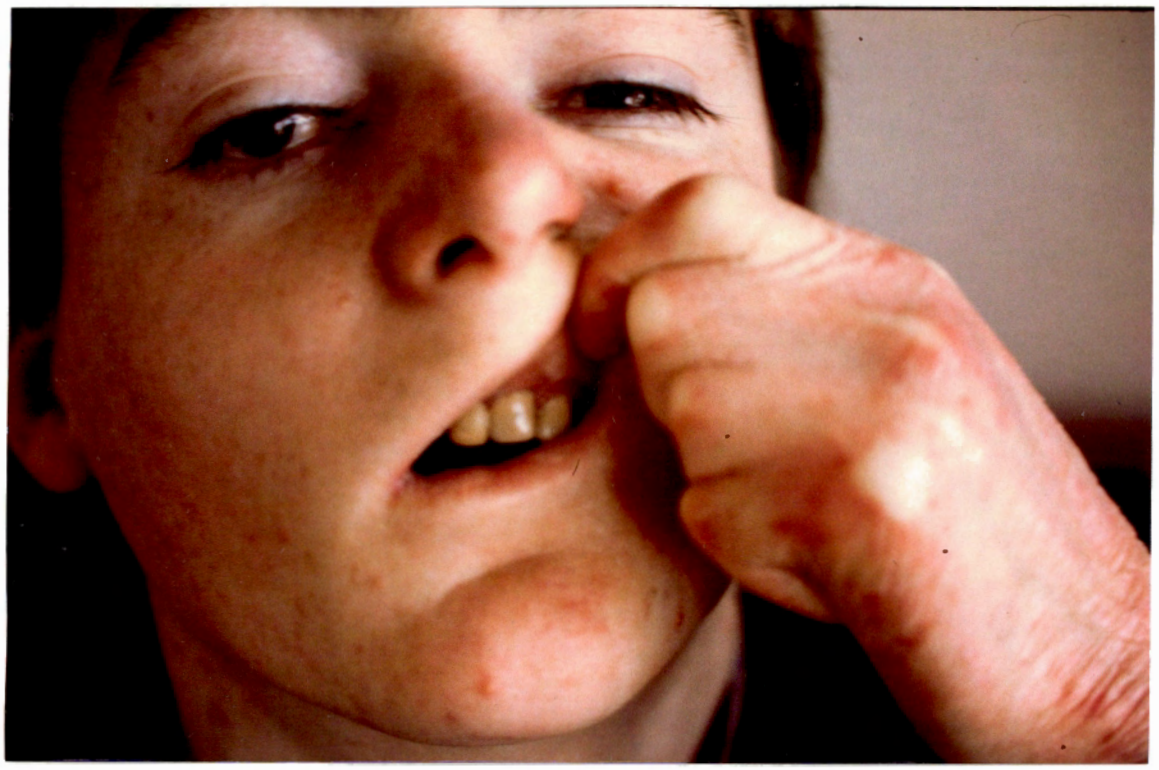


Figure 21. Blister on the gingiva of a man with RDEB Hallopeau Siemens (mutilans)

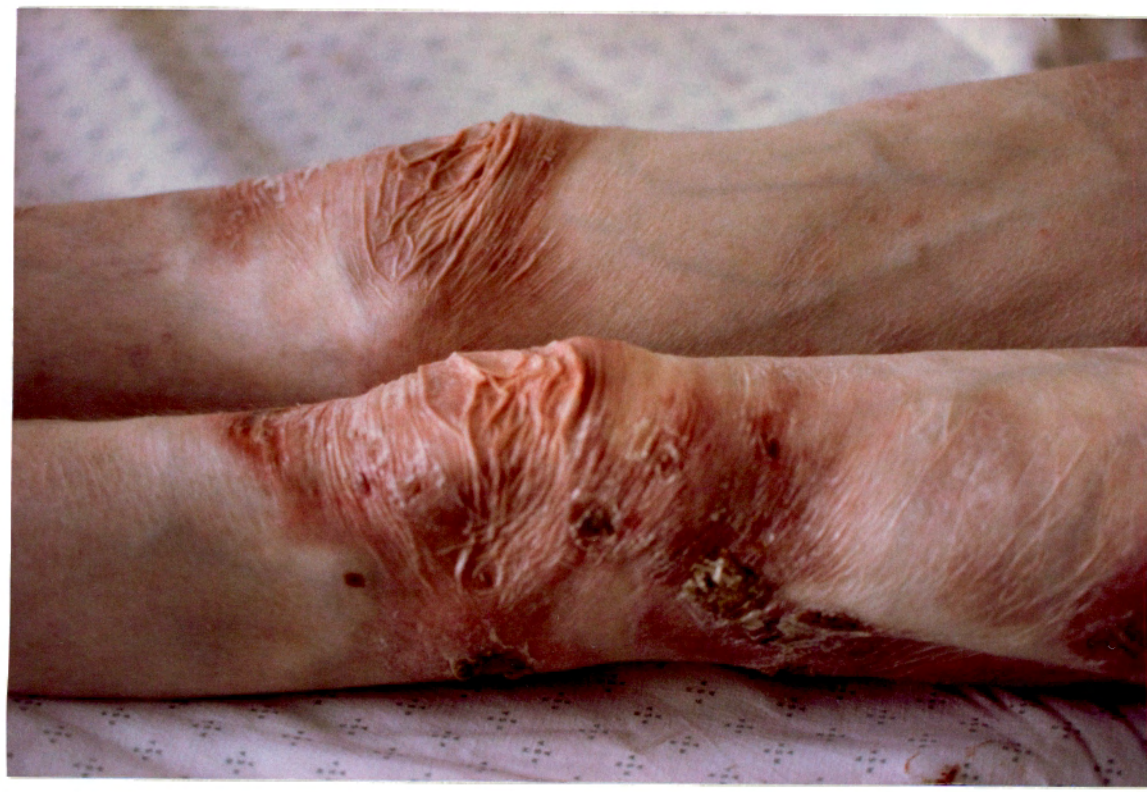


Figure 22. Skin lesions with atrophy and scarring in RDEB Hallopeau Siemens (mutilans)

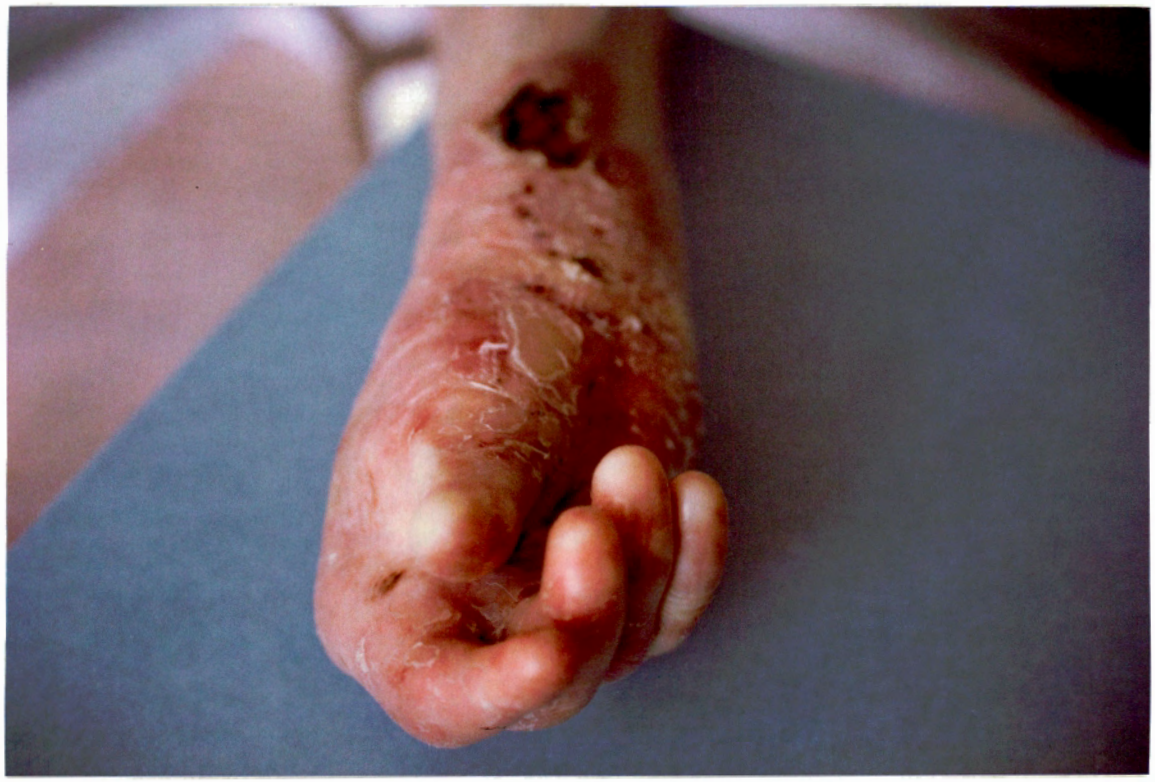


Figure 23. Mitten deformities of the hands of a man with RDEB Hallopeau Siemens (mutilans)

previous studies had demonstrated two significant strictures of the oesophagus. These had been dilated several years previously without recurrence of his symptoms.

Blistering of the oral mucosa and gingiva were evident (Fig 21). Full examination of the teeth was not possible as a result of prosthodontic intervention; those teeth not yet treated displayed hypoplastic enamel and exaggerated caries. Scarring was evident and perioral contractures prevented full opening of the mouth.

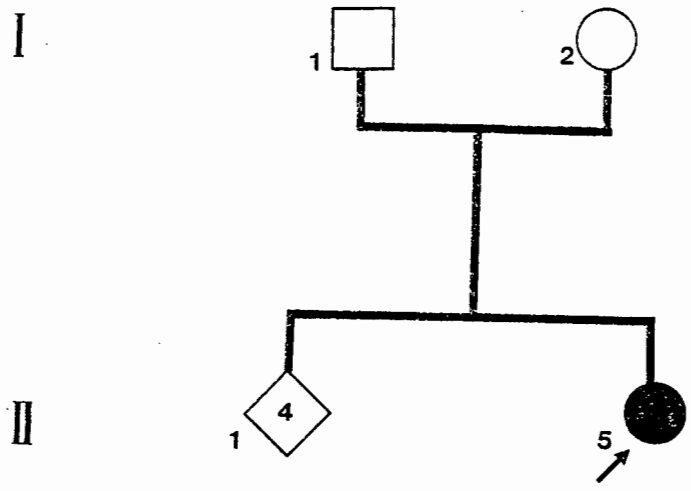
His height was significantly below the average. Bullae, both intact and deroofed, were noted throughout the skin especially on the pressure areas where the skin was atrophic. (Fig 22). Flexion contractures and secondary soft tissue fusion of digits (syndactyly) had resulted in mitten deformities of his hands and feet, with significant loss of function. No nails were present (Fig 23).

Comment: The symptoms and signs were those of **Epidermolysis Bullosa Dystrophica Hallopeau Siemens (Mutilans)**. The pedigree data confirmed autosomal recessive inheritance and his sister who had died of unrelated causes had the characteristic lesions of this subtype.

4.4.2.1.2.3 FAMILY 3

The parents of this child of mixed ancestry were non-consanguineous and were both young.

Pedigree:



The proband was a four year old girl who presented with blistering of the skin at the time of birth. These bullous lesions occurred throughout the body, and resulted in the formation of scars. Similar subungual involvement caused the nails of her fingers and toes to be shed, and the regrowth of dystrophic nails had occurred on only three fingers.

Examination revealed a child who was below third centile for all parameters of growth, and had pallor consistent with an iron-deficient anaemia. She had a generalised distribution of the typical acute lesions and scars of dystrophic EB. In addition, those nails present on the fingers were dystrophic. Her dentition was poor, with hypoplastic enamel and severe caries.

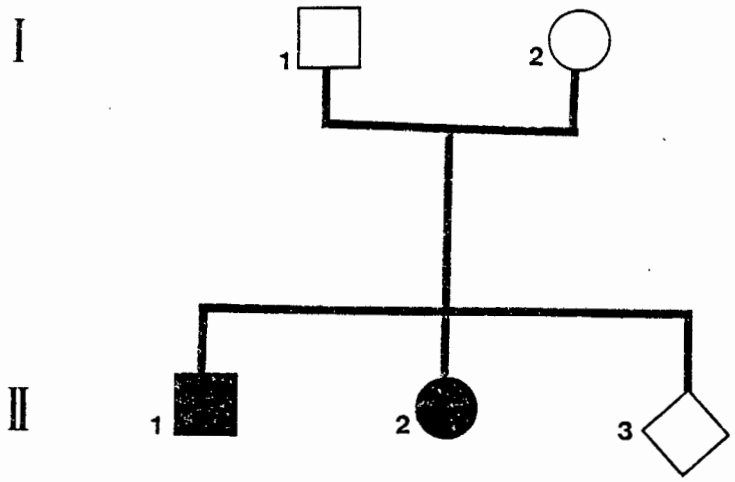
Complications of scarring were fixed flexion contractures of the left foot and the fingers of the right hand, although no fusion of her digits had occurred.

Comment: The features were consistent with **RDEB Hallopeau Siemens**. The very early development of the severe flexion deformities was consistent with the **mutilans** subgroup.

4.4.2.1.2.4 FAMILY 4

This family were Asians residing in Natal.

Pedigree:



These persons had been lost to follow-up, and were not examined during this study. The two affected siblings had been documented by a medical practitioner as having the typical manifestations of EB Dystrophica. Scarring lesions had been complicated by mutilating deformities of the hands, and clinical records confirmed the diagnosis of **REDB Hallopeau Siemens (Mutilans).**

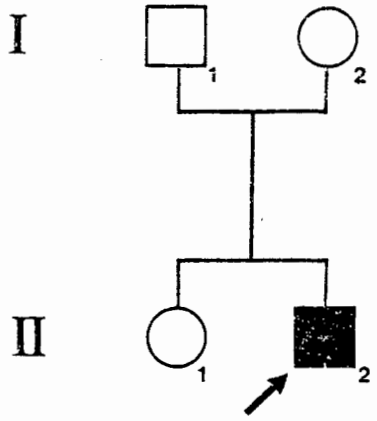
Comment: Their manifestation of EBD demonstrated that the gene is present in persons of Asian stock. Inheritance, as seen in the pedigree, was autosomal recessive.

4.4.3 Unclassified subtypes of EB Dystrophica

4.4.3.1 FAMILY 1

This family were South African Blacks, of the Tswana tribal group. Both parents were young. No positive family history for EB was obtained.

Pedigree:



The proband was a five year old boy who was noted at the time of birth to have absence of skin of the left foot and a raw area at the top of his tongue. Soon thereafter blistering of the skin began. The distribution of lesions was localised to the arms, legs and oral mucosa and tongue. Blisters were slow to heal with resultant scarring. His nails were all shed and did not regenerate. His dentition was not affected.

On examination he was noted to have bullae and scars on the distal extremities (Fig 24) No nails were present on his fingers or toes Several blisters were evident on his tongue.

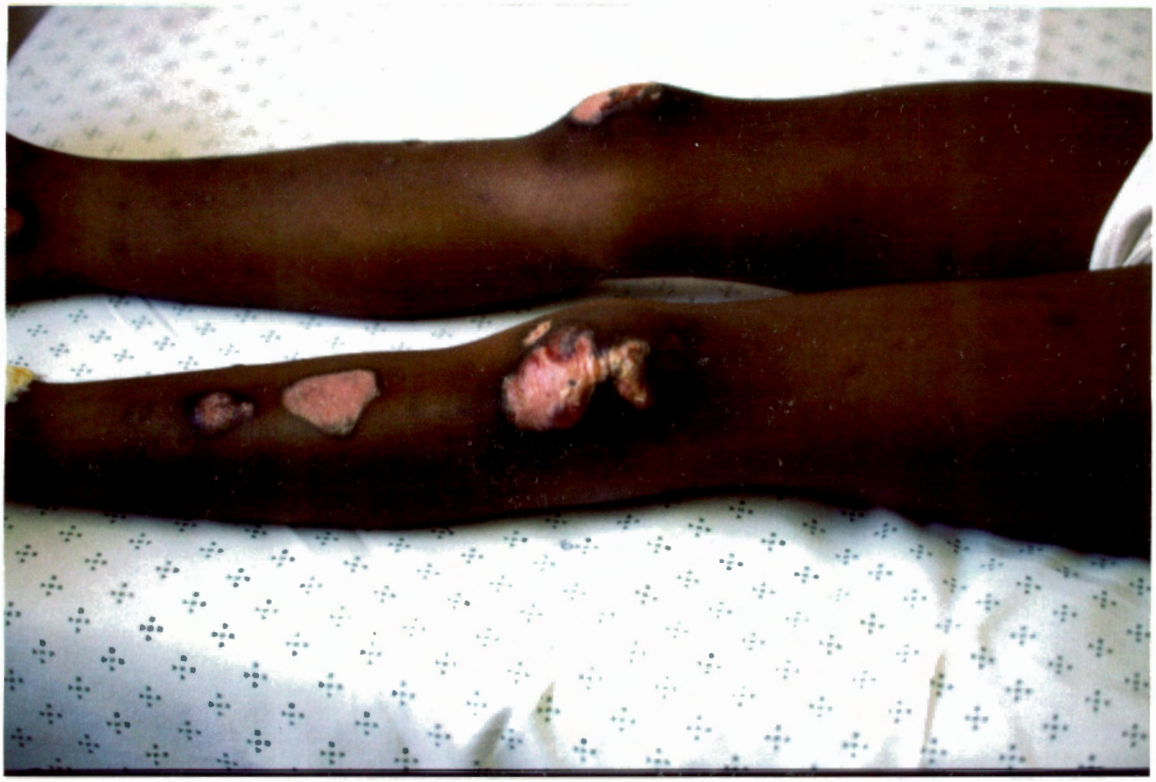


Figure 24. Scarring lesions in a child with unclassifiable EB Dystrophica

His clinical condition was not severe and, the distribution of bullae mainly on the extremities, was consistent with a diagnosis of **DDEB Cockayne Touraine.** This phenotype however is also characteristic of **RDEB Hallopeau Siemens (local)** and differentiation between these two subtypes was impossible.

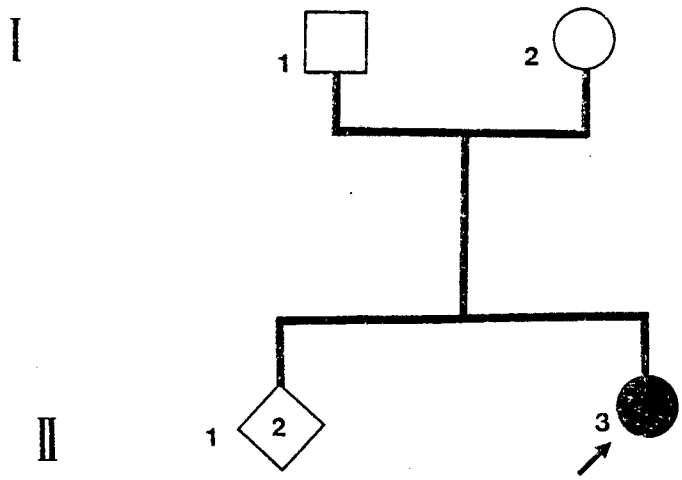
Comment: The pedigree data was unhelpful. In this tribal group traditional inbreeding is known to exist and consanguinity is considered desirable. This knowledge may support autosomal recessive inheritance, although a fresh mutation of the gene for DDEB may have occurred. This sporadic presentation of the milder form of EB Dystrophica poses a problem in terms of genetic counselling.

One cannot positively differentiate on clinical or ultrastructural grounds between autosomal recessive and autosomal dominant dystrophic EB. Clinically the phenotype of the autosomal dominant subgroups is less severe than the autosomal recessive form, although similar to **RDEB Hallopeau Siemens (local)** dystrophic subtype presents sporadically, a dilemma exists in establishing whether the inheritance is recessive, or a new mutation of a dominant gene. This aspect of genetic counselling will be further discussed in Section VII.

4.4.3.2 FAMILY 2

This family of South African Blacks were from the Xhosa tribal group. There was no consanguinity.

Pedigree:



The proband, a three year old girl, presented with blistering skin lesions from the age of two months. The distribution of lesions was widespread and minimal scarring resulted from blisters.

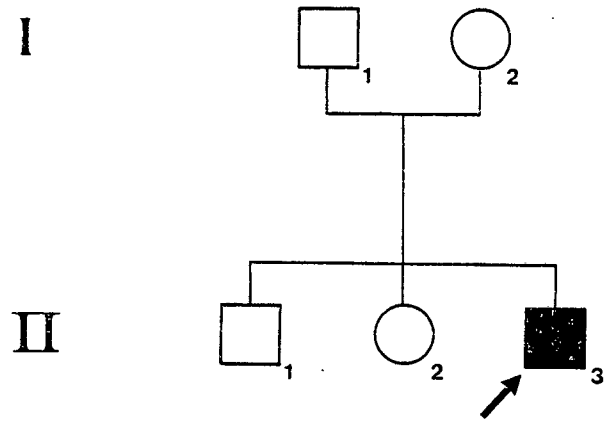
Examination of the skin revealed bullous lesions distributed mainly on the hands and feet, legs, forehead and sacrum. Healed lesions were present as areas of depigmentation and some scarring was present.

Comment: The sporadic manifestation of EB could be consistent with autosomal recessive inheritance or a new mutation of an autosomal dominant form. Phenotypically, this milder form of EBD is more in keeping with the autosomal dominant subgroup.

4.5 A NEW SOUTH AFRICAN SUBTYPE OF EB: EBD GENERALISATA GRAVIS

The pedigree below shows an Afrikaner kindred from the Southern Cape. The proband was the only affected member of the family. There was no parental consanguinity, and paternal age was not advanced.

Pedigree:



The proband, a 20 year old male, presented with severe generalised blistering of his skin at birth. In addition, the nails of his fingers and toes were absent and did not develop.

His history followed of a stormy course with severe bullae developing on minor trauma such as light contact with the skin, with the result that he had been unable to attend school or acquire employment. Lesions occurred at any site on the body including the scalp, the eyelids and the genitalia. The oral mucosa frequently blistered but he had not suffered dysphagia. Hair growth had been severely impaired since infancy.

Figure 25. A man with EBD Generalisata Gravis beside his mother, who is of normal stature

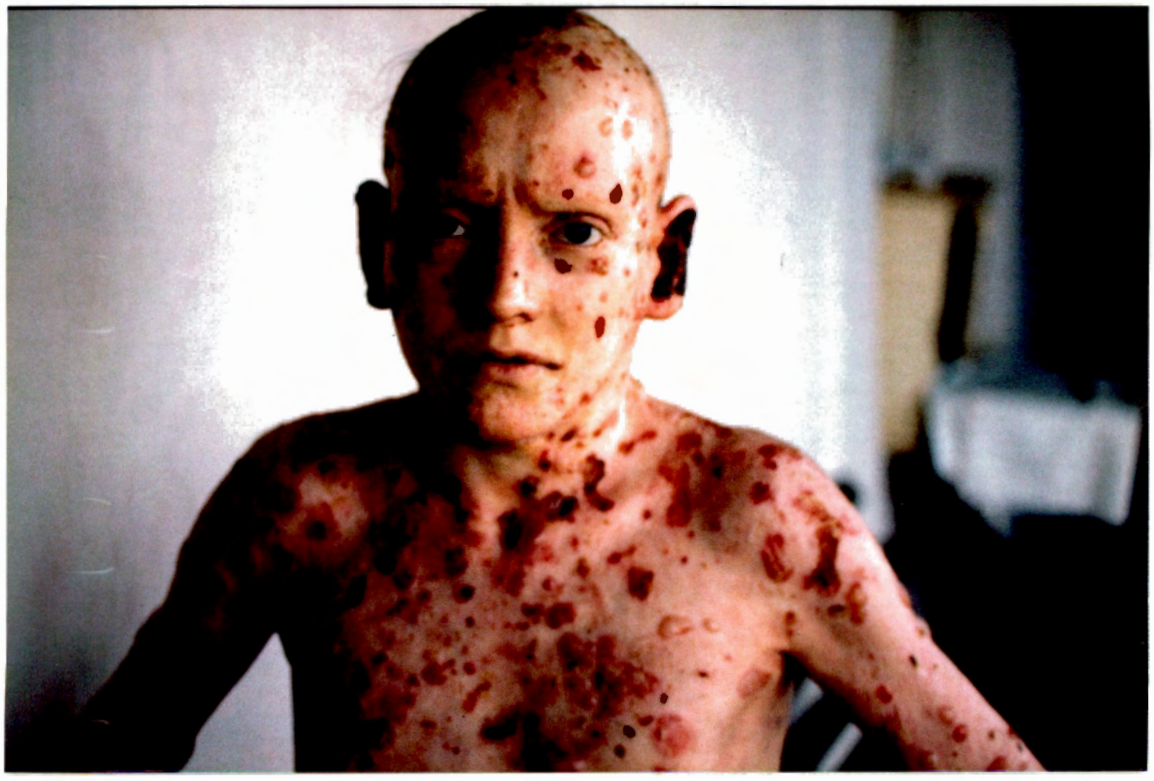


Figure 26. Severe lesions in a widespread distribution in EBD Generalisata Gravis

At the age of 16 years his condition deteriorated markedly, but over the four years preceding this investigation, his skin lesions had shown a very slight improvement. An additional problem was a longstanding iron deficiency anaemia.

Numerous treatment modalities had been attempted including topical and oral vitamins, steroids and antibiotics, and latterly Phenytoin. No significant relief of symptoms had been achieved.

This young man was of very short stature as demonstrated in Figure 25 which shows him beside his mother, who is of average stature.

The skin was very severely affected with an almost confluent pattern of bullae at varying stages of development distributed (Fig 26) throughout the body. Most of these blisters were filled with blood. No area of the skin was spared and bullae were present on the upper eyelids and on the genitalia. There were no acute lesions in the oral mucosa and his the corneas were unaffected.

The nails of all his fingers and toes were absent (Fig 27). His teeth were markedly carious despite good oral hygiene. Body hair was minimal despite otherwise normal secondary sexual

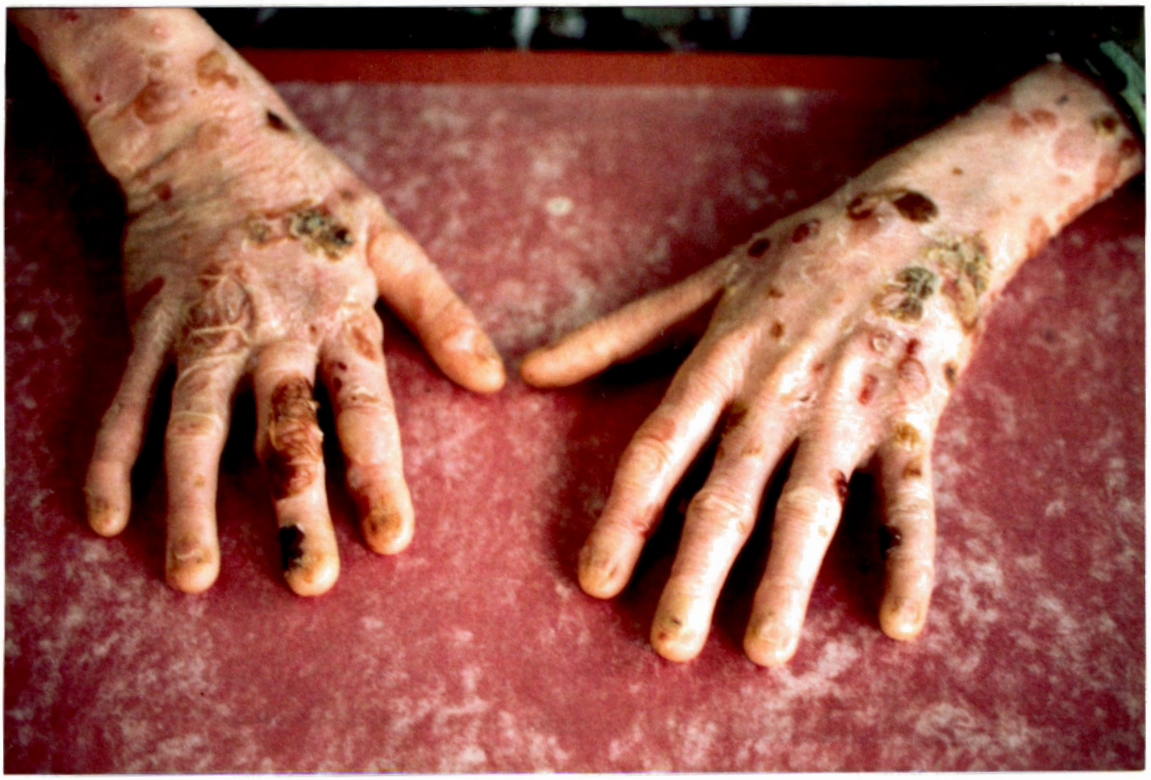


Figure 27. Absence of all finger nails in EBD Generalisata Gravis

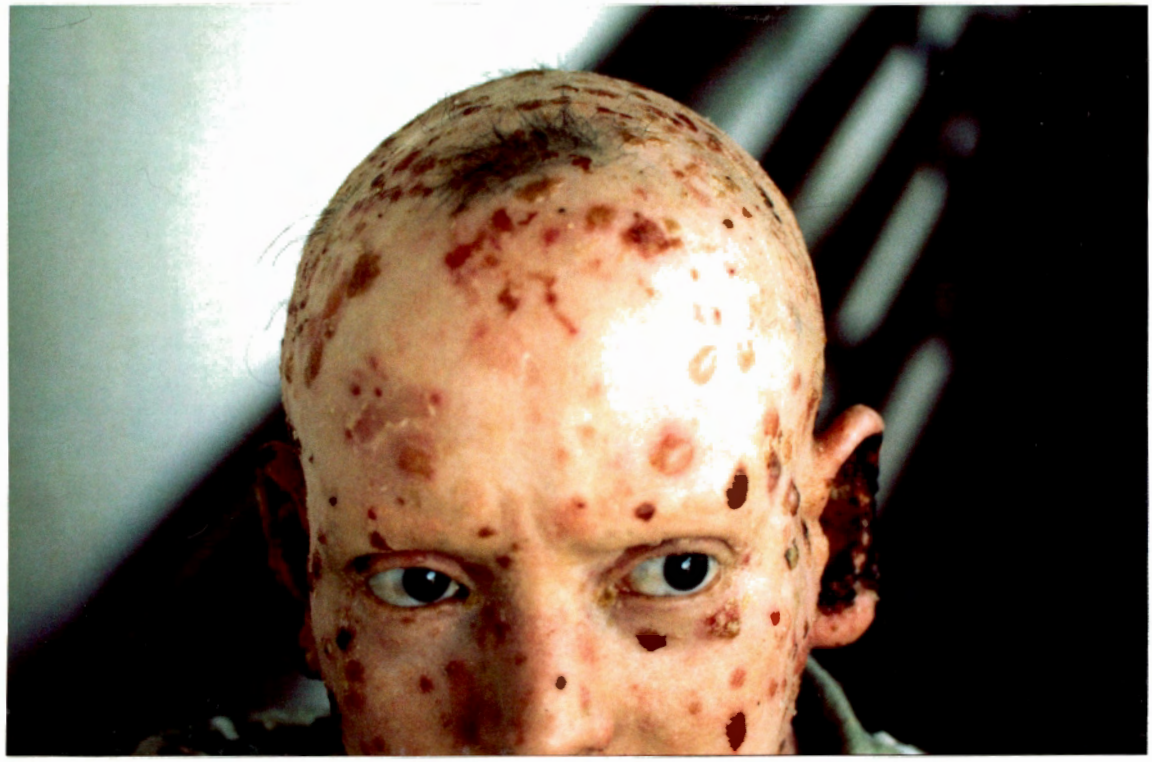


Figure 28. Alopecia is almost complete in the man with EBD Generalisata Gravis

characteristics. He had almost complete alopecia and his scalp was covered by fresh blisters, open sores and some scar tissue (Fig 28). In addition he had flexion contractures of the digits, elbows and knees consequent upon repeated bullae, scarring and fibrosis.

Comment: This young man did not have the phenotype of any previously documented type of EB. The fact that this was a new entity was verified by Tobias Gedde Dahl in personal communication.

This subtype varies from **RDEB Hallopeau Siemens (mutilans)** in that despite very severe scarring lesions, no secondary soft tissue fusion of digits occurred. In addition the distribution of lesions is quite unlike any reported cases of **RDEB Hallopeau Siemens**. Almost complete alopecia is a compelling finding in this man. While it is true that scarring would prevent hair growth, he has never had a normal distribution of hair on his head or body. In addition, the degree of scarring of his scalp is not sufficient to postulate that this was the sole cause of his alopecia.

The nomenclature **RDEB Generalisata Gravis** has been chosen in view of the widespread distribution of lesions in this very severe dystrophic subtype. In accordance with the use of eponyms, this form will be designated **EBD Generalisata Gravis (Winship).**

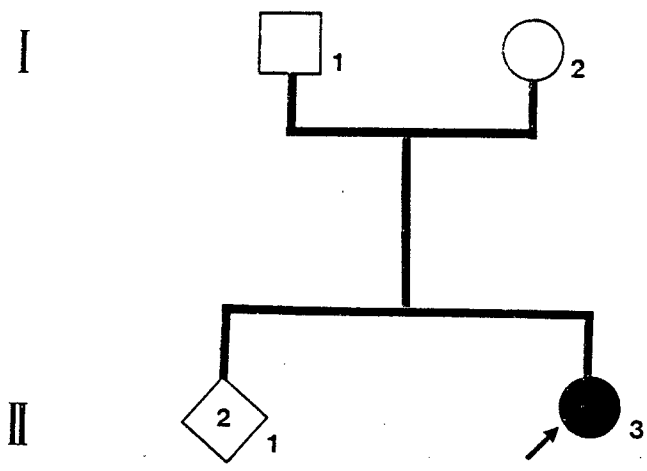
4.6 UNCLASSIFIABLE EB

The families described in this subsection were resident in rural communities. Documentation of EB was made in the infants, who were then lost to follow-up, without further investigation. For this reason, no specific diagnosis of a subtype of EB could be made.

4.6.1 FAMILY 1

This negro family of the Shangaan tribe were non-consanguineous. There was no parental age effect.

Pedigree:



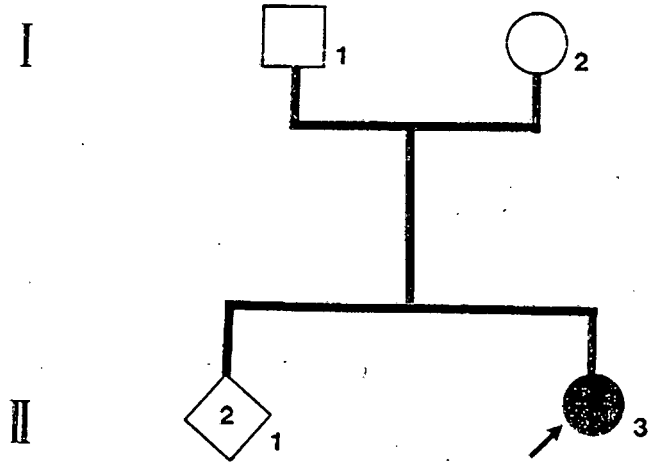
This infant was noted to have blisters on the abdomen with peri-umbilical involvement soon after birth. The lesions became progressively worse and widespread involvement of the trunk and all four limbs occurred. In addition, she was found to have a patent ductus arteriosus.

Comment: The investigator did not personally examine this infant. In the absence of further clinical or histological information, no classification of her EB to a specific subtype could be made.

4.6.2 FAMILY 2

The family were non-consanguineous South African Blacks of the Zulu tribal group from Natal. The father of the child was 45 years of age.

Pedigree:



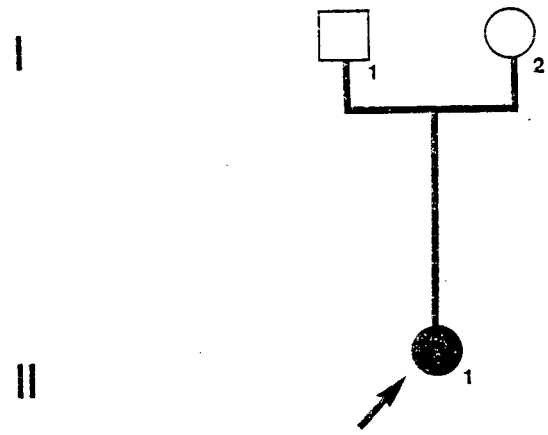
The infant presented soon after birth with widespread bullous lesions typical of EB. These lesions appeared to heal well and it was not yet unknown with which type of EB she was affected. The family were resident in a remote rural area and no specialist opinion or histopathological studies had been possible.

Comment: The presence of EBA is well documented in the Zulu people of South Africa. Clinically, this infant was more likely to have one of the simplex or dystrophic subtypes, as the reporting practitioner had discharged her home, without fear that she would not thrive. The advanced paternal age may be of significance, where a new mutation of an autosomal dominant gene cannot be excluded.

4.6.3 FAMILY 3

This family were Zulus, non-consanguineous, both parents being teenagers.

Pedigree:



The infant presented with bullous lesions from the time of birth. Blistering was mainly on the hands and arms, and the nails were dystrophic.

Comment: As was the case with 4.1.4.5.2, this infant had not been fully investigated due to geographical constraints. Her dystrophic nails were suggestive of EB Dystrophica.

CHAPTER 5 RESULTS OF SPECIAL INVESTIGATIONS

5.1 HISTOPATHOLOGICAL FINDINGS

5.1.1 EB Simplex

5.1.2 EB Atrophicans

5.1.3 EB Dystrophica

5.1.3.1 Autosomal dominant EB Dystrophica

5.1.3.2 Autosomal recessive EB Dystrophica

5.2 GENETIC STUDIES

5.2.1 HLA typing

5.2.2 Red cell enzymes

5.2.3 Restriction fragment length polymorphisms

5.3 BIOCHEMICAL STUDIES

5.1 HISTOPATHOLOGICAL FINDINGS

The histopathological findings are constant within each subset of EB, and for this reason specific details of each patient will not be listed. In order to avoid repetition, the ultrastructural changes of the major subgroups, EBS, EBA and EBD will be described as a whole. Any significant additional factors, or unusual individual findings will follow. Features specific to the minor subtypes of the four main groups will be elaborated thereafter.

Conventional light microscopic examination of skin was not relied upon for diagnosis; apart from an obvious cleavage plane, the ultrastructural changes are not visible at low magnification. Light microscopy is however essential for the study of survey sections, in the localisation of the area of interest for ultrathin sections for electron microscopy.

These survey sections, simply stained with methyl blue, were inadequate for detailed study of the dermo-epidermal junction. Bullae may be visible intra- or sub- epidermally at a magnification of X 40, but no further ultrastructural localisation of the dermo-epidermal junction is possible. The ultrastructural changes of the skin in the persons studied were therefore delineated by electromicroscopy. The findings in each type are elaborated in the following subsections.

5.1.1 EB Simplex

UNDAMAGED SKIN

Samples from undamaged skin, i.e. skin without acute or recent bullae, had normal ultrastructure (Fig 29).

BULLOUS LESIONS

Samples taken from the edge of a fresh blister showed cytolysis of basal cells. Light microscopy revealed occasional dyskeratosis and acanthosis.

Intra-epidermal cleavage planes were noted, within the basal layer. The floor of the blister was the cytoplasm of the basal cells while the roof consisted of epidermis and stratum corneum (Fig 30).

The basal lamina was undisturbed, and hemidesmosomes, basal lamina, anchoring fibrils and collagen were qualitatively and quantitatively normal.

SPECIFIC FINDINGS

None of the skin samples examined in this way demonstrated any unusual or remarkable ultrastructural changes. The findings

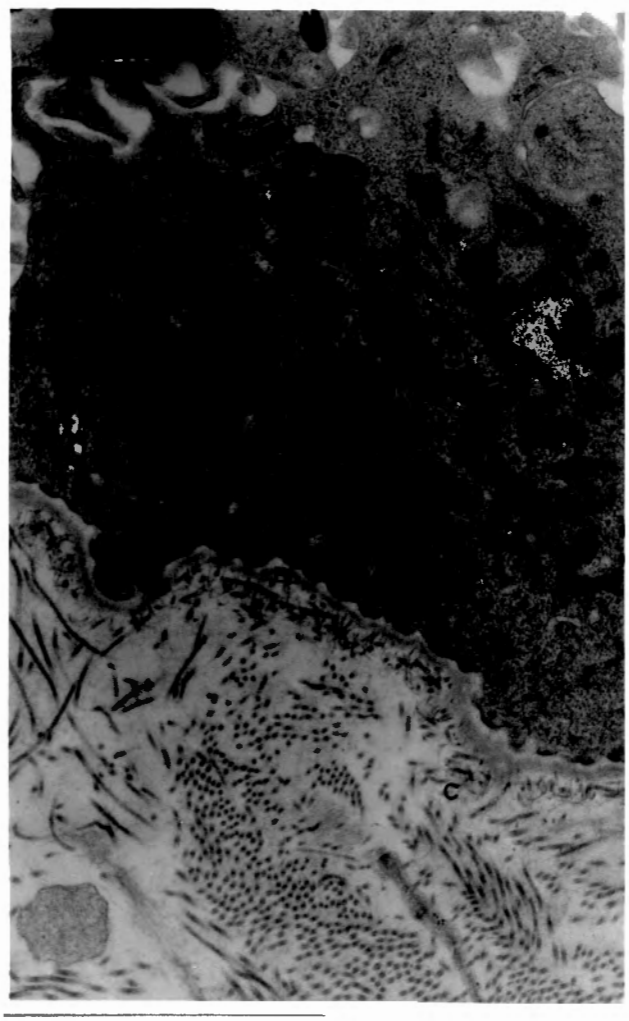


Figure 29. Normal ultrastructure at the dermoepidermal junction of the undamaged skin of a patient with EB Simplex (x 8200) (BL = basal lamina ; C = dermal collagen ; H = hemidesmosomes ; B = basal cell)

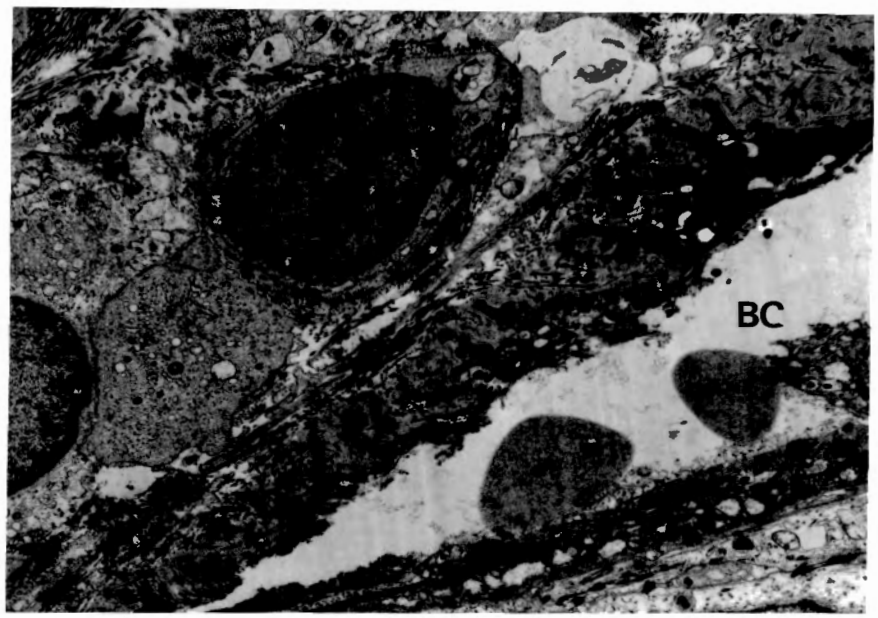


Figure 30. Intraepidermal blister in EB Simplex (x 30000). The cleavage is within the basal cell layer
(BC = blister cavity)

were identical in persons with **EBS Koebner** or **EBS Weber Cockayne.** In the family with **EBS with mottled pigmentation** there was cytolysis of hyperpigmented basal cells. There was no significant inflammatory infiltrate in the epidermis of the 2 persons with **EBS Dowling Meara.**

5.1.2 EB Atrophicans

The majority of patients in this study with the lethal form of EBA were born in rural areas, and skin biopsies were not immediately performed. The life span of many of these patients was too short for the investigator in this study to personally obtain such specimens.

Histopathological studies were therefore performed on only nine of the twenty patients. Three of these were post-mortem.

UNDAMAGED SKIN

The 'normal' skin was not studied.

BULLOUS LESIONS

Blister formation was within the lamina lucida (Fig 31). The blister roof was formed by the basal cells. Vacuolation occurred, with resultant disruption of tonofilaments. The basal lamina, which formed the blister floor, was undisturbed The structure and number of anchoring fibrils, as well as collagen fibrils, was unaltered. The hemidesmosomes were sparse in number and were markedly hypoplastic (Fig 32).

The histological changes in the 11 year old girl with the non-lethal **EBA Generalisata Gravis** were identical (Fig 33), as were those of the child with **EBA Generalisata Mitis.**

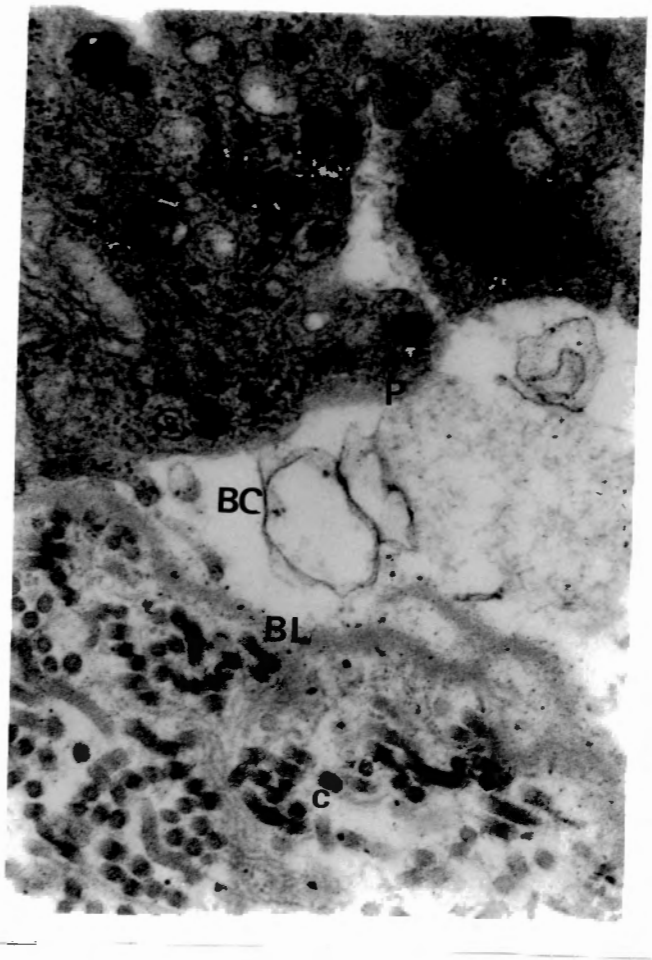


Figure 31. A blister has formed within the lamina lucida in EB Atrophicans (x 49000)
(BL = basal lamina ; BC = blister cavity ; P = plasma membrane of basal cells ; C = dermal collagen)

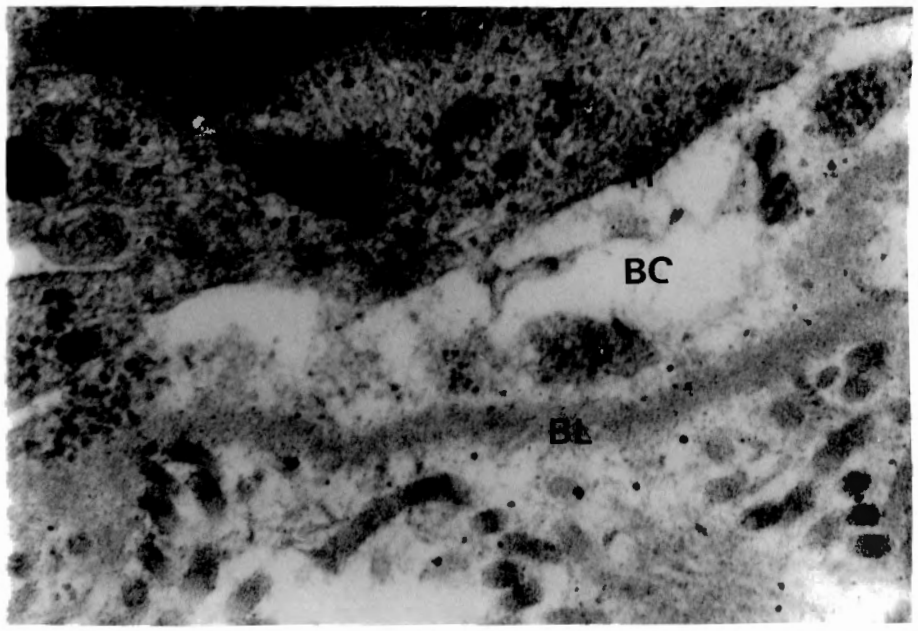


Figure 32. Hemidesmosomes are markedly hypoplastic when present in EB Atrophicans (x 49000)
(H = hemidesmosomes ; BC = blister cavity ; BL = basal lamina)

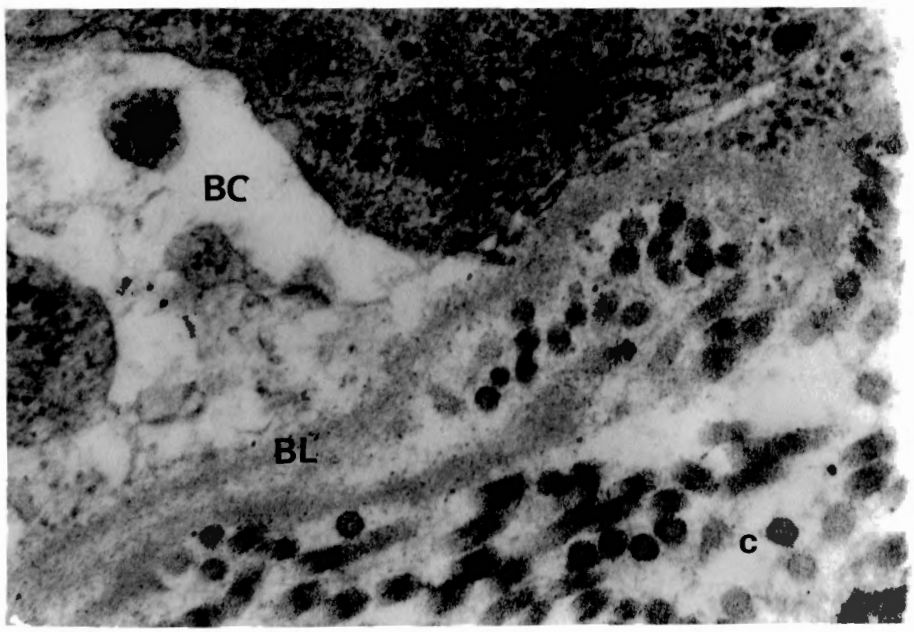


Figure 33. A typical " junctional" bulla in the child with non-lethal EBA Generalisata Gravis (x 49000)
(BC = blister cavity ; B = basal cell ; BL = basal lamina ; C = dermal collagen)

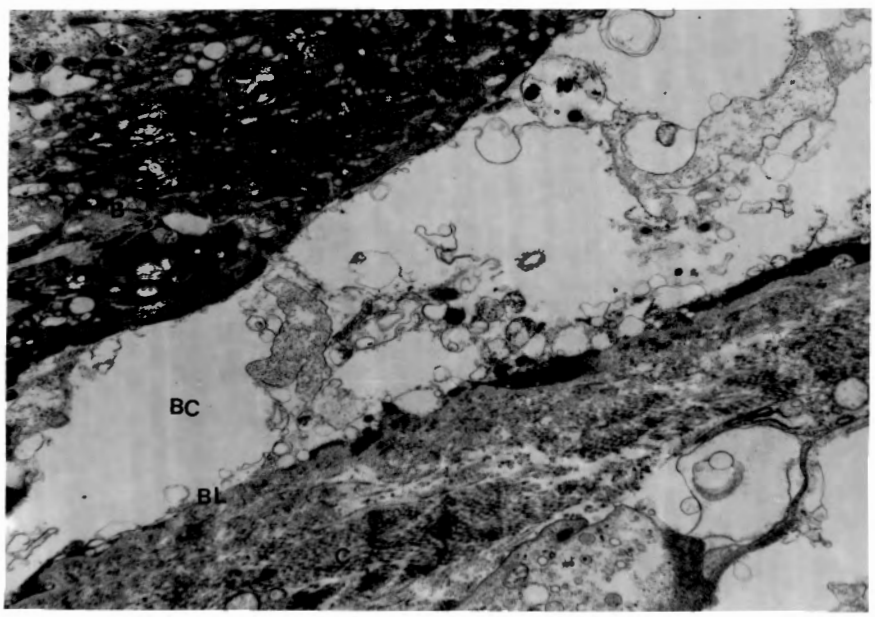


Figure 34. The typical ultrastructural changes of EB atrophicans in the abortus who had co-incidental Turner's syndrome (x 30000) (B = basal cell ; BC = blister cavity ; C = dermal collagen)

POST-MORTEM STUDY

Autopsy was performed on two infants as well as the abortus (4.3.1.5). The histological features were identical to those described in 5.1.2 (Fig 34). In addition to these findings, areas of unaffected skin adjacent to bullae were examined. Festooning and basal cell vacuolation was prominent in these normal areas.

Many of the bullae had become ulcerated with denudation of the epithelium. The base of these ulcers was composed of fibrinoid necrosis and numerous subacute inflammatory cells. Areas adjacent to ulceration showed hyperkeratosis with an increase in the number of rete pegs.

5.1.3 EB Dystrophica

In accordance with other chapters of this thesis, the histological findings in persons with EB Dystrophica will be divided into Autosomal Dominant and Autosomal Recessive sections. EBD.

5.1.3.1 DOMINANT EB DYSTROPHICA

NORMAL SKIN

Skin samples taken from undamaged areas demonstrated ultra-structurally normal skin. This finding was consistent in the so-called predilection sites, i.e. the dorsal surfaces of the extremities, as well as the other areas of the body (Fig 35).

Blister formation was subepidermal (Figure 36). The roof of the blister was the basal lamina, and all structures superficial to this region were unaffected. The blister floor comprised the dermis. Collagen fibres were normal in structure and in number. The anchoring fibrils were structurally abnormal (X 49 000), and were decreased in number. The fibrils were slim, peglike and did not turn back to the basal lamina. There was loss of the cross-banding pattern. (Fig 37).

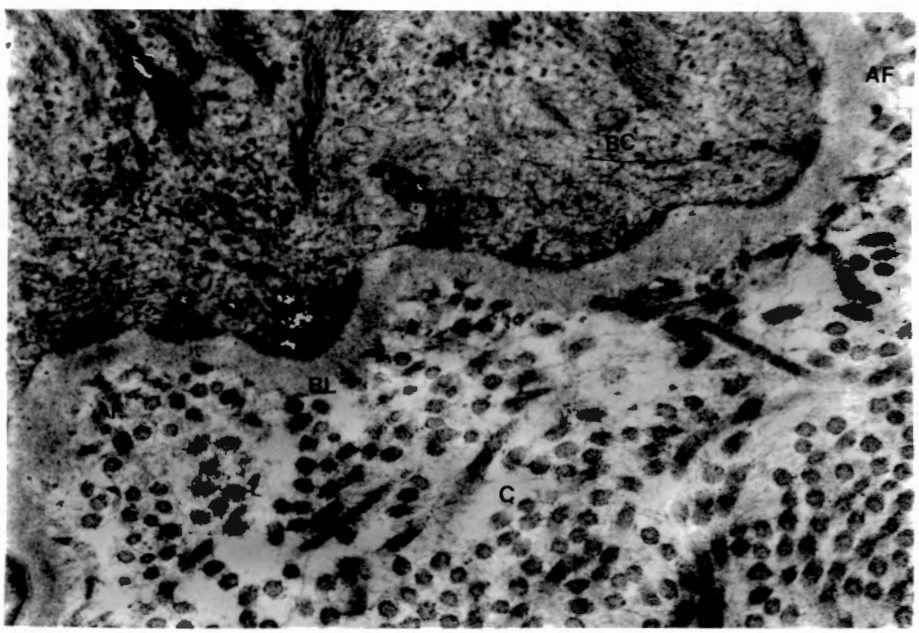


Figure 35. Undamaged skin in the non predilection areas in DDEB Cockayne Touraine (X 36000)
(BC = basal cell ; H = hemidesmosome ; BL = basal lamina
AF = anchoring fibrils ; C = dermal collagen)

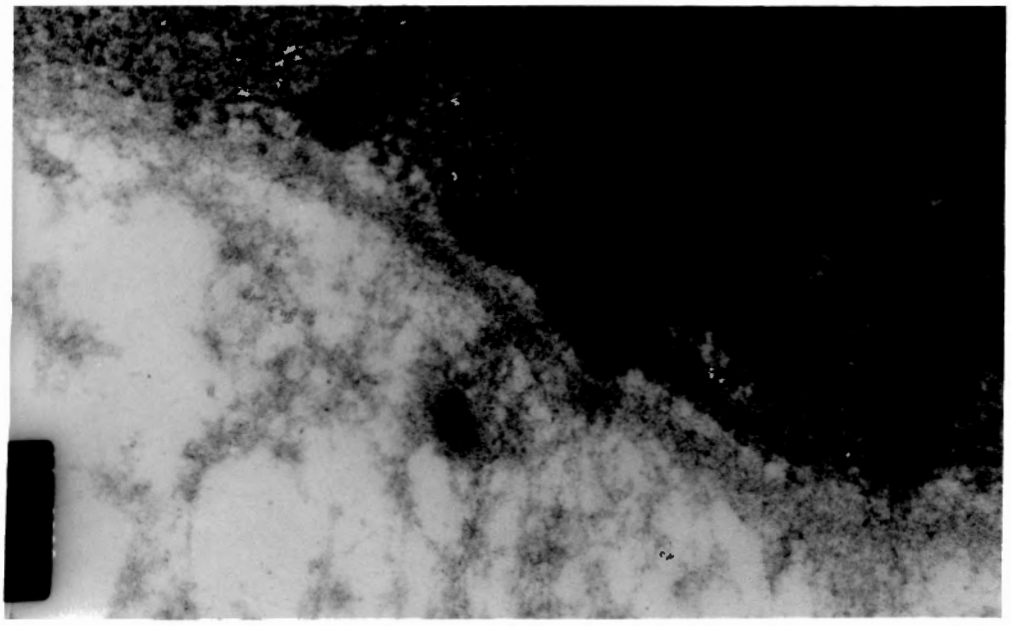


Figure 36. A subepidermal bulla in autosomal dominant EB Dystrophica (x 49000)
(PM = basal cell plasma membrane ; H = hemidesmosome ; BL = basal lamina ; BC = blister cavity)

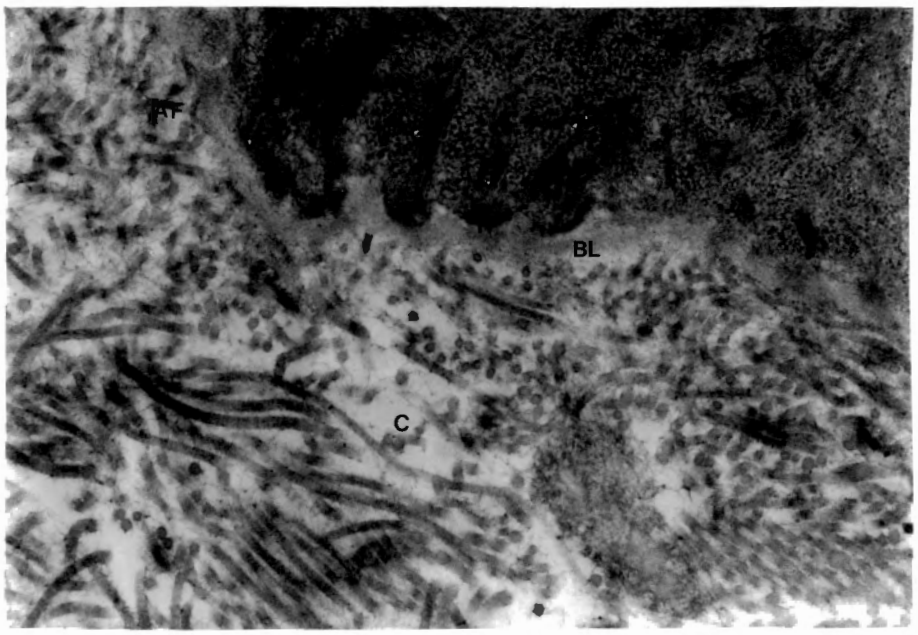


Figure 37. Quantitative and qualitative abnormalities are seen in the anchoring fibrils of traumatised skin in DDEB (x 18500)
(BL = basal lamina ; H = hemidesmosomes ;
AF = anchoring fibrils ; C = dermal collagen)

SPECIFIC FINDINGS

The histological changes were identical in the **Cockayne Touraine** and the **Pasini** subtypes. Depigmented scar tissue examined from one person with **EBD Pasini** showed classical histologic changes compatible with scar formation.

5.1.3.2 AUTOSOMAL RECESSIVE EB DYSTROPHICA

NORMAL SKIN

Specific abnormalities were noted on samples of undamaged skin. There was a diminution or even absence of the anchoring fibrils (Fig 38). The basal lamina was intact, and there were no alterations in collagen or epidermal structures.

BULLOUS LESIONS

As in DDEB, blister formation was in the dermis in the area normally occupied by the anchoring fibrils. The roof consisted of intact basal lamina while the floor comprised dermal structures, largely collagen. These changes were indentical to those found in DDEB (Fig 36).

Anchoring fibrils were absent in areas of blister formation, and in the adjacent area were markedly reduced in number , if indeed present (Fig 39). Those anchoring fibrils which were present were abnormal in structure, short and straight, with loss of the normal curved shape and the tendency to turn back to the basal lamina. The usual cross banding pattern was altered.

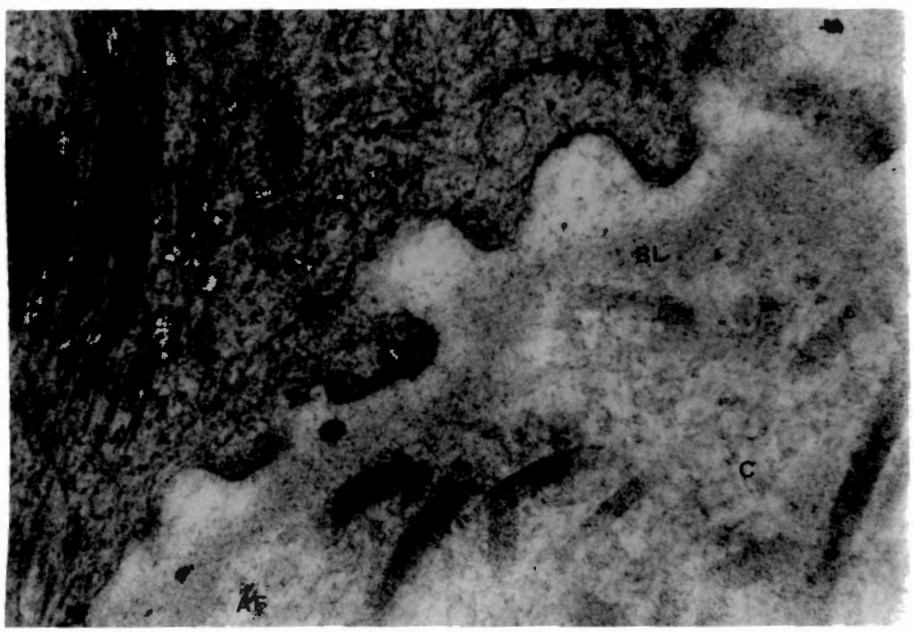


Figure 38. Diminution of anchoring fibrils in undamaged skin in RDEB (x 49000)
(BL = basal lamina ; AF = anchoring fibrils ;
C = dermal collagen)

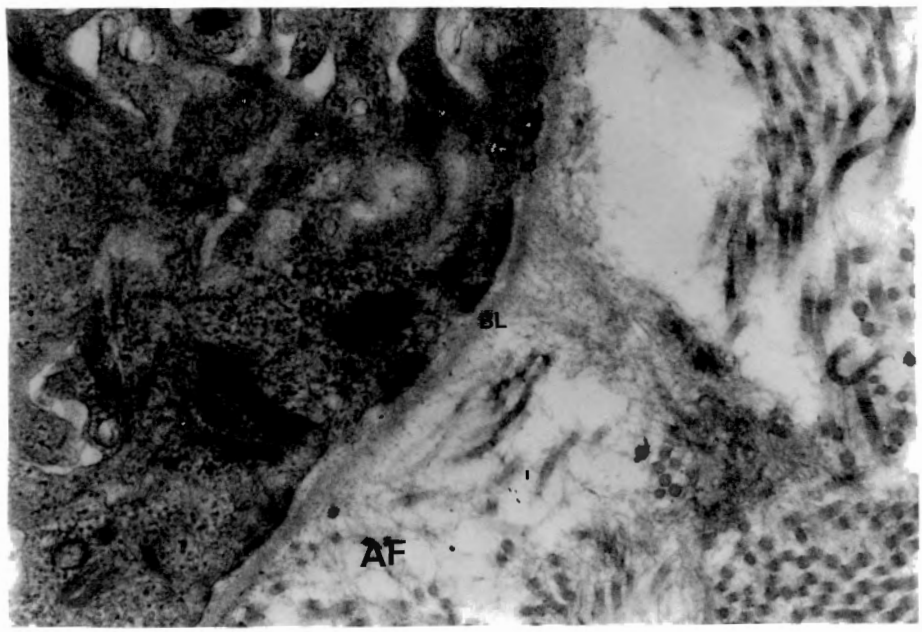


Figure 39. Anchoring fibrils are reduced in number, and when present are structurally abnormal (x)
(B = basal cell ; BL = basal lamina ; AF = anchoring fibril)

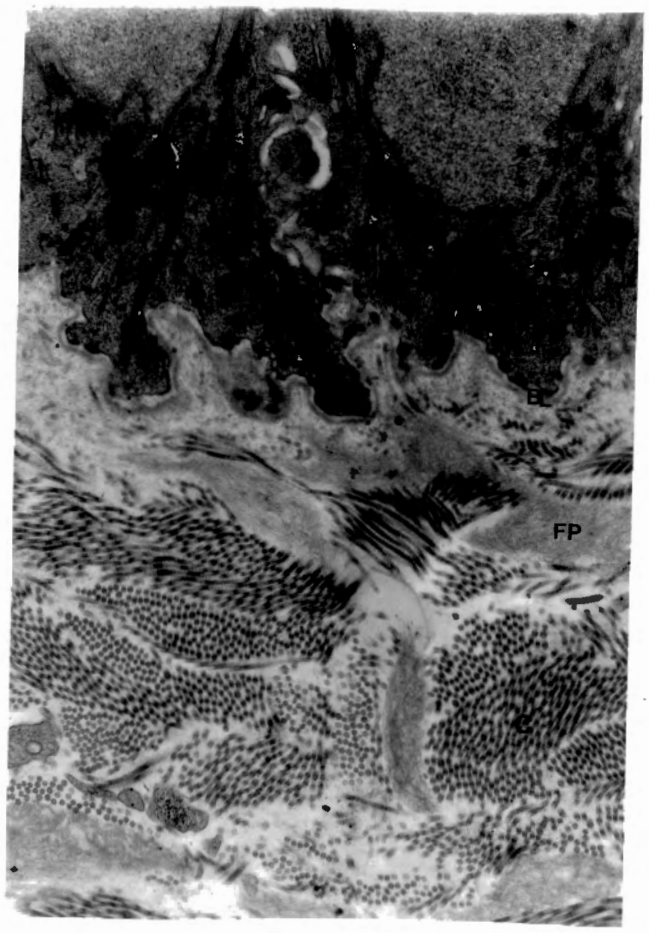


Figure 40. "Finger print" clumping of microfibrillar collagen (x 8200) (B = basal cell ; BL = basal lamina ; C = dermal collagen ; FP = finger print clumping)

Collagen fibrils appeared normal structurally. Areas of moderately sparse distribution of collagen fibrils were present deep to the blister floor, although this was not a strikingly consistent finding. "Finger print" clumping of microfibrillar structures immediately below the basal lamina and among the collagen fibrils was seen in the skin samples of several persons with RDEB (Fig 40).

SPECIFIC FINDINGS

No specific changes were noted in the minor subtypes, and the electron microscopic changes of all the skin sampled from persons with EB dystrophica were identical. This lack of differentiation between the bullous lesions of RDEB and DDEB is an important feature of EB and will be dealt with more fully in subsequent sections. It is notable however that the undamaged skin of DDEB is of normal ultrastructure, while anchoring fibril abnormalities may be seen in unblistered skin in RDEB. The ultrastructural changes of the young man with **RDEB Generalisata Gravis** were identical to those of the other forms of EBD.

5.2 GENETIC STUDIES

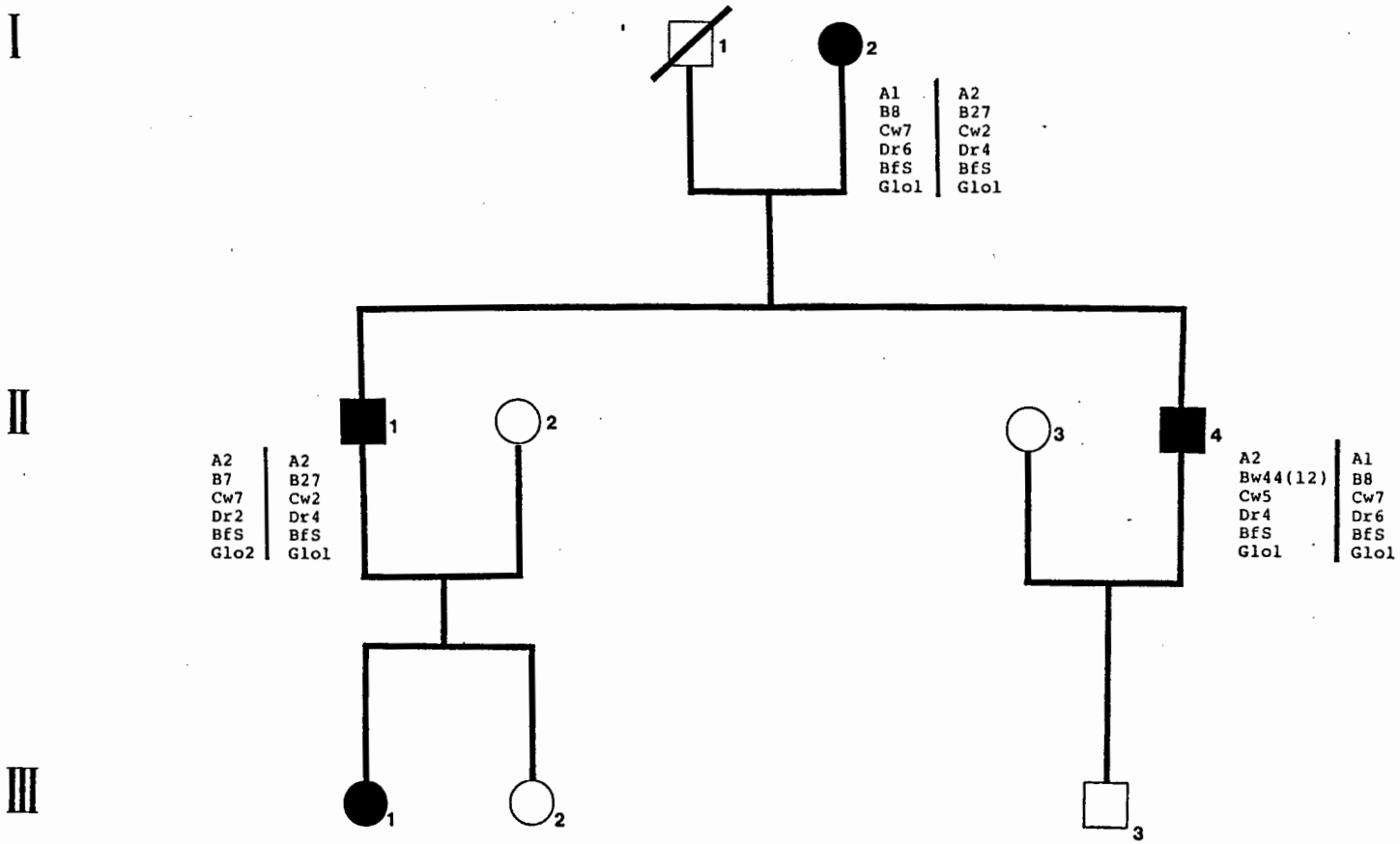
LINKAGE STUDIES

5.2.1 HLA typing

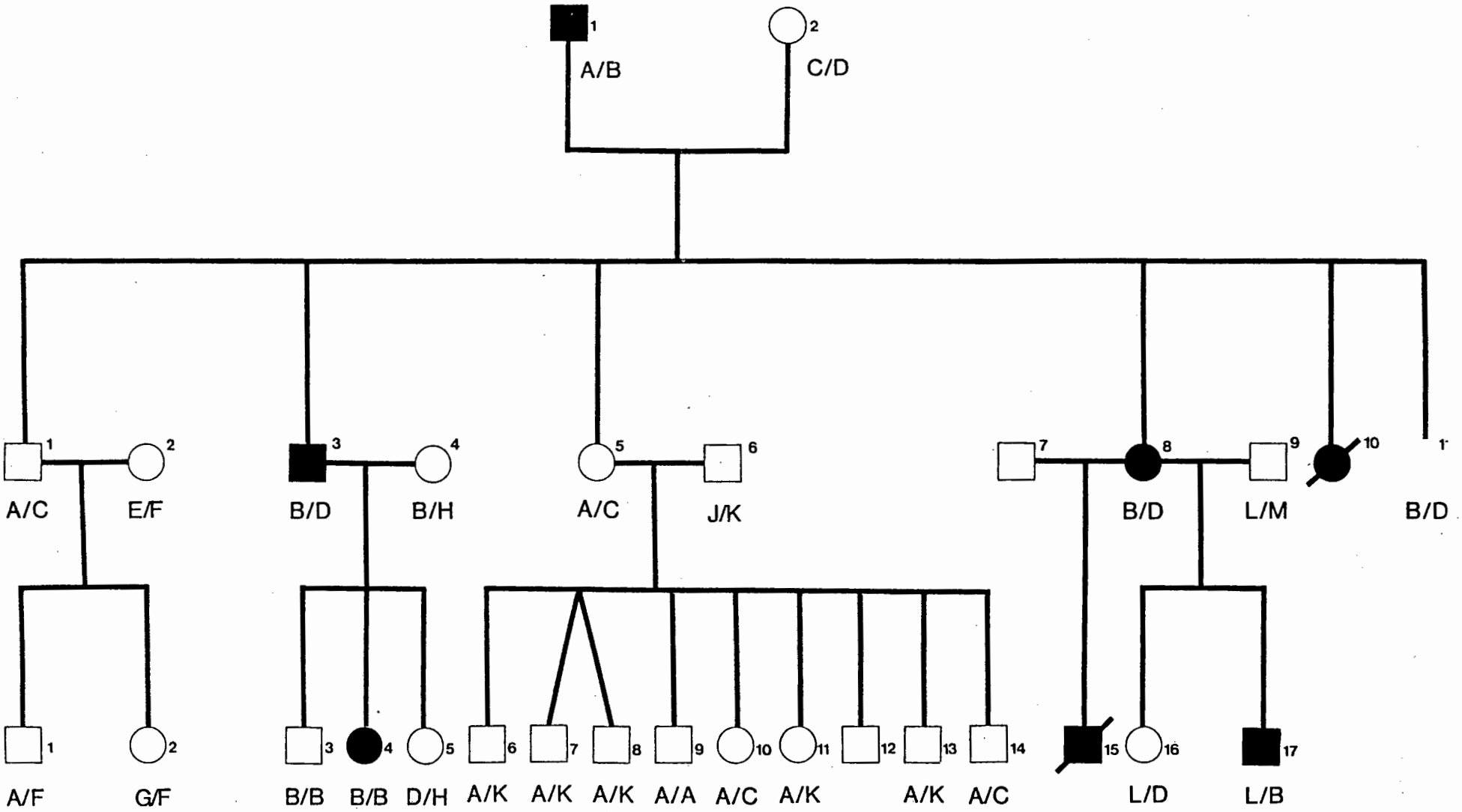
Results are presented in the form of pedigrees, illustrating the haplotypes of family members. Figures 41 to 44 are the pedigrees of 4 kindreds with autosomal dominant inheritance of EB. Complete studies were prevented in the families shown in Figures 41 to 43 by the fact that members were deceased or unable to be traced. Figure 42 shows the haplotypes of the kindred with **EB Simplex with mottled pigmentation.** The haplotype A1; B8; Cw7; Dr3 was found to segregate in six affected family members and one non-affected. Using the standard formula for Lod score analysis where $\theta = 0,1$ (phase known), a Lod score of 1,3 was found.

A kindred with **EB Simplex Koebner** is shown in Figure 41. No information can be gained from their haplotype analysis.

Figure 43 demonstrates the incomplete pedigree of a family with **EB Dystrophica Cockayne Tourraine.** No specific HLA associations were found.



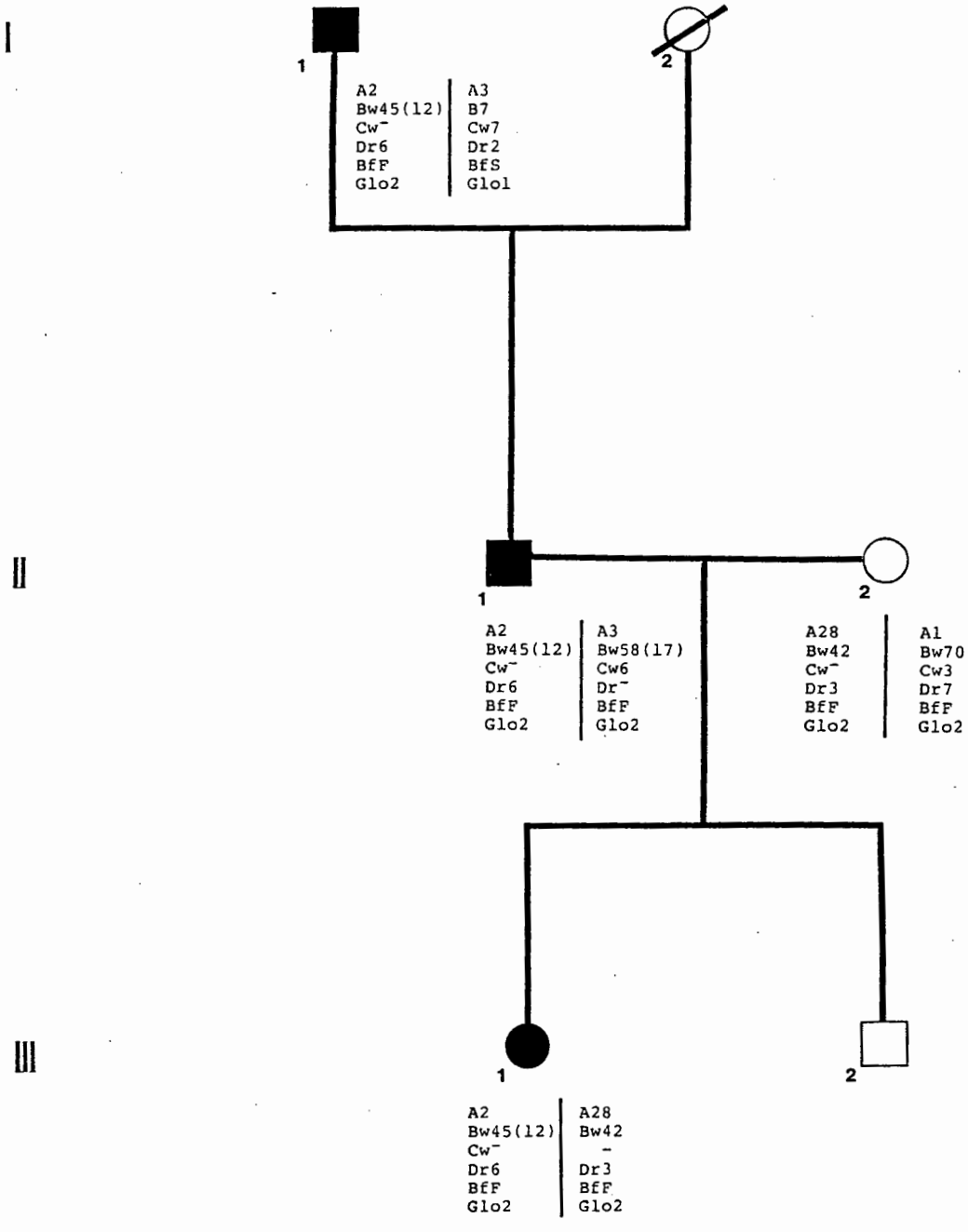
41 Pedigree data illustrating haplotype of the family with EBS KOEBNER



42 Pedigree data illustrating haplotype of the family with EBS with mottled pigmentation

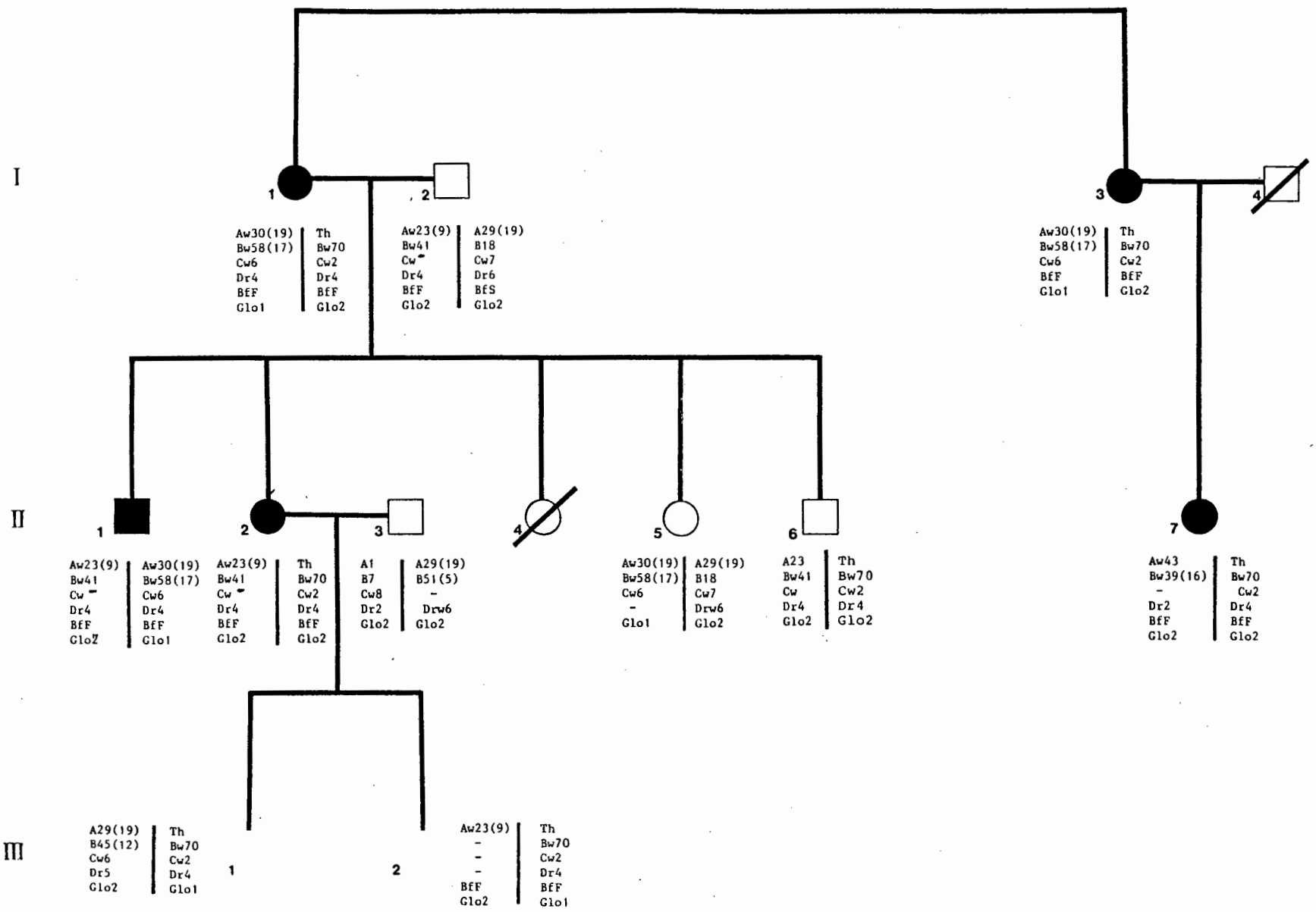
B A1 B8 Cw7 Dr3 Glo1	A A1 B37 Cw6 Dr5 Glo1	G A11 B7 Cw7 Drw6 Glo2	K A3 B8 Cw7 Drw8 Glo2
C Aw30 B44(12) Cw4 Dr2 Glo2	D Aw23(9) B8 Cw2 Dr4 Glo2	H A3 Bw51(5) Cw5 Dr4 Glo2	L A3 BW5(15) Cw2 Dr1 Glo2
E A3 Bw57(17) Cw6 Dr5 Glo2	F A29 B13 Cw6 Dr1 Glo2	J A30 Bw70 Cw6 Dr2 Glo2	

KEY TO FIGURE 42



43 Pedigree data illustrating haplotype of the family with EBD COCKAYNE TOURAINE

44 Pedigree data illustrating haplotype of the family with EBD PASINI



The data shown in Figure 44 are that of the Sotho family with **EB Dystrophica Albopapuloidia (Pasini).** The haplotype Th; Bw70; Cw2; Dr4 was studied in this family. Where $\theta = 0,05$ (phase unknown) the resultant Lod score was 70-0,907, demonstrating non-linkage. Non paternity is evident in III-1 and III-2.

5.2.2 Red cell enzyme and genotype studies

The results of red cell enzyme and blood group studies in the form of the standard paternity testing protocol is tabulated in the same four families where complete or near-complete studies were possible. (Tables 5-II to 5-IV).

Loose linkage was demonstrated in the family with EBS with mottled pigmentation and the enzyme glyoxalase I (Glo-1)-1. Using the usual formula where $\theta = 0,2$ the Lod score was 0,124.

5.2.3 Restriction fragment length polymorphisms

The linkage studies performed were non-informative. Southern blot analysis of genomic DNA using the probe L7 showed no linkage between the polymorphism and Autosomal Recessive Epidermolysis Bullosa Dyptrophica (RDEB).

TABLE 5 - II LINKAGE RESULTS IN FAMILY K

PATIENT (NUMBERED AS PER PEDIGREE)	RED CELL ENZYMES											SERUM PROTEINS				RED CELL SYSTEMS				
	AP	ADA	AK	ESD	G6PD	6PGD	PGM_1	PGM_2	CA I	CA II	GLO	Hp	Tf	Bf	Gc	ABO	MNSs	RHESUS	KELL	DUFFY
I - 2	CB	1	1	1	B	A	1	1	1	1	1	2-1	C	S	*	*	*	*	*	*
II - 1	B	1	1	1	B	A	1	1	1	1	2-1	1	C	S	2-1S	O	MN$\bar{s}$	$\bar{c}$DE$\bar{e}$	K+$\bar{k}$+	Fy (a+b+)
II - 2	B	1	1	1	B	A	1	1	1	1	1	1	C	S	*	O	N$\bar{s}$	C$\bar{c}$D$\bar{e}$	K+$\bar{k}$+	Fy (a-b+)

* = Unable to be typed

TABLE 5 - III LINKAGE RESULTS IN FAMILY C

PATIENT (NUMBERED AS IN PEDIGREE)	RED CELL ENZYMES												SERUM PROTEINS				RED CELL SYSTEMS				
	AP	ADA	AK	ESD	G6PD	6PGD	PGM_1	PGM_2	CA I	CA II	GLO	GPT	Hp	Tf	Bf	Gc	ABO	MNSs	RHESUS	KELL	DUFFY
I - 1	B	1	1	1	B	A	1	1	1	1	1	2-1	2	C	S	2-1S	A_1	MS	$C\bar{c}D\bar{e}$	$K+\bar{k}+$	Fy(a+b-)
I - 2	B	1	1	1	B	AC	2-1	1	1	1	2	1	2-1	C	FS	2-1S	O	$M\bar{S}$	$C\bar{c}D\bar{e}$	K-	Fy(a+b-)
II - 1	B	1	1	1	B	AC	1	1	1	1	2	*	2	C	S	2	O	$MS\bar{s}$	$C\bar{c}D\bar{e}$	$K+\bar{k}+$	Fy(a+b-)
II - 2	AB	1	1	1	B	A	2-1	1	1	1	2	*	2	C	S	2	A_1	$MNS\bar{s}$	$C\bar{c}D\bar{e}$	K-	Fy(a+b-)
II - 3	B	1	1	1	B	AC	1	1	1	1	2-1	*	2-1	C	FS	2-1S	O	$MS\bar{s}$	$C\bar{c}D\bar{e}$	$K+\bar{k}+$	Fy(a+b-)
II - 4	B	1	1	1	B	AC	2	1	1	1	2-1	*	2	C	FS	1S	A_1	$MNS\bar{s}$	$C\bar{c}D\bar{e}\bar{e}$	K-	Fy(a+b+)
II - 5	AB	1	1	1	B	A	2-1	1	1	1	2	*	2-1	C	FS	1S	O	$M\bar{s}$	$CD\bar{e}$	K-	Fy(a+b+)
II - 6	B	1	1	1	B	A	1	1	1	1	2	1	2	C	S	2	A_1	$MS\bar{s}$	$C\bar{c}D\bar{e}$	$K+\bar{k}+$	Fy(a+b-)
II - 8	B	1	1	1	B	A	1	1	1	1	2-1	1	2	C	FS	2-1S	O	$MS\bar{s}$	$CD\bar{e}$	$K+\bar{k}+$	Fy(a+b-)
II - 9	B	1	1	1	B	A	1	1	1	1	2	1	2-1	C	S	2-1S	O	$MS\bar{s}$	$CD\bar{e}$	K-	Fy(a+b+)
II - 11	B	1	1	1	B	A	2-1	1	1	1	2-1	2-1	2-1	C	FS	2-1S	A_1	$MS\bar{s}$	$C\bar{c}D\bar{e}$	$K+\bar{k}+$	Fy(a+b-)
III - 1	B	1	1	1	B	AC	2-1	1	1	1	2	*	1	C	S	2	A_1	MS	$\bar{c}D\bar{e}$	$K+\bar{k}+$	Fy(a+b-)
III - 2	A	1	1	1	B	A	1	1	1	1	2	*	2-1	C	1S	2-1S	A_1	$N\bar{s}$	$C\bar{c}D\bar{e}$	K-	Fy(a-b+)
III - 3	B	1	1	1	B	AC	2-1	1	1	1	1	*	2	C	S	2-1S	O	MS	$CD\bar{e}$	K-	Fy(a+b-)
III - 4	B	1	1	1	B	A	2-1	1	1	1	1	*	2	C	S	1S	O	$MN\bar{s}$	$CD\bar{e}$	K-	Fy(a+b-)
III - 5	B	1	1	1	B	AC	2-1	1	1	1	2	2-1	1	C	F	2-1S	O	$MNS\bar{s}$	$CD\bar{e}$	$K+\bar{k}+$	Fy(a+b+)
III - 6	AB	1	1	1	B	A	1	1	1	1	2	1	2-1	C	S	2-1S	O	$MS\bar{s}$	$CD\bar{e}$	$K+\bar{k}+$	Fy(a-b+)
III - 7	AB	1	1	1	B	A	1	1	1	1	2	1	2	C	S	2-1S	O	$MS\bar{s}$	$CD\bar{e}$	K-	Fy(a+b+)
III - 8	AB	1	1	1	B	A	1	1	1	1	2	1	1	C	S	2-1S	O	$MS\bar{s}$	$CD\bar{e}$	K-	Fy(a+b+)
III - 10	AB	1	1	1	B	A	2-1	1	1	1	2	1	2	C	FS	2-1S	A_1	$MS\bar{s}$	$CD\bar{e}$	K-	Fy(a+b-)
III - 11	AB	1	1	1	B	A	1	1	1	1	2	1	2	C	S	2-1S	A_1	$MS\bar{s}$	$CD\bar{e}$	K-	Fy(a+b+)
III - 13	AB	1	1	1	B	A	1	1	1	1	2	1	1	C	S	2-1S	A_1	$MS\bar{s}$	$CD\bar{e}$	K-	Fy(a+b-)
III - 14	B	1	1	1	B	A	1	1	1	1	2	-	2-1	C	FS	2-1S	A_1	$MS\bar{s}$	$D\bar{e}D\bar{e}$	K-	Fy(a-b+)
III - 16	B	1	1	1	B	A	1	1	1	1	2	1	2-1	C	FS	*	O	$NS\bar{s}$	$CD\bar{e}$	$K+\bar{k}+$	Fy(a+b-)
III - 17	B	1	1	1	B	A	1	1	1	1	2-1	1	2-1	C	FS	*	O	MS	$CD\bar{e}$	K-	Fy(a+b-)

* = Unable to be typed.

TABLE 5 - 1V LINKAGE RESULTS IN FAMILY H

PATIENT (NUMBERED AS PER PEDIGREE)	RED CELL ENZYMES											SERUM PROTEINS				RED CELL SYSTEMS				
	AP	ADA	AK	ESD	G6PD	6PGD	PGM_1	PGM_2	CA I	CA II	GLO	Hp	Tf	Bf	Gc	ABO	MNSs	RHESUS	KELL	DUFFY
I - 1	AB	1	1	2-1	B	A	1	1	1	1	2-1	1	C	FS	1S	A1	$N\bar{s}$	$\bar{c}D\bar{e}$	K-	Fy (a+b-)
II - 1	RB	1	2-1	2	B	A	1	1	1	1	2	2	C	F	1S	A1	$N\bar{s}$	$C\bar{c}D\bar{e}$	K-	Fy (a+b-)
II - 2	AB	1	1	1	B	A	1	1	1	1	2	2	C	F	2-1S	O	$N\bar{s}$	$C\bar{c}D\bar{e}$	K-	Fy (a+b-)
III - 1	AR	1	1	1-2	B	A	1	1	1	1	2	2	C	F	2-1S	A1	$N\bar{s}$	$\bar{c}D\bar{e}$	K-	Fy (a+b-)

* = Unable to be typed

TABLE 5 - V LINKAGE RESULTS IN FAMILY P

PATIENT (NUMBERED AS PER PEDIGREE)	RED CELL ENZYMES												SERUM PROTEINS				RED CELL SYSTEMS					
	AP	ADA	AK	ESD	G6PD	6PGD	PGM_1	PGM_2	CA I	CA II	GLO	GDT	Hp	Tf	Bf	Gc	ABO	MNSs	RHESUS	KELL	DUFFY	OTHER
I - 1	B	1	1	1	B	A	1	1	1	2-1	2	1S	2	C	FS	1S	B	$M\bar{s}$	$\bar{c}D\bar{e}$	K-	Fy(a-b-)	Le (a-b+)
I - 2	B	1	1	1	B	A	2-1	1	1	1	2-1	1S	2	CD	F	1S	O	$MN\bar{s}$	$\bar{c}D\bar{e}$	K-	Fy(a-b+)	Le (a-b-)
I - 4	B	1	1	1	B	A	2-1	1	1	1	2-1	1S	2-1	CD	F	1S	A2	$MNS\bar{s}$	*	K-	Fy(a+b+)	Le (a-b+)
II - 1	B	1	1	1	B	A	2-1	1	1	2-1	2-1	1S	2	CD	F	1S	*	*	*	*	*	
II - 2	B	1	1	1	*	A	1	1	1	2-1	2	1	2-1	D	F	2-1S	O	$MS\bar{s}$	$\bar{c}D\bar{e}$	K-	Fy(a-b-)	
II - 3	B	1	1	1	B	A	1	1	1	1	2	1S	2	CD	F	1S	B	$M\bar{s}$	$\bar{c}D\bar{e}$	K-	Fy(a-b+)	Le (a-b-)
II - 5	B	1	1	1	B	A	1	1	1	1	2-1	1	2	CD	FS	1S	B	$M\bar{s}$	$\bar{c}D\bar{e}$	K-	Fy(a-b-)	
II - 6	B	1	1	1	B	A	1	1	1	2-1	2	1	*	C	F	1S	O	$MN\bar{s}$	$\bar{c}D\bar{e}$	K-	Fy(a-b-)	
II - 7	RB	1	1	1	B	A	2	1	1	1	2	2-1S	2	CD	F	2-1S	*	*	*	*	*	
III - 1	B	1	1	1	B	A	2-1	1	1	1	2-1	1	2-1	CD	F	1S	O	$MN\bar{s}$	$\bar{c}D\bar{e}$	K-	Fy(a-b+)	
III - 2	B	1	1	1	B	A	1	1	1	1	2-1	1S	2-1	C	F	1S	B	$MNS\bar{s}$	$C\bar{c}DE\bar{e}$	K-	Fy(a-b+)	Le (a-b-)

* = Unable to be typed.

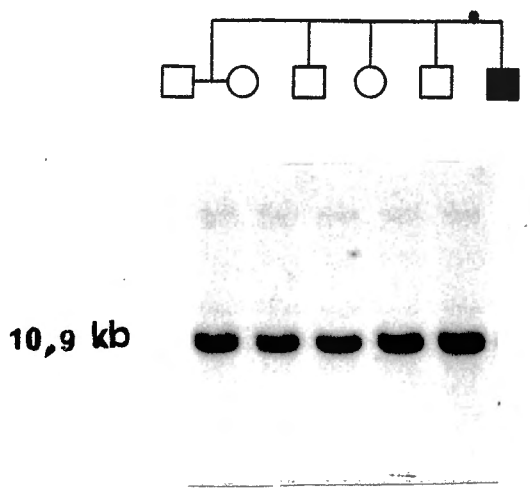


Figure 45. Autoradiograph of a family with RDEB Hallopeau Siemens (mutilans) demonstrating that L7 is not polymorphic

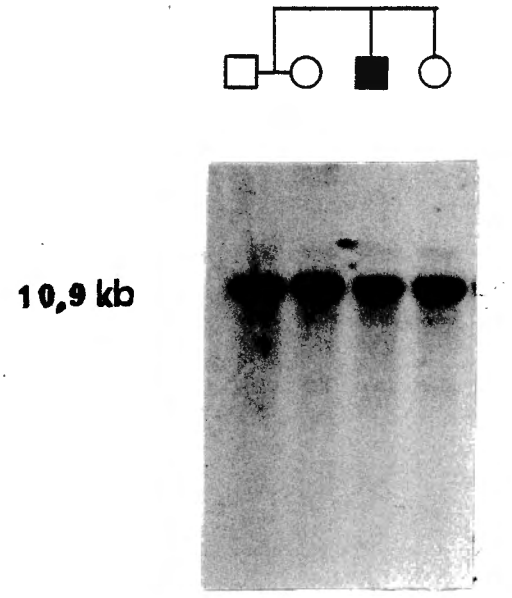


Figure 46. Autoradiograph of a family with RDEB Hallopeau Siemens (general) : this is non informative as L7 is not polymorphic

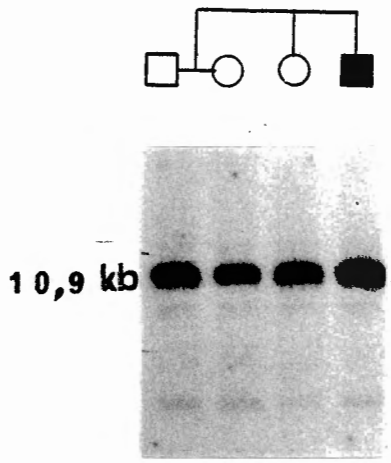


Figure 47. Non polymorphic L7 represented as an autoradiograph in a family with RDEB Hallopeau Siemens (mutilans)

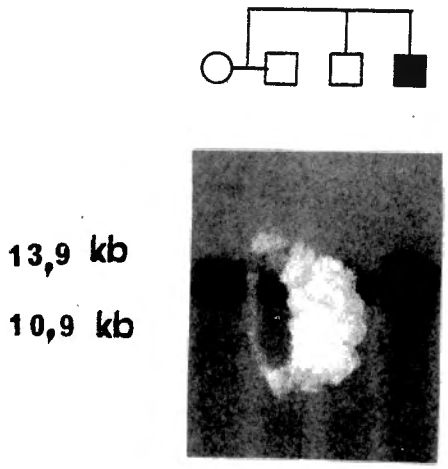


Figure 48. Autoradiograph of a family with RDEB Hallopeau Siemens (general) This family is too small to draw any conclusions regarding L7

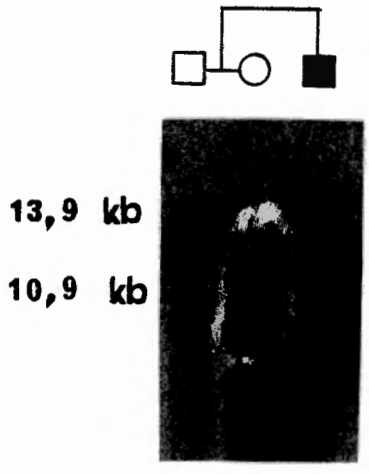


Figure 49. Autoradiograph of the family with RDEB Generalisata Gravis. All children of these parents will be heterozygous for L7

Figures 45-49 below illustrate that the allele was polymorphic in two families, and not polymorphic in three families. In the two families where the allele was polymorphic, the former was too small to draw conclusions regarding L_7 as a marker for RDEB (Fig 48). In the family with **RDEB Generalisata Gravis**, all offspring of these parents will be heterozygous for L_7, so that no linkage of this probe to EB is possible.

5.3 BIOCHEMICAL STUDIES

Investigation of the assay of collagenolytic activity of fibroblasts from RDEB yielded neither positive nor negative results. A number of technical problems were encountered, which will be elaborated in Chapter 10. A typical example of the data obtained is shown in Table 5-I.

TABLE 5 - I

System	Time(hours)	Total DPM*	% Total **
Cells:			
P.N.	24	4700	9.2%
L.S.	24	3240	6.3%
Control	24	2710	5.3%
Medium:			
P.N.	48	1560	3.1%
L.S.	48	1720	3.4%
Control	48	1000	2.0%

* DPM = Disintegrations per minute

** Refers to total released by 50 g */ml collagenase type IV (Sigma) incubated for 12 hours, with no further radioactivity releasable after such time.

Clearly there is little release collagenous peptide material when this data is expressed in relation to the amount released by 50 μg/ml of collagenase. When conditioned medium was exposed to trypsin (100 μg/ml) prior to addition to ECM coated dishes, the collagenolytic activity was enhanced very slightly. Normal control medium also showed increased activity.

SECTION IV

SECTION 4 REVIEW OF LITERATURE AND ANALYSIS OF RESULTS

CHAPTER 6. CLINICAL FEATURES AND COMPLICATIONS

6.1 INTRODUCTION

The clinical features and complications of EB are summarised in this chapter and the world literature is reviewed. Additional points of interest established by this investigation will be discussed at the end of each subsection under the heading "Comment".

The involvement of the oesophagus in EB will not be included in this section. This aspect has been of particular interest and importance to this study following the successful dilatation of severe oesophageal strictures in a young man with **EBD Hallopeau Siemens.** For this reason an entire chapter, Chapter 7, has been devoted to this aspect of EB.

6.2 GENERAL MANIFESTATIONS

There are few extracutaneous changes in EB except in **RDEB Hallopeau Siemens Mutilans.** In this subtype moderately severe growth retardation may ensue, as measured by standard percentile charts for age. There are no biochemical factors to explain this feature but it has been suggested that the metabolic stress of constant damage and healing of skin might lead to stunting of stature and poor weight gain. In addition, an iron-deficient anaemia is invariably present in these patients. Severe dental involvement in this subtype, with or without oesophageal complications may contribute to dietary deficiencies, accounting for anaemia and poor weight gain. This problem will be further outlined in 6.4 and Chapter 7.

6.3 SKIN AND APPENDAGES

The skin manifestations are the major clinical stigmata of EB. These and their complications have been discussed at length in all sections of this thesis, and will not be elaborated further in this subsection to avoid unnecessary repetition.

Nail changes are unusual in EB Simplex. In EB Dystrophica, the nails when shed, may regenerate as dystrophic stubs, but more frequently do not regrow.

Abnormalities of the hair have been reported in the difficient subtypes of EB. Alopecia is accepted as a syndromic component of the non-lethal forms of EB Atrophicans. By contrast localised areas of hypertrichosis may complicate EB Dystrophica (Fitzpatrick 1971).

Comment: Almost total absence of hair was a notable feature in the child with **EBA Generalisata Mitis**. By contrast the girl with **non-lethal EBA Generalisata Gravis** had normal healthy hair.

In the patient designated **EBD Generalisata Gravis (Winship)** there was almost total alopecia of the scalp. Despite a relatively normal puberty, no body hair had developed. The degree of scarring of the scalp was not enough to account for almost complete baldness. These hair changes are a compelling point for the diagnosis of **RDEB Generalisata Gravis** in preference to the other subtypes of RDEB.

6.4 DENTAL CHANGES

The teeth are rarely involved in EB Simplex and autosomal dominantly inherited EB Dystrophica.

In EB Atrophicans, there are clinical and histological abnormalities in the teeth. These teeth may be rudimentary decreased in number or irregularly spaced. The enamel may be defective and the teeth are soft. Caries are marked. Histological examination confirms poor mineralisation and thinned 'cobblestone' enamel.

Hypoplastic enamel is the most consistant dental anomaly in RDEB. The teeth may develop normally initially, but rampant caries often results in early destruction of teeth. This is largely compounded by poor oral hygiene; painful intra-oral lesions and poor fine hand control restrict the effective manipulation of a toothbrush. The puréed diets that many persons with RDEB follow, adhere to the teeth and afford no masticatory cleaning.

Comment: The two surviving persons with EBA had carious teeth. The child with **EBA Generalisata Mitis** has hypoplastic enamel. The dental anomalies outlined above were evident in all the patients with **RDEB Hallopeau Siemens Mutilans** investigated in this survey. There was no involvement of the teeth in any of the other subtypes.

6.5 INTRA-ORAL INVOLVEMENT

Bullae of the oral mucosa, tongue and palate develop frequently in EB Simplex. Healing is rapid and without scarring or long-term sequelae. These lesions occur almost entirely in infancy, as a consequence of suckling. In the atrophic, or junctional forms of EB the mouth is similarly affected.

The most significant oral lesions occur in the dystrophic subgroups of EB. In the autosomal dominantly inherited subtypes, this involvement is confined to infancy and early childhood. RDEB is that form of EB where the oral mucosa is most frequently affected. Intra-oral bullae are seen in most infants with this condition, and because of this high frequency, in the past it was traditional for infants with RDEB to be hospitalised and fed by nasogastric tube. Unlike the lesions of the Simplex and Atrophicans forms, blisters become denuded, and heal slowly, with scarring. Contractures may result, and in severe cases of oral EB, ankyloglossia of the tongue and binding of the mucosa with resultant microstomia may occur.

Comment: Intra-oral bullae were reported in almost all persons in this study, irrespective of the subtype of EB. Notable is the fact that these lesions only persisted beyond infancy in persons with RDEB. The patients with RDEB Hallopeau Siemens had limitation to the opening of their mouths as a result of repeated bullae and scarring.

The use of naso-gastic tubes is not advocated in any subtype of EB. The oral mucosa heals adequately in all forms. By contrast, the trauma of the passage of a naso-gastric tube results in blistering in the narrow naso-pharynx and oesophagus, and may contribute significantly to the lesions which later result in severe stricture formation.

6.6 GASTROINTESTINAL INVOLVEMENT IN EB

Involvement of the gastrointestinal tract (GT) varies in the different subtypes of EB. These manifestations are discussed under headings of anatomical site; intra-oral lesions were described in 6.5 and this section deals with the stomach, small intestine and the colon. In view of special implications as previously stated, the oesophagus will be discussed in Chapter 7.

6.6.1 The stomach and small intestines

Pyloric atresia has become recognised as a syndromic component of a specific type of autosomal recessive Epidermolysis Bullosa Atrophicans. Approximately 20 cases have been reported in world literature, and similarly affected siblings have been described (Bull 1980, Egan 1985).

Pyloric atresia exists as an occlusive luminal web in these cases. Atretic segments are represented by fibrous cords. Diaphragmatic membranes may be present, and there may be intrinsic proliferation of connective tissue. It is unclear whether the atresia is a primary phenomenon, or a secondary obstruction resulting from bullae and scarring leading to

secondary obstruction of the pylorus, on the same pathogenetic basis as the oesophageal strictures. Interestingly, oesophageal lesions rarely occur in junctional EB, and pyloric atresia has not been reported in conjunction with RDEB.

An infant with EB Atrophicans was reported to have acquired a pyloric obstruction at the age of one month (Honig 1983). By contrast to the EBA and pyloric atresia entity, where the obstruction is noted in the first few days of life, this obstruction at the age of one month represents postnatally-acquired intrinsic obstruction, probably secondary to his primary disease, EBA.

Comment: During the two years of this study, a single infant with the association of pyloric atresia and EB was born in a rural community. It was referred, moribund, to the nearest major hospital where the diagnosis was confirmed shortly before the infant's demise.

6.6.2 The colon

There have been no consistent associations between large bowel pathology and EB. The non genetic EB acquisita, by contrast, is frequently a complication of inflammatory bowel disease. Ano-rectal fistulae and proctitis, as well as minor alterations in bowel function have been noted, but not in

significant proportions to assign these lesions as syndromic components. One person with EB Dystrophica was investigated for recurrent diarrhoea which paralleled exacerbations of skin lesions (Sehgal 1977). Radiological examination revealed narrow segments of the descending and transverse colon with ulcer craters and loss of haustra from the transverse to sigmoid colon.

Comment: One patient in this study complained of constipation. Investigation of his bowel revealed no abnormalities.

6.7 GENITO-URINARY SYSTEM

Genito-urinary involvement occurs infrequently in EB Dystrophica. Hydronephrosis secondary to urethral strictures have been reported (Kretkowski 1973, Eklof 1984). In the former case, stenotic ulcerative meatitis resulted in bladder hypertrophy and bilateral hydronephrosis and hydro-ureter. The latter case had a contraction of the bladder neck with a posterior urethral valve and diverticula which led to secondary dilatation. Scarring of the external genitalia in a female child resulted in chronic vaginal and uterine reflux with retention of urine (Shackleford 1980).

In the investigation and treatment of genito-urinary EB, instrumentation may exacerbate ulceration and stricture formation, on the same basis as in oesophageal disease. Successful treatment of meatal stenosis has been achieved by meatotomy.

Comment: None of the cases in this study had urinary involvement. The man with **EBD Generalisata Gravis** had widespread blistering on the external genitalia, but no meatitis nor stricture formation had developed.

6.8 OCULAR CHANGES

Ocular involvement was not regarded as a feature of EB Simplex until 1980 when Granek (1980) described corneal involvement in two generations of an affected family. Ring-like configurations of fine bullous lesions in the deep corneal epithelium, superficial to Bowman's membrane, paralleled the level of blister formation in their skin. Rupture of bullae through to the corneal epithelial surface resulted in symptoms. The initial presentation had been an unresponsive blepharo-conjunctivitis. Aurora (1975) reported on a patient with EBA who had oedema of the cornea uveal tract, trabecular network, lens and optic tract. Oedematous cysts of the iris as well as detachment and focal necrosis of the retina were found at post-mortem examination. The aetiology of these changes was postulated to be the activity of a proteolytic enzyme, possibly collagenase.

Dystrophic EB is well recognised as having moderate to severe ocular changes. Gedde Dahl (1971) noted that 14% of patients studied with EBD had erosive keratitis and kerato-conjunctivitis. Manifestations of ocular involvement include blepharitis, pterygia, conjunctivitis, symblepharosis and bullous keratitis. Subepithelial blisters may cause ulceration, complicated by corneal or scleral opacity or,

more severely, proliferation. Blistering of the eyelids occurs and circumferential scarring may result in severe complications.

Comment: Intra-ocular changes were not encountered in this study, although, one person with EBA, and the adult male with EBD Generalisata Gravis, had severe blistering of the eyelids with loss of eyelashes.

6.9 OTORHINOLARYNGEAL INVOLVEMENT IN EB

While oesophageal lesions in EB are a recognised syndromic component, laryngeal involvement is virtually unknown. One report (Ramadass 1978) describes a male child with narrowed vestibules of the nose due to concentric stenosis. Laryngoscopic investigation of a hoarse voice by demonstrated cicatricial stenosis of the larynx at the anterior commissure and in the inter-arytenoid region, with irregular margins of the vocal cords. Subglottic oedema with an inflammatory membrane has been seen in a child with persistent stridor (Shackleford 1980).

These unusual complications are potentially lethal and tracheostomy may be necessary if the stenosis worsens, leading to respiratory distress. Anaesthesia is hazardous as the effects of incubation are traumatic to a sensitive larynx.

Comment: Apart from minor blistering around the nostrils, no ear, nose or throat manifestations of EB were seen in this study.

6.10 NEOPLASIA AND EB

Epidermolysis Bullosa has an association with malignancy, although no malignancy has been shown to segregate with any of the subtypes.

The most consistent association in EB is in EBD Cockayne Touraine, where malignant skin tumours may occur within scars as a late complication. These are generally squamous cell carcinomas. Less commonly, similar skin tumours have been reported in persons with autosomal recessive EB dystrophica. No specific association has been described between neoplasia and EB Simplex nor EB Atrophicans.

In addition to skin neoplasia, malignant tumours of other organs, especially the tongue, oesophagus and respiratory tract, have been reported in persons with the different dystrophic forms of EB. None of these associations have been of statistically significant proportions.

Comment: In this study, malignancy was not observed in the skin or internal organs of any patient.

CHAPTER 7 OESOPHAGEAL INVOLVEMENT

7.1 INTRODUCTION

A separate chapter has been devoted to the description of oesophageal involvemnt in EB. This subject was of particular therapeutic importance to the study as a patient suffering from severe dysphagia caused by oesophageal strictures underwent the successful dilatation of his oesophagus. For this reason, a large amount of emphasis was placed on this infrequent complication of EB.

The involvement of the oesophagus in EB Dystrophica is an unusual but well recognised manifestation of the condition. In his thesis Gedde Dahl (1971) reported that only three out of fifty-five persons with RDEB had oesophageal involvement. A study of world literature has yielded approximately 60 cases of oesophageal EB. Such involvement is rare in the dominant dystrophic and atrophic forms, and to the best of my knowledge has not been reported in EB Simplex.

Dysphagia is the major symptom of oesophageal involvement. Bullae of the oral mucosa and tongue are evident from the first few days of life and generally persist during the months of suckling. Blistering of the oesophageal mucosa also begins early, but starts with an insidious course, with symptoms usually appearing long after onset of the lesions.

The aetiology of oesophageal pathology parallels that of the skin lesions, namely trauma. Repeated insult to the mucosa of the oesophagus in the form of rough, dry or very hot foodstuffs results in the formation of bullae in certain individuals with RDEB. The effect of the trauma is to separate the epithelium from the lamina propria. As acute inflammatory tissue becomes replaced by scar tissue, oesophageal involvement becomes irreversible; more permanent stricture formation occurs and the motility of the affected area is diminished.

In keeping with the heterogeneity of EB, oesophageal involvement in RDEB is variable, and a wide range of oesophageal lesions may occur. Diagnosis can be made radiographically and/or endoscopically. Barium studies of the oesophagus are effective and non-invasive, and form the baseline investigations of dysphagia in EB. There are limitations and acute bullae may not be well visualised on barium swallow. Oesophagoscopy is potentially hazardous, but when performed with prior radiographic description of the size of constrictions, yields very precise descriptions of the lesions. The effect of the trauma induced by the sheer width of an endoscope may be to exacerbate stricture formation.

Involvement of the oral mucosa rarely persists beyond infancy, and it is thought that oesophageal blistering parallels that of the mouth. The young man in this study with severe involvement of the oesophagus had ongoing acute bullae in his mouth and oesophagus.

7.2 PRIMARY LESIONS

The earliest primary pathology is areas of non-specific inflammatory infiltrate with mucosal oedema. Bullae are seen endoscopically but have been reported radiographically as tiny filling defects on barium study. Ulceration of the damaged mucosa may occur. Scarring results from acute lesions and healing may be very slow. An unusual early complication is the formation of a pseudodiverticulum within the area of healing.

A complication of early scar formation is impairment of motility of the oesophagus, by segmental stenosis. Peristaltic activity is decreased proximal to the area of narrowing. Gastro-oesophageal reflux may occur as a result of shortening of a stenotic oesophagus (Agha 1983).

7.3 SECONDARY LESIONS

The later manifestations of oesophageal EB are webs and strictures. Progression of transverse webs leads to circumferential lesions as a phenomenon of post-inflammatory healing and accounts for the subjective symptom of worsening dysphagia despite a solid, pureed and ultimately even liquid diet.

Bullae occur throughout the length of the oesophagus, but certain predilection sites are more vulnerable to the trauma and friction of swallowed foods and resultant lesions. The site of stricture formation varies, with at least half the reported cases having had strictures in the upper third, which is the narrowest and least distensible part. The lower third is less commonly involved. The middle third is largely spared, though up to 25% of persons presenting with dysphagia and Epidermolysis Bullosa have two or more strictures, affecting multiple sites of the oesophagus. The length of the narrowed segment in these persons varies, with the range extending from the narrow web, to an average stricture covering 2-6 cm., to narrowing of the entire length of the oesophagus. Dilation proximal to each area of narrowing is usually noted.

Oesophageal strictures, without medical intervention, are irreversible. As is the case with the webs, which are progressive, strictures may worsen in severity with repeated trauma. This becomes a vicious cycle with minor trauma having disproportionately significant effects on an ever-narrowing lumen. However, anecdotal evidence suggests that the majority of these strictures tend to remain unchanged for long periods of time.

7.4 COMPLICATIONS OF OESOPHAGEAL LESIONS

Obstruction of the oesophagus, whether partial or complete is a well documented complication of EB. Acute severe blistering and ulceration of the oesophagus may lead fairly rapidly to an area of complete stenosis. Long segments, or multiple segments of stricture may extend inwards to approximate and so block off the lumen. Similarly a wedged bolus of food in an area of narrow constriction, may cause total occlusion.

Less common, but potentially lethal, is perforation of the oesophagus. This may occur with a relatively minor insult to an already damaged oesophagus. Within an area of obstruction, intraluminal pressure may increase to the point where tearing of the oesophagus occurs to relieve this pressure. Massive haemorrhage may accompany perforation.

Significant long term sequelae are unusual, but may occur. Malnutrition and/or nutritional anaemias may be consequent upon severe dysphagia. Aspiration may occur following regurgitation of entrapped foodstuffs, causing so-called 'dysphagia pneumonitis'. Carcinoma of the oesophagus arising in areas of damaged tissue is rare.

7.5 DIFFERENTIAL DIAGNOSIS

Where dysphagia and the typical oesophageal lesions co-exist with skin and nail signs of RDEB, it is accepted that the oesophageal lesions are a consequence of this genodermatosis. Unrelated conditions may present with similar symptoms and radiographic and endoscopic appearances. The co-existence of other medical conditions causing these lesions can be excluded with little difficulty clinically; scleroderma, peptic ulceration and the strictures caused by the ingestion of caustic substances do not pose a problem with the diagnosis of oesophageal EB.

7.6 MANAGEMENT OF OESOPHAGEAL EB

The management of the oesophagus in EB comprises two major approaches; initially, prevention, and later palliative and where possible curative procedures. Dietary manipulation is important in all stages of management. Prevention of acute lesions in susceptible patients is attempted by avoidance of hard, dry, rough or hot foodstuffs which may be swallowed with any degree of difficulty. The avoidance of any trauma to the sensitive oesophagus, may be best achieved by a soft, semi-puréed, or even liquid diet.

In the longer term, the same dietary measures are appropriate, where the consequences of trauma, namely webs and strictures, do not permit passage of solid foodstuffs through the narrowed lumen.

In the acute blistering phase, corticosteroids are used with limited success. Latterly, oral phenytoin has been given, applying the same principles as those used in the treatment of skin lesions. The inhibition of collagenase may serve to decrease the vulnerability of the oesophageal epithelium to detachment with trauma.

Webs and strictures, are not easily managed. Dilatation of these lesions is usually a traumatic process, and it is trauma which has specifically to be avoided. Forceful dilatation by standard measures, using the tangential shearing forces of bougienage, may offer initial relief but the trauma of this procedure may initiate a viscous cycle of bullae and then complications.

Following the example of successful dilatation of vascular stenosis by transluminal angioplasty, endoscopic balloon dilatation of oesophageal strictures is now proving to be valuable in the management of oesophageal stenosis. In EB this is the preferable manner of dilatation, because the vertical pressure exerted by the inflatable balloon causes far less damage to tissue than conventional bougienage.

The procedure consists of passing of the balloons, upon a 7 French catheter, over a guidewire to the point of stricture. This is carried out under fluoroscopic control and guidewires as well as the proximal and distal ends of the balloons are are radio-opaque. The balloons range in width from 4 to 20 millimetres and in length from 2 to 10 centimetre.

The balloon, once in situ, ideally in the middle of the stricture, is inflated, using air, water, or radiographic contrast, and pressure is exerted between 20 and 60 psi. This is then withdrawn, maintaining pressure, through the stricture. Sequential dilatations may be performed, using increasingly larger diameter balloons.

Combination of balloon and bougie type dilatation may also be performed. In the treatment of the patient in this study, the very narrow stricture was initially opened by sequential balloon dilatation, and thereafter conventional bougienage was employed.

Dilatation of stenoses are purely palliative measures and the recurrence of lesions so treated is frequent. A last resort in the management of very severe obstructions and an attempted cure is the resection of that segment of the oesophagus and replacement with a graft of the patient's colon.

Ideally, management of the oesophageal strictures in EB combines pharmacological and endoscopic procedures. Corticosteroids or Phenytoin may be administered for a period prior to, and following, dilatation, in order to minimise susceptibility to the trauma caused by dilatation. Post-dilatation, the use of an anti-ulcerogenic coating agent, such as Sucralfate, may be of value, where slow sipping of the

dissolved tablets forms a protective layer on the sensitive oesophageal endothelium. This approach was used empirically, and with good effect in the patient treated in this study.

The outlook in oesophageal EB is largely unfavourable. Lesions are generally irreversible, and stricture formation in post-traumatic areas is virtually inevitable. Strictures need frequent repeated dilatation, and even after successful dilatation, the affected person is recommended a lifelong diet of puréed foodstuffs.

7.7 COMMENT

The successful management of the patient discussed in Chapter 4 demonstrates that the dilatation of strictures may not be as hazardous as previously believed. The procedure was uncomplicated and follow up over 1 year has confirmed that there has been no recurrance of his symptoms.

CHAPTER 8 EPIDEMIOLOGY

8.1 DEFINITIONS AND OUTLINE

At the outset of this study, the frequency of EB in South Africa was unknown, and an endeavour was therefore made to achieve total ascertainment of affected persons in the pursuit of epidemiological data.

These data are traditionally divided into three major categories, as defined by Barker (1982).

Incidence is the frequency of the disease in relation to the size of the population at a specified time

Prevalence is the proportion of people affected by a disease at any particular time

Mortality is the number of deaths from a given cause per time unit per unit population.

In a chronic genetic condition such as EB, where the incidence and prevalence are parallel parameters, it is practical to consider the two together, encompassed by the term frequency. The gathering of this data may be retrospective, current or prospective.

An epidemiological study of Epidermolysis Bullosa is complicated by practical and mathematical pitfalls. Heterogeneity is well recognised in EB and 26 different types have been delineated. Each subtype is clearly demarcated as a separate disease entity, and for this reason the analysis of epidemiological data regarding EB as a whole is meaningless. Each subtype has therefore to be considered as an entity in its own right.

Retrospective studies of frequency are impractical as the record sources, subjective and objective, that must be tapped, are often inaccurate and unreliable. As discussed in a prior section, not all persons with EB come to medical attention. At the milder end of a large spectrum, affected persons may not consider their increased blistering tendency as pathologically significant, especially in the dominantly inherited variety, where generations of the family know and understand the condition. At the other extreme, the so-called lethal form of EB Atrophicans may be rapidly fatal, and in a large country with disproportionate distribution of medical care, these infants may not survive to receive hospital treatment. Such figures may be lost to statisticians, or incorrectly represented.

8.2 DEMOGRAPHY

8.2.1 Area studied

The study was conducted throughout Southern Africa. The cummulative area of this territory is 1 220 000 km^2. The country is divided into four major provinces - the Cape, Transvaal, Natal and the Orange Free State - the Black "homelands" and the independent Black states - Transkei, Ciskei, Venda and Bophutatswana, as shown in Figure 50.

8.2.2 Population

The total population according to the 1980 census is 32 585 960. The people of South Africa have widely differing ethnic origins and the population comprises four main groups. These are:

a) European (White)

b) African negro (Black)

c) Asian

d) Mixed ancestry

The African negro population may be sub-divided according to tribal groups, namely, Zulu, Ndabele, Xhosa, Sotho, Twsana, Pondo, Venda, Shangaan.

The population by ethnic group and population density in the major regions of South Africa according to the 1980 census is shown in table 8-I.

TABLE 8-I

POPULATION DISTRIBUTION IN SOUTH AFRICA

	Total	Whites	Blacks	Asians	Mixed Ancestry
Cape	5 091 360	1 264 040	1 569 040	32 120	2 226 160
Natal	2 676 340	561 860	1 358 120	665 340	91 020
Transvaal	8 350 500	2 362 060	5 644 660	115 560	228 220
O.F.S.	1 931 860	326 220	1 549 600	0	56 040
Black Homelands	6 835 900	13 920	6 802 340	8 300	11 340
Transkei	2 000 000		2 000 000		
Ciskei	1 600 000		1 600 000		
Bophuta-tswana	3 500 000		3 500 000		
Venda	600 000		600 000		
TOTAL	32 585 960	4 528 100	24 623 760	821 320	2 612 780
		(14%)	(75,5%)	(2,5%)	(8%)

8.3 FREQUENCY OF EPIDERMOLYSIS BULLOSA

8.3.1 Frequency of EB outside of South Africa

Disease frequencies outside of South Africa are based mainly on a retrospective study in Norway (Gedde Dahl 1971). Apart from the Scandinavian countries, little has been published of the incidence or prevalence of EB worldwide, and only anecdotal estimates of patient frequencies are known at most major academic institutions. Isolated reports of EB have emanated from all parts of the world including Europe, the United Kingdom, the United States of America, the Far East, West Africa and India.

Table 8-II shows frequency rates based on retrospective screening of 2,74 million births from 1913 to 1962 and the prospective study of 1,04 million births from 1963 to 1979 in Norway (Gedde Dahl). In addition to these figures, data are available in Table 8-III from a study of 44 109 births in Lund (Book) and 29 092 births in Umea (Beckman) in Sweden.

TABLE 8-II

EB FREQUENCY AT BIRTH ($\times 10^{-6}$) IN NORWAY

EB Subtype	1913-1962	1963-1979
EB Simplex Koebner	>1,5	2,9
EB Simplex Weber Cockayne	>13,1	19,2
EB Simplex Ogna	20,1	14,4
EB Herpetiformis Dowling Meara	>0,8	1,0
EB Bart	>0,4	1,9
Dystrophica Bullosa Hereditaria, Mendes da Costa	Single Dutch family	
EB Atrophicans Generalisata Gravis Herlitz	5,3	1,9
EB Atrophicans Inversa	>1,8	2,9
EB Progressiva	>1,5	>1,0
EB Dystrophica Cockayne Touraine	>1,1	1,9
EB Dystrophica Albopapuloidea Pasini	>1,8	2,9
EB Dystrophica Hallopeau Siemens	>4,4	4,8
EB Dystrophica Inversa	>2,2	<1,0

TABLE 8-III

FREQUENCY OF EB IN SWEDEN

	Lund (1927-1946)	Umea (1950-1972)
EB Atrophicans Generalisata Gravis Herlitz	45	170
EB Dystrophica Hallopeau Siemens		103

8.3.2 Frequency of Epidermolysis Bullosa in South Africa

Eighty-two affected persons in forty families were investigated during the two year concurrent study, 1984-1986. The population of the country according to the 1980 census was 32 585 960.

The estimated minimum frequency of EB in South Africa is therefore of the order of one in 40 000.

8.3.2.1 Sex Ratio in EB

The incidence of EB was roughly equal in males and females, as shown below:

Male	Female
43	39
(52.4%)	(47.6%)

Further breakdown shows male:female ratio in the different major groups (Table 8-IV).

The sex ratios in the Simplex Atrophicans and Dominant Dystrophic forms were roughly equal, while there was a marked male preponderance in RDEB, with a ratio of 3:1.

TABLE 8-IV

MALE : FEMALE RATIOS IN MAJOR SUBTYPES OF EB

	Male	Female
EB Simplex	13	11
EB Atrophicans	9	10
EB Dystrophica	21	15
DDEB	11	11
RDEB	9	3
Indeterminate EBD	1	1
Unclassified	0	3

8.3.2.2 Geographic Distribution

There was no geographic limitation on this study and persons from all parts of the country were seen. The map in Figure 51 shows the distribution of patients. Population figures for that province are shown in parentheses.

The majority of patients were from the Cape Province (45.1%) and the Transvaal (40.3%) which are the largest provinces with the highest population densities. There was no evidence that environmental factors in the form of geographic location had any significant effect on the frequency nor manifestations of EB. The fact that the study was based in the Cape Province may have introduced some observer bias, thus the higher figures in this region.

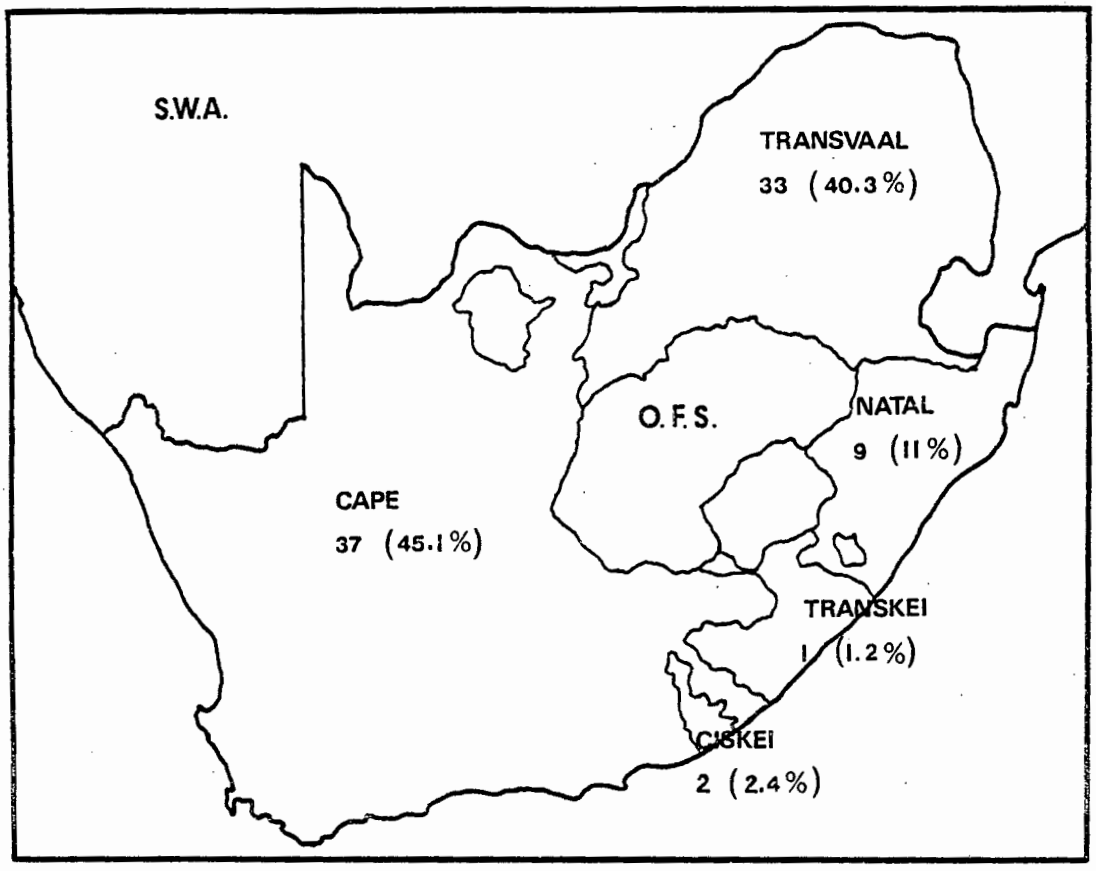


Figure 51. The geographic distribution of EB in South Africa

8.3.2.3 Ethnic Distribution

EB was present in all the ethnic groups of South Africa. Table 8-V shows that in both patient and kindred numbers 42.7% and 37.5% respectively, the largest group of persons with EB was from the White (European) population. These persons were of British, German, and Madeiran origin, as well as families of several generations of South African Afrikaner stock. This proportion indicates over-representation as only 14% of the population of South Africa are White.

The population of mixed ancestry was over represented, (29.3%), since this group forms only 8% of the total population. The numbers of South African blacks appear to be adequate and persons were seen from Xhosa, Tswana, Zulu, Sotho, and Shangaan tribal groups, with no preponderance of EB in general in any one tribe. When this number is compared with the fact that more than 75% of the population, or almost 25 million South Africans are Blacks, this is gross under-representation as compared with the White group. This discrepancy may be due to a low gene frequency. Conversely the paucity of negroes with EB may be an ascertainment error, as discussed in other relevant sections of the thesis.

TABLE 8-V

ETHNIC PREVALENCE N = 82 (40)

	White	Black	Coloured	Indian
Patient numbers	35 (15)	20 (13)	24 (10)	3 (2)
Percentages	42.7 (37.5)	24.4 (32.5)	29.3 (25)	3.6 (5)

Figures in parentheses represent the number of families.

Asians, who form the smallest sector of the diverse population structure, were under-represented, with only two affected families (5%) encountered.

The different subtypes of EB will be discussed according to the ethnic origins of the population in a part of this chapter so designated.

8.4 EPIDERMOLYSIS BULLOSA IN SOUTH AFRICA - SUBTYPES REPRESENTED

Each kindred was assigned to a subtype of EB according to the clinical findings as described in a preceding chapter of this thesis.

EB in South Africa can thus sub-divided and analysed according to standard parameters, i.e. male:female ratio, as well as the distribution of the different subtypes and the prevalence of these among the various ethnic groups. The population of South Africa is very heterogeneous and the documentation of the different forms of EB in members of each gene pool was sought.

8.4.1 Major subtypes

EB, though uncommon in South Africa, was well represented in all major subtypes (Table 8-VI). The numbers of affected persons and families are tabulated; in the analysis of these data, frequency will be considered in terms of kindreds rather than affected individuals.

EB Dystrophica was most commonly encountered, with 36 persons (43.9%) in 15 families (37.5%) being affected. An equal number of families with EBA were studied, although the total number of affected persons in these 15 families was 19, 23.2% of the total. Only 17.5% of families had EB Simplex. Within

TABLE 8-VI

MAJOR SUBTYPES OF EB IN SOUTH AFRICA

SIMPLEX vs ATROPHIC vs DYSTROPHIC	N = 82 Patients		N = 40 Families	
EB Simplex	24	(29.3%)	7	(17.5%)
EB Atrophicans	19	(23.2%)	15	(37.5%)
EB Dystrophica	36	(43.9%)	15	(37.5%)
Unclassified	3	(3.6%)	3	(7.5%)

the dystrophic subtype, there was a preponderance of RDEB, 9 of 15 families, as compared with the under-represented DDEB. Only four families in the study has this form of EBD. Comparing patients rather than families, however, there were roughly equal numbers in the DDEB and RDEB groups.

8.4.1.1 Major subtypes according to ethnic origins

The frequency of the major subtypes of EB has been analysed according to the ethnic origins of the patients in order to establish relative prevalence of the subtypes in the different gene pools. Table 8-VII lists the numbers of persons and kindreds, as well as percentages of the various forms of EB in the population groups.

An excess of persons of White and mixed ancestry manifest both the EB Simplex form and RDEB, while no Black persons were documented as having the non scarring EB Simplex. This may be due to a number of complex socio-economic reasons; an unsophisticated rural family may not present for medical care for a seemingly unimportant skin disease which may be interpreted as a familial tendency to be borne stoically. The distribution of medical care in the urban and rural settings is disproportionate and outside of the cities, limited medical expertise is available; in these circumstances the diagnosis of uncommon conditions such as EB may not readily be made.

EBA was seen in Black persons of the Tswana, Shangaan, Venda, Zulu, Xhosa, and Sotho tribes, suggesting the existence of EBA in the African negroes before their division into different tribal groups. Indeed, there was a paucity of EBA in White and Asian people, while representation of the mixed ancestry group was proportionately appropriate.

TABLE 8-VII

MAJOR SUBTYPES OF EB ACCORDING TO ETHNIC ORIGINS N = 82 (40)

	White		Coloured		Black		Asian	
Simplex	16 19.5%	(6) (15%)	8 9.8%	(1) (2.5%)	- -	- -	- -	- -
Atrophicans	3 3.7%	(3) (7.5%)	8 9.8%	(5) (12.5%)	7 8.5%	(6) (15%)	1 1.2%	(1) (2.5%)
Dominant Dystrophic	9 11%	(1) (2.5%)	5 6%	(1) (2.5%)	8 9.8%	(2) (5%)	- -	- -
Recessive Dystrophic	7 8.5%	(5) (12.5%)	3 3.7%	(3) (7.5%)	2 2.4%	(2) (5%)	2 2.4%	(1) (2.5%)
Unclassified	-	-	-	-	3.7%	(7.5%)	-	-

Figures in parentheses represent families

EB Dystrophica was seen in members of all ethnic groups, with a slightly higher frequency in the White sector than predicted.

8.4.2 Minor Subtypes

8.4.2.1 EB Simplex

Twenty four persons in seven families had EBS. Two of these persons were sporadic and examination of family members verified the negative family history. Inheritance in the other families was autosomal dominant.

Two of the families had the Koebner type EBS with generalised distribution of lesions. Three families had lesions confined to the extremities compatible with a diagnosis of **EBS Weber Cockayne.** A family was encountered with two affected individuals displaying the classical herpetiformis grouping of lesions **EBS Dowling Meara.** One large kindred comprising eight affected members had the rare subtype as **EBS with mottled pigmentation.** (Table 8-VIII).

The clinical histories in all of these patients were similar, irrespective of subtype. Onset was at birth or early infancy and bullous formation worsened with the onset of ambulation. All adult patients described a subjective improvement in their condition after puberty. Persons from each family described occasional dystrophic fingernails; in all such instances damage to nails had been traumatic.

TABLE 8-VIII

EPIDERMOLYSIS BULLOSA SIMPLEX N = 24 (7)

	Patients	Families
EBS Koebner	5	2
EBS Weber Cockayne	9	3
EBS Dowling Meara	2	1
EBS with mottled pigmentation	8	1
Total	24	7

Apart from the fundamental similarities in all types of EBS, the characteristic differentiating factors which distinguish the 'minor' subtypes were classical within a group. A notable observation is that penetrance was complete and expressivity was constant within each kindred studied.

A constant observation among persons with **EBS Koebner** was an increased tendency to blistering in the hot summer months. This factor was encountered in all the geographical areas of the country where temperature and humidity levels vary greatly.

ETHNIC DISTRIBUTION

EB Simplex was seen only in persons from the White and mixed ancestry population groups (Table 8-IX). This may represent a spurious paucity in the Black and Asian groups where full ascertainment of patients may not have been achieved for reasons as outlined in Section 8.4.1.1

TABLE 8-IX

EB SIMPLEX : ETHNIC DISTRIBUTION N = 24 (7)

	White	Black	Mixed Ancestry	Asian
EBS Koebner	5 (2)	-	-	-
EBS Weber Cockayne	9 (3)	-	-	-
EBS Dowling Meara	2 (1)	-	-	-
EBS With Mottled Pigmentation	-	-	8 (1)	-
Total	16 (6)	0	8 (1)	0

Figures in parentheses represent families

8.4.2.2 EB Atrophicans

In this study, information was gained concerning nineteen persons in fifteen families with Epidermolysis Bullosa Atrophicans.
Table 8-X shows the representation of the subtypes seen in South Africa.

LETHAL EBA

Initial presentation was similar in all cases, with onset at birth of bullous lesions and areas of denuded skin. The mean age of death of the fourteen infants with lethal **EBA Generalisata Gravis** (EBAGG) and the child with an associated pyloric atresia was approximately one month. The cause of death in these infants was cited as dehydration, septicaemia or respiratory failure as complications of EB.

Two of the cases of EBAGG were foetuses terminated at the mid-trimester of the pregnancies. The former was the foetus of a mother at risk, having had two previously affected babies. The latter (Section 4.3.1.5) had a termination of pregnancy after abnormalities resembling a cystic hygroma had been observed during a routine antenatal ultrasound scan. The foetus was found at autopsy to have macroscopic and histological features of EB Atrophicans as well as Turner syndrome. Chromosomal analysis confirmed the karyotype XO. Coincidence can be the only likely explanation for the existence of two unassociated conditions in the same foetus.

TABLE 8-X

EB ATROPHICANS N = 19 (15)

	Patients	Families	Lethal	Non-lethal
EBA Generalisata Gravis	17	13	16	1
EBA with pyloric atresia	1	1	1	-
EBA Generalisata Mitis	1	1	-	1
Total	19	15	17	2

NON LETHAL EBA

The two children encountered with non-lethal EBA are noteworthy and warrant further discussion at this juncture. A female child with the features of **EBA Generalisata Mitis** is described in Subsection 4.3.3. She is the only person affected with this subtype of EB in South Africa. 'Mitis' would appear as a misnomer in her case; despite her survival thus far, her disease is severe, and quality of life is markedly impaired.

A child with the typical features of **EBA Generalisata Gravis** or **Herlitz syndrome** is discussed in section 4.3.4. She is now 11 years of age and, despite moderately severe lesions, enjoys a relatively normal lifestyle. Her survival can be explained by a variety of possibilities. Impeccable nursing care through the most vulnerable stage of infancy cannot alone be responsible, since more survivors of this condition would be anticipated following sophisticated neonatal care. She may be a manifestation of a genetic compound of two allelic autosomal recessive traits. Conversely her condition may be confirmation of still further heterogeneity in EB.

ETHNIC DISTRIBUTION

Table 8-XI shows the ethnic distribution of EBA; there is an equal incidence in the mixed ancestry group and Black persons. This represents over representation in persons of mixed ancestry, who form 8% of the population, compared with 75.5% blacks.

TABLE 8-XI

EB ATROPHICANS : ETHNIC DISTRIBUTION N = 19 (15)

	White	Black	Mixed Ancestry	Asian
EBA Generalisata Gravis	2 (2)	6 (5)	8 (5)	1 (1)
EBA with pyloric atresia	-	1 (1)	-	-
EBA Generalisata Mitis	1 (1)	-	-	-

Figures in parentheses represent families

TABLE 8-XII

EB DYSTROPHICA N = 36 (15)

Autosomal Dominant	22	(4)
Autosomal Recessive	12	(9)
Unclassified	2	(2)

Figures in parentheses represent number of families

8.4.2.3 EB DYSTROPHICA

The largest major subgroup represented was EB Dystrophica. Thirty-six persons in fifteen families had the scarring form of EB. As seen in Table 8-XII, twenty-two of the persons had DDEB and twelve had RDEB; of the fifteen kindreds represented, four had DDEB and nine had RDEB. Four families were not further classified because of inadequate information.

As in previous sections of this thesis, EBD will again be discussed under the two headings of DDEB and RDEB.

8.4.2.3.1 Dominant EB Dystrophica

Twenty-two persons in four families had the autosomal dominant form of EB Dystrophica. The patterns of inheritance were clear cut and with the exception of one family penetration and expressivity were complete. A kindred in whom persons were affected with **EBD Cockayne Touraine** had questionable lack of penetrance in a generation; however, the family member said to be unaffected was unable to be traced for examination and, without objective proof, no assumption of a skipped generation may be made.

TABLE 8-XIII

AUTOSOMAL DOMINANT EB DYSTROPHICA : SUBTYPES N = 22 (4)

	Patients	Families
EBD Cockayne Touraine	15	3
EBD Pasini	7	1

TABLE 8-XIV

AUTOSOMAL DOMINANT EB DYSTROPHICA : ETHNIC DISTRIBUTION
N = 22 (4)

	White	Black	Mixed Ancestry	Asian
EBD Cockayne Touraine	4 (1)	1 (1)	4 (1)	-
EBD Pasini	-	7 (1)	-	-

Nine persons in three families had lesions confined to the distal parts of the extremities, consistent with the diagnosis of **DDEB Cockayne Touraine.** None of the persons had any signs of malignancy within scar tissue. (Table 8-XIII.)

The fourth family had the small papules in the sebaceous areas which are classical of **DDEB Pasini.** This is an important observation as this condition has not been previously recognised in Negroes. It is important that in these persons the papules were morphologically identical to the Pasini patches, though not ivory coloured, as is found in Caucasians. The limited laboratory facilities available did not allow for assay of the biochemical defect previously reported in this subtype.

ETHNIC DISTRIBUTION

Table 8-XIV shows the ethnic distribution in DDEB. Family numbers in each type are too small to draw statistical conclusions from these figures.

8.4.2.3.2 Autosomal Recessive EB Dystrophica

Twelve patients in nine families who had manifestations of dystrophic EB were designated autosomal recessive EBD because of sporadic occurrence or, in two cases, affected relatives with or without consanguinity. This assumption was supported by conformity of the phenotype characteristic of RDEB where

there was doubt, the persons was considered as unclassified EB Dystrophica.

RDEB is divided into six subsets, namely **EBD Inversa**, with distribution of lesions, particularly in the peri-anal and perivulvar areas, **EBD Progressiva**, a late onset type associated with perceptive deafness, and **EBD Hallopeau Siemens**. **EBD pretibial** is a late onset form and **EBD Fine** with centripetal symmetrical blisters are uncommonly encountered. **EBD Generalisata Gravis (Winship)** is a previously unrecognised entity.

The classical form of**EBD Hallopeau Siemens**, may be further divided into three sections depending on the distribution of lesions and the clinical severity and complications. These are designated localised, generalised and mutilans.

Eleven patients had **EBD Hallopeau Siemens** (Table 8-XV), Five had moderately severe blistering in a widespread distribution compatible with diagnosis of **EBD Hallopeau Siemens (General)**. Four persons were very severely affected, having stunted stature, and the repeated scarring, had led to flexion contractures, syndactyly and in two persons mitten deformities of hands and feet. These latter four persons designated **EBD Hallopeau Siemens (mutilans)** suffered involvement of the teeth with severe enamel hypoplasia.

TABLE 8-XV

AUTOSOMAL RECESSIVE EB DYSTROPHICA N = 12 (9)

		Patients	Families
EBD Hallopeau Siemens	Generalised	5	4
	Mutilans	6	4
EBD Generalisata Gravis (Winship)		1	1

Significant oesophageal strictures, were noted in two persons of this group, and in one with the general subtype. The former who had been previously treated, were asymptomatic, while the latter who had symptoms of dysphagia, underwent a successful dilatation of his oesophageal webs.

The only patient with RDEB that did not belong to the subtype Hallopeau Siemens has a hitherto undelineated form of RDEB. This was confirmed by personal communication with Gedde Dahl. This has been designated **EBD Generalisata Gravis (Winship)** in accordance with the standard use of eponyms in EB and has been is described in Section 4.5.

ETHNIC DISTRIBUTION

Table 8-XVI shows the ethnic distribution of RDEB in its subtypes. White persons were over-represented while no black families were diagnosed in this group.

TABLE 8-XVI

AUTOSOMAL RECESSIVE EB DYSTROPHICA : ETHNIC DISTRIBUTION
N = 12 (9)

		White	Black	Mixed Ancestry	Asian
EBD Hallopeau Siemens	Generalised	2 (1)	-	2 (2)	2 (1)
	Mutilans	3 (2)	-	1 (1)	-
EBD Generalisata Gravis (Winship)		1 (1)	-	-	-
Total		7 (5)	0	3 (3)	2 (1)

8.5 MORTALITY DUE TO EB IN SOUTH AFRICA

The Central Statistical Services, based in Pretoria, accrue information arising from all deaths registered within South Africa. This data is coded according to a three digit number which represents a specific diagnosis within the 9th Edition of the International Statistical Classification of Disease, Injuries and Causes of Death (ICD). Epidermolysis Bullosa has been assigned the code 757 which it shares with hereditary oedema of legs, Icthyosis Congenita, dermatoglyphic abnormalities and the specific anomalies of skin, hair, nails, breasts and integument.

In South Africa, the registration of deaths under this coding system does not differentiate between the other conditions designated to this three digit number. However, since the other conditions listed are rarely the cause of death in an individual, it is assumed that the majority, if not all persons listed under the code 757 had Epidermolysis Bullosa.

Statistical information for the different ethnic groups is compiled by various bodies. Data regarding Whites, Asians and persons of mixed ancestry was extracted from the Death Register (B.1.7) filed at the Department of Home Affairs. Figures concerning African Negroes are filed as 'Information of a Black Death' (B.A.676) by the Department of Co-operation and Development.

EB is an uncommon condition and it is only in the so-called lethal form, **EB Atrophicans Generalisata Gravis**, that this condition is cited as the cause of death.

The number of deaths in South Africa under the code 757 for the years 1980 to 1984 are shown in Table 8-XVII. These are given according to ethnic group.

In addition to these figures, EB caused at least one other death in 1983, with a further two in 1984 and four in 1985. In addition the termination of a pregnancy at risk for lethal EBA was performed in 1983.

These figures, as confirmed by the average age of death of less than two months, represent primarily lethal 'junctional' EB, **EB Atrophicans Generalisata Gravis.** This is known to be fatal in early infancy. An exception was an African negro infant born in 1985 who had EBA associated with pyloric atresia, which is also a lethal form of EBA.

The number of deaths listed per year in South Africa ranges from one to five, with a mean of 2.8 in the period 1980-1984. Of the fourteen persons listed in the Table 8-XVII , none were White, one of Asian stock and two of mixed ancestry. The

TABLE 8-XVII Deaths due to Epidermolysis Bullosa 1980-1984

Year	Total	White	Coloured	Asian	Black
1980	3	0	0	0	3
1981	2	0	1	0	1
1982	1	0	1	0	0
1983	5	0	0	0	5
1984	3	0	0	1	2

over-riding majority of deaths due to EBA Letalis were in Black infants. This observation is strengthened by subjective comment from the major neonatal units, who report a relative increase in the number of Black babies with the lethal form of EB as compared with the other population groups.

It would appear that the mortality data for EB are not entirely accurate, born out particularly by the fact that at least one out of the six cases known to have died in 1983 was unreported, representing at least 16% error. This inaccuracy of documentation of the cause of death may be due in part to the inadequate completion of death certificates by medical practitioners. Compounding this problem is the fact that a proportion of infants, born to unsophisticated parents in rural areas remote from medical attention, who have the lethal form of EB, may die before a diagnosis can be made. To the untrained eye, severe EB with areas of aplasia of skin closely resembles burns, and it is feasible that this erroneous assessment may be made.

Mortality figures, therefore, do not give a reliable assessment of the incidence of EB and the actual mortality rate reported is regarded in the light of possible discrepancies.

CHAPTER 9 GENETICS

9.1 PATTERNS OF INHERITANCE

9.1.1 EB Simplex

9.1.2 EB Atrophicans

9.1.3 EB Dystrophica

9.1.3.1 EB Dystrophica Autosomal Dominant

9.1.3.2 EB Dystrophica Autosomal Recessive

9.2 LINKAGE ANALYSIS

9.2.1 Lod scores

9.2.2 HLA typing

9.2.3 Restriction fragment length polymorphisms in genetic linkage

9.2.4 Review of linkage in EB

9.2.4.1 EBS Ogna

9.2.4.2 EBS Koebner

9.2.4.3 EB Dystrophica

9.2.5 Genetic linkage in EB in South Africa

9.2.5.1 HLA and red cell enzymes

9.2.5.2 Restriction Fragment length polymorphisms

9.3 GENE FREQUENCY, CARRIER AND MUTATION RATE IN EB

9.1 PATTERNS OF INHERITANCE

The genetics of Epidermolysis Bullosa are complex and it is meaningless to consider the condition as a whole as a single genetic defect. Each major group, EBS, EBA and EBD, is so different in so many aspects that it is very unlikely that Epidermolysis Bullosa Hereditaria is one single gene defect. Indeed, within the minor subtypes of each group, the phenotype varies to such an extent that it is improbable that a single mutation is responsible, for example, in all forms of dominantly inherited dystrophic EB. The possibility exists that the genes responsible for certain subgroups of EB may be alleles of those causing similar subtypes, and this may ultimately be true for all forms of this genodermatosis.

In this section of the thesis, the genetics of EB will be discussed according to the major subtypes and the minor groups within these.

9.1.1 Epidermolysis Bullosa Simplex

EB Simplex is inherited in an autosomal dominant manner. This pattern of inheritance has been established in all forms of EBS except the single Dutch family with the **Mendes da Costa** subtype, who exhibit X-linked recessive transmission. More recently a rare autosomal recessive subtype with early infantile death has been reported. (Salin 1985).

Most of the persons in this study with EBS fell into the **Koebner** or **Weber Cockaynê** subgroups. Two generations of one family had the typical signs of **EB Herpetiformis Dowling Meara.** In addition the eight affected members of a large kindred manifest the characteristic signs of EBS with mottled pigmentation.

The pedigree data of all families with EBS were consistent with AD inheritance. Penetrance was complete in these kindreds irrespective of subtype. Expressivity was constant, and there was no instance of a family with EBS manifesting signs of the Atrophicans or Dystrophic variety, or indeed of another minor subtype within EB Simplex.

In addition to these families, two persons with EBS Koebner were sporadic, i.e. there was no family history. Advanced paternal age which would weigh the balance in favour of a fresh mutation was not a factor in either case. Neither proband had consanguineous parents which might suggest autosomal recessive inheritance. In view of the very typical phenotype of **EBS Koebner,** and the absence of any unusual family history, the probability is that new mutations of the autosomal dominant gene had occurred in each instance.

Gedde Dahl (1971) has suggested that the genes responsible for **EBS Koebner** and **EBS Weber Cockayne** might be allelic. No evidence emanating from this study could prove or disprove this hypothesis.

9.1.2 EB Atrophicans

The accepted mode of inheritance of all forms of EBA is autosomal recessive. It is unlikely that all the subtypes of EBA are caused by one 'faulty' gene, although it is possible that a **EBS Generalisata Gravis** and **EBA with pyloric atresia** are allelic, or pleotropic expressions of a single gene defect.

Most of the children affected had no family history of EBA and only three families reported affected siblings thus confirming autosomal recessive inheritance. Neither consanguinity nor advanced paternal age was known. In the Tswana population, consanguineous marriage is a favoured tradition; conversely, to the Zulu and Xhosa people intermarriage is taboo. In a lethal condition, however, such as EBA, it is more likely that autosomal recessive inheritance is consistent rather than a fairly high mutation rate of an autosomal dominant gene.

EBA Generalisata Gravis, also known as **EBA Letalis** or **Herlitz syndrome**, is usually rapidly fatal. One girl the classical clinical and histological manifestations of this subtype had survived to the age of 11 years. The reason for her favourable outcome, as discussed in other chapters, should be attributed to a primary level, and it is postulated that her EB may be a consequence of a genetic compound. Alternatively, her condition is suggestive of further heterogeneity in EB.

9.1.3 EB Dystrophica

Unlike the other two major subgroups, EBD is divided into two distinct groups according to the mode of inheritance. These differ to an extent, phenotypically; the autosomal dominant forms tend to be less severe than the autosomal recessive group, although an area of overlap exists. Of the nine recognised subtypes of EBD, four are autosomal dominant and five are autosomal recessive.

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9.1.3.1 Dominant EB Dystrophica

The different subtypes inherited as an autosomal dominant trait are tabulated in Chapter 4, preceding clinical results. All are known to have complete penetrance and constant expression.

In this study, three kindreds with DDEB were seen. One family had the classical phenotype of **EBD Pasini** and displayed an autosomal dominant pedigree consistent with this subtype. Two families were diagnosed as **EBD Cockayne Touraine**, and had the typical distribution of lesions localised to the distal extremities. In the former, penetrance was complete and expression was comparable in all affected persons. A discrepancy exists in the pedigree of the second family with **EBD Cockayne Touraine**. Figure 52 shows a female (II-1) reported to be unaffected, who should, according to the laws of autosomal dominant inheritance, have EBD. This suggests the possibility of non-penetrance. She could not be traced for examination and without objective proof of her clinical status, this putative 'skipped generation' cannot be substantiated.

One sporadic black female had the classical phenotype of EBD Cockayne Touraine, and therefore represents a new mutation. It is of interest that none of her five children are affected.

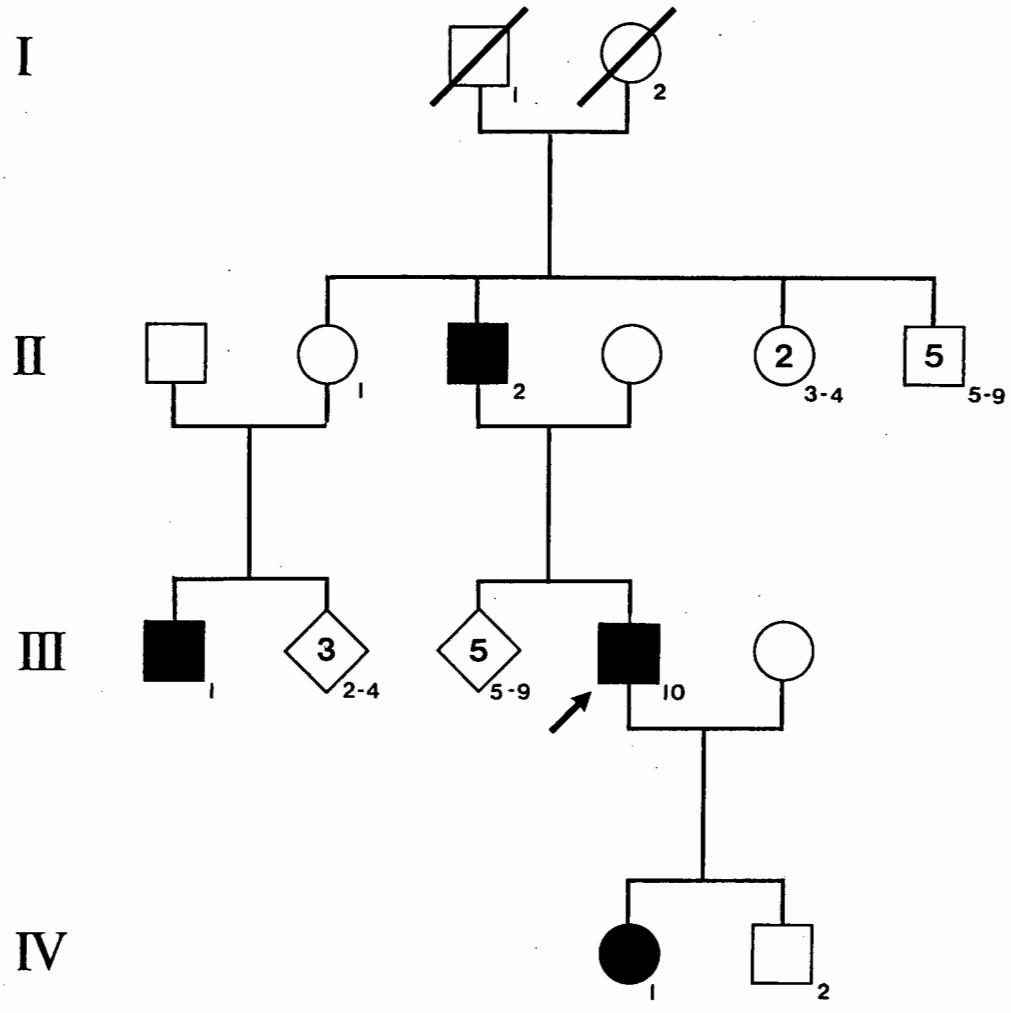


Figure 52. An autosomal dominant pedigree where a generation may have been skipped

9.1.3.2 Recessive EB Dystrophica

The **Inversa, Progressiva** and **Hallopeau Siemens** forms of RDEB are all established as autosomal recessive traits.

Apart from one male patient designated **EBD Generalisata Gravis (Winship)**, all the persons studied in this group were of the subtype **EBD Hallopeau Siemens**, either **generalised** or **mutilans**. Two families had two affected siblings, thus confirming autosomal recessive inheritance. The remaining persons were sporadic, and no details in the family histories suggested that they might be new mutations. Their categorisation into RDEB was on the basis of the appropriate phenotype.

One Tswana boy with a fairly mild form of EBD posed a genetic dilemma. Clinically he was more suggestive of AD **EBD Cockayne Touraine** although AR **EBD Hallopeau Siemens (local)** could not be excluded. Neither family history nor histopathological studies helped to differentiate between DDEB and RDEB.

The male patient with the newly recognised **EBD Generalisata** Gravis had no significant family history. While autosomal recessive inheritance seems most likely, X-linked recessive inheritance cannot be definitely excluded.

9.2 GENETIC LINKAGE ANALYSIS

Introduction

Genetic marker traits that are useful for linkage studies should ideally be dominantly or co-dominantly expressed, highly polymorphic, with a large number of alleles, and expressed in easily accessible body tissues or fluids. The various genetic markers which can be used for linkage studies are listed in Table 9-I. Family data are analysed for linkage by segregation analysis. Statistical analysis for the determination of linkage is calculated almost exclusively by means of 'lod scores'.

TABLE 9-I

POLYMORPHIC LOCI FOR FAMILY STUDIES FOR LINKAGE ANALYSIS

1. Antigenic markers
   a) Histocompatibility antigens (HLA)
   b) Blood group systems

2. Electrophoretic markers
   a) Serum proteins and enzymes
   b) Salivary proteins and enzymes
   c) Soluble red cell enzymes
   d) Mitochondrial enzymes
   e) Lysosomal enzymes
   f) Urinary proteins

3. Chromosomal variants
   a) Heterochromatin size and inversion
   b) C-band fluorescence
   c) Satellite size and staining
   d) Fragile sites

4. Restriction fragment length polymorphisms.

9.2.1 Lod scores

A 'lod score' is the acronym for the 'logarithm of the odds' that the two loci under study are linked. This concept in linkage analysis was proposed by Haldane and Smith (1947).

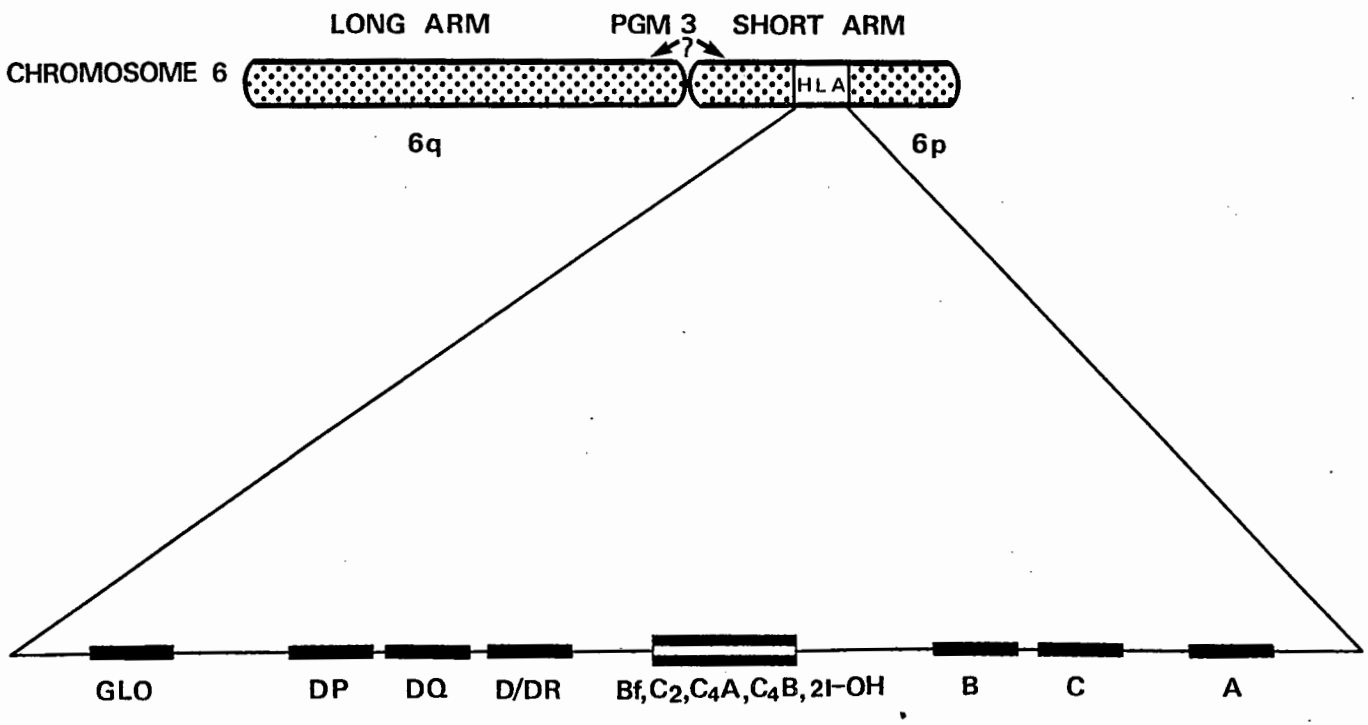
Lod scores are calculated separately for different recombination fractions. If the lod score is 3, this indicates that the odds are 1 000 to 1 in favour of linkage. The scores derived from different families can be added provided the same genetic loci are under study. Mathematical formulae and computer programs have been designed to analyse such data, and allowances can be made for genetic heterogeneity, multilocus models, incomplete penetrance, as well as late onset disorders.

The lod scores calculated in this study are a measure of the probability of linkage.

9.2.2 HLA typing

HLA antigens are glycoproteins located on the membranes of nucleated cells and in the serum. There have been semantic changes in the designation of the term "HLA" over the past 20 years, but now the entire human major histocompatibility complex is referred to as HLA. There are five loci for HLA on the 6th chromosome, with a series of allelic genes at each

Fig. 53 SCHEMATIC REPRESENTATION OF THE HLA LOCI ON CHROMOSOME 6 IN MAN



locus on analogous chromosomes. To date, 23 HLA-A genes, 46 HLA-B genes, 8 HLA-C genes, 19 HLA-D genes and 14 HLA-DR genes have been identified in each respective locus. In addition 3 DQ, 6 DP and antigens have been recognised. Figure 53 shows a schematic representation of the HLA loci on Chromosome 6.

HLA types are inherited as an autosomal dominant traits in accordance with Mendelian laws. An HLA haplotype is the specific series of HLA alleles in one chromosome, and an HLA phenotype is the specific array of HLA antigens on the cell membrane. The phenotype of an individual can be establihed by HLA typing. Family studies are necessary to identify the haplotype of that individual, and once the two haplotypes are known, the genotype can be established.

Phenotype frequencies of each HLA antigen vary according to race, country, immigration pattern. In the context of this study, this factor is highly significant as the population of South Africa is so heterogeneous.

HLA typing has been useful in many disease processes, including approximately 50 disorders of the skin. The practical application of HLA typing, i.e. the relative risk, is defined as how much more likely a person who has a particular antigen is to have or develop a specific disorder, than one who does not have that same HLA antigen.

9.2.3 Restriction fragment length polymorphisms (RFLP's) in genetic linkage

RFLP's are variations in an individuals DNA which create or eliminate restriction enzyme sites. They are not associated with the disease process but can be used as markers when linked to a specific locus for a disorder.

Different forms of the RFLP if co-inherited with the disorder can be used to predict homozygosity or heterozygous carrier status.

9.2.4 Review of genetic linkage in Epidermolysis Bullosa

9.2.4.1 EB Simplex Ogna

Gedde Dahl and Olaisen (1973) demonstrated close linkage between the loci of erythrocyte soluble glutamic-pyruvic transaminase (GPT) and **EBS Ogna.** The map distance was calculated to be approximately 5 centimorgans (cM). In males the distance was longer, 8 cM, than in females, 3 cM.

9.2.4.2 EB Simplex Koebner

Mulley (1984) studied linkage in a single family with **EBS Koebner.** There was a hint of linkage between this subtype and Duffy blood group on Chromosome 1. The lod score was 1,2 at $\theta = 0.2$.

Linkage between Fy and **EBS Weber Cockayne** was proposed Hauge (1962), and subsequently a lod score of 0,6 at $\theta = 0,2$ has been established by Gedde Dahl (1971).

There have been suggestions that the Koebner and Weber Cockayne types of EBS are allelic and this linkage may be a further evidence in favour of this theory.

9.2.4.3 EB Dystrophica

Weak linkage has been found in certain forms of EBD. A low

positive lod score was found between **EBD Cockayne Touraine** and IgK, the Kappa light immunoglobulin chain in one family (Gedde Dahl, 1971; Mulley, 1985). The same workers found a suggestion of linkage between **EBD Pasini** and Sr, the ABH secretor gene. Joensen (1979) found possible linkage between **EBD Bart** and PGM1, phosphoglucomutase-1.

Despite these weak associations, no firm evidence for linkage of any of the genes for EB Dystrophica has been established.

9.2.5 Genetic linkage and EB in South Africa

9.2.5.1 HLA and red cell enzymes

As was outlined in Chapter 5.2.1 and 5.2.2, no positive evidence for light linkage in EB was found.

Segregation of the A1B ^{8} haplotype with affected members in a large kindred with EB Simplex with mottled pigmentation was noted, with only 1 recombinant. Despite a lod score of 1,3, this is not significant as this is the most common haplotype in persons of mixed ancestry (Δ value = 0.0167; haplotype frequency = 0.0207).

Loose linkage is shown in the same family with the red cell enzyme Glyoxalase I-1 (Glo I-1) with a lod score of 0.124 was obtained from the third generation. No further information

can be gained from this pedigree however, as I-1 is homozygous for Glo I-1.

Studies of the family with EBD Pasini show that there is no linkage between HLA, red cell enzymes or proteins, and this form of EBD. The two families studied with **EBD Cockayne Touraine** were too small to derive statistical information regarding linkage. In the absence of positive linkage studies, this form of testing cannot contribute towards the prenatal diagnosis of EB.

9.2.5.2 Restriction Fragment Length Polymorphisms

Both the genes for collagenase and for RDEB have been assigned to Chromosome 11; Church (1983) concluded that the autosomal collagenase produced by RDEB cells was a structural mutation of the normal collagenase gene.

Studies of linkage in EB with the L_7 probe were non-informative. In three families the allele was not polymorphic.

In one family as demonstrated in Chapter 5.2.3, the allele was polymorphic but with only one affected member, it was impossible to draw any absolute conclusion from this data.

In one family it was not possible to determine linkage as all the offspring of these parents will be heterozygous. This is

because one chromosome concerned comes from each parent, who are different types of homozygotes.

The conclusion is that L$_7$, the probe that localises to 11q23, is a random polymorphism in this population and was not linked to the gene for RDEB, in this study.

TABLE 9-II

MUTATION RATES IN EB IN NORWAY

Mutation rate per gamete ($\times 10^{-6}$)

EB Subtype	1913-1962	1963-1979
EB Simplex Koebner	>0,18	0,96
EB Simplex Weber Cockayne		1,92
EB Simplex with mottled pigmentation		<0,48
EB Simplex Ogna		0,48
EB Herpetiformis Dowling Meara	>0,36	<0,48
EB Bart	>0,18	<0,48
EB Dystrophica Cockayne Touraine	>0,36	<0,48
EB Dystrophica Pasini	>0,36	<0,48

9.3 GENE FREQUENCY, CARRIER AND MUTATION RATE IN EB

Estimation of the gene frequency of EB as a whole, as well as the carrier rate in the autosomal recessive subgroups would be a meaningless figure; each major subtype has been considered in its frequency in Chapter 8, entitled Epidemiology, and to prevent repetition, shall not be discussed in this chapter.

Gedde Dahl has postulated mutation rates in various subtypes of EB based on retrospective screening 1913-1962 (2,74 million births) and the prospective study 1963-1979 (1,04 million births) in Norway. These figures are listed in Table 9-II.

In the EB Simplex category, two persons were sporadic, and a third was the first member of her three-generation family to be affected. Therefore three of the fourteen affected persons were probably fresh mutations. These persons differed in age from sixty-eight years to one week of age, and this fact, coupled with the probability of incomplete ascertainment and further heterogeneity, renders the accuracy of a calculated mutation rate meaningless in the South African context.

In the DDEB group there were inadequate numbers of persons who manifest mutations of an autosomal dominant gene, so that no accurate mutation rate could be calculated.

CHAPTER 10 THE HISTOPATHOLOGY OF EPIDERMOLYSIS BULLOSA

10.1 INTRODUCTION

The discussion of the histopathological changes in EB will be divided into the Simplex, Atrophicans, and Dystrophic EB groups. The established histopathological findings in EB will precede analysis of results of this study, which will be in the form of a "Comment". This investigation was undertaken in as many affected persons as was possible using election microscopy, as outlined in Chapter 3, Methodology. Light microscopy was used for survey sections as a step in preparation of ultra thin sections for electron microscopy, to orientate the specimen and ensure that the dermo-epidermal junction was isolated accurately.

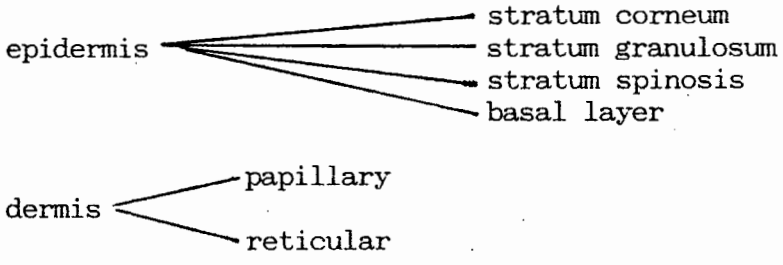
For the sake of clarity a description of the structure of normal skin precedes the review of the histopathological changes in EB.

10.2 NORMAL SKIN

The skin consists of two main regions; outermost, the epidermis, and inner, the dermis, with skin appendages projecting through these layers. The embryological derivation of the epidermis is primarily ectodermal, but cells of neural crest and bone marrow mesenchymal origin are also involved. The dermis is derived from the mesenchyme.

Proportionately, epidermis makes up 5% of the thickness of the skin, with dermis forming the major portion.

The layers of the skin are:-



This is shown diagrammatically by Figure 54, while Figure 55 depicts the dermo-epidermal junction.

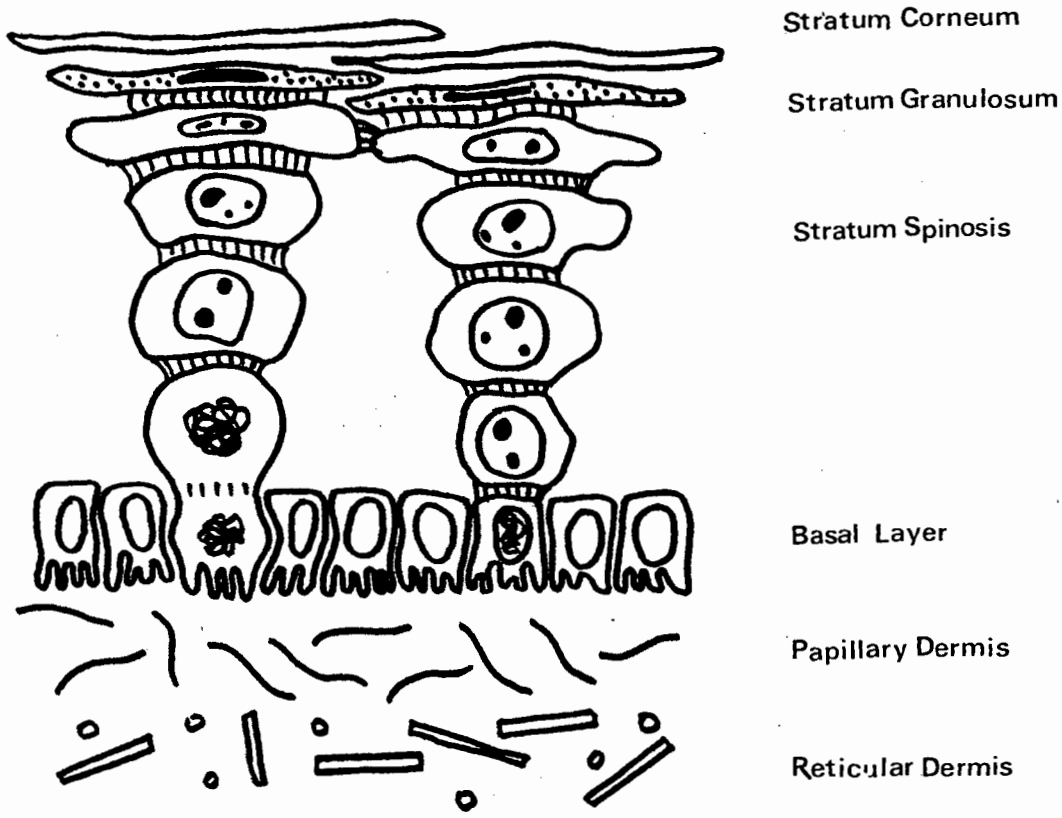


Figure 54. Diagramatic representation of the layers of the skin

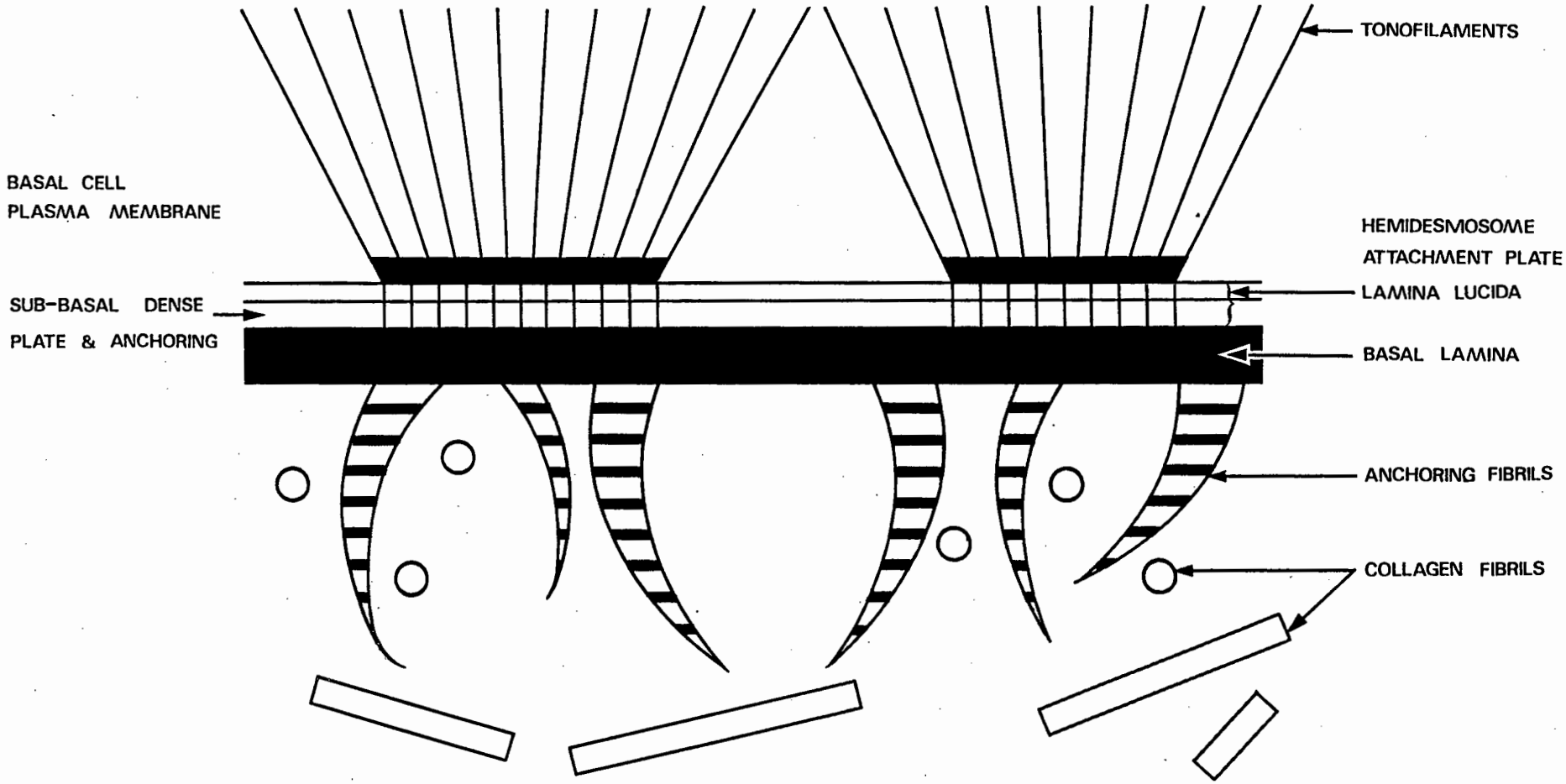


Fig. 5 5 DERMO – EPIDERMAL JUNCTION

10.2.1 The epidermis

Epidermis is keratinised, stratified squamous epithelium. This tissue is multilayered, consisting of cornified, granular, spinous and basal layers. The epidermis is in a state of progressive differentiation, replacing itself from epidermal stem cells, a process which takes 28 days to complete.

The basal layer comprises the epidermal stem cells and basal cells. Basal cells contain keratin and actin filaments, and microtubules, as well as other cell organelles, such as mitochondria and endoplasmic reticulum which are associated with cells undergoing metabolic activity. These basal cells are joined to each other by desmosomes; hemidesmosomes link them to the basal lamina below. The basal lamina is a continuous electron-opaque layer, 30-35 mm. thick. Hemidesmosomes comprise tonofilaments, the lamina lucida and anchoring fibrils. Tonofilaments are associated with the dense plate of the basal membrane and are filaments of keratin. The lamina lucida (lamina rara), which lies between the plasma membrane and the basal lamina (lamina densa) is crossed by anchoring filaments. Anchoring fibrils originate from the basal lamina and extend downward into the dermis (Figure 55).

Anchoring fibrils are a structural component of the dermo-epidermal junction, and are of particular importance in the pathogenesis of dystrophic EB. They range from 200-750 A in diameter and are cross-banded structures that have an alternating pattern of thick, dense and thinner, less dense bands. Proximally, they extend from 250-300 mm. inside the papillary dermis, while their distal ends insert into the basal lamina. The embryological origin of anchoring fibrils is dermal cellular elements; by contrast, the basal lamina originates from epidermis. Recent unpublished data (Burgerson) has suggested that Type VII collagen is the major structural component of the anchoring fibrils.

The spinous layer, or stratum spinosum, consists of 3-5 layers of differentiating cells. The number of desmosomes associated with these cells are greater than in the basal layer, as are numbers of keratin filaments. In the process of migration to the surface, flattening of the spinous cells occurs and ovoid lamellar granules develop. The next step of differentiation is into one or two layers of granular cells. This is the uppermost viable epidermal cell layer. The major cell content is keratohyalin granules within an interlacing network of keratin filaments. Large numbers of the lamellar granules are present at the cell border, particularly that border facing the adjacent cornified cells. These granules

release their contents into the extracellular space; this process results in the formation depositing sheets of lipid which play a role in the barrier function of the epidermis.

The cornified, or outermost layer, consists of flattened dehydrated cells in which the nuclei and cytoplasm have dissolved. Several layers of these cells, with the occasional transitional cell, form the stratum corneum.

10.2.2 The dermis

The dermis comprises a matrix in which connective tissue fibres are embedded. There are two main structural layers, papillary and reticular, the latter being the deeper.

The papillary dermis comprises fine collagen fibrils which are organised into small fibre bundles. These fibres are composed primarily of Types I and III collagen. Immature elastic fibrils, oxytalan fibres, are found perpendicular to the epidermis. This layer is the more cellular of the dermal layers, with an abundance of fibroblasts.

The reticular dermis consists of a pattern of coarse collagen fibrils of large diameter, consisting mainly of Type I collagen. Elastic fibres, elaunin fibres, which are more mature than oxytalan, and contain larger amounts of elastin matrix, are found in the upper part of this layer. Deeper than this are fully mature elastic fibres, in association with collagen fibres and sweat glands. This layer is less cellular than the papillary dermis.

In addition, appendages of the skin, viz. nails, hair follicles, sebaceous, apocrine and eccrine glands, as well as nerves and blood vessels, pass through the dermis.

10.3 EPIDERMOLYSIS BULLOSA SIMPLEX

Light microscopy

Light microscopy of an area of freshly blistered skin demonstrates a split in the epidermis at the level of the basal cells. This is not well defined and light microscopy alone is insufficient for the histologic differentiation of EB.

Electron microscopy

Blister formation begins in the subnuclear region of the basal layer of the epidermis.

The blister floor consists of the basal cytoplasm of the basal cells and the dermo-epidermal junction. The hemidesmosomes, basal lamina and anchoring fibrils are unaffected, as are melanocytes and Langerhans cells and only occasional damaged pyknotic cells are observed. The blister roof consists of the epidermis and stratum corneum.

By contrast to the bullae of EBS, the blisters caused by exaggerated friction in normal persons are found in the upper epidermis, in the upper or mid-portion of the stratum spinosum, developing from perinuclear intracellular oedema of the Malpighian layer. Pearson (1971) reported blisters in **EBS Weber Cockayne** in the upper spinous epidermis. It was for this reason that the Weber Cockayne subtype, in which localised lesions occur, was thought to be an exaggeration of the blistering induced by friction to the skin of healthy persons.

Various workers have examined the blistered skin in the various types of EBS. It is now widely accepted that the ultrastructural changes in the skin are identical in the **Koebner** and **Weber Cockayne** types of EBS (Haneke and Anton Lamprecht (1982), Pearson (1921)). In both of these subgroups undamaged skin is histologically normal.

Niemi (1983) described a new form of EBS with intra-epidermal cytolosis and "clumping" of tonofilaments. These clumps are seen as large irregular darkly staining masses. The hemidesmosomes and basal lamina are unaffected. There is a degree of cellular irregularity above the basal membranes, where macrophages or Langerhans cells are seen. Keratinocytes showed clumping of tonofilaments and pyknotic nuclei. Above

the level of cleavage, keratinocytes have cytolytic cytoplasm with swollen rough endoplasmic reticulum and degenerative mitochondria. The tonofilaments are retracted and clumped, and desmosomes are missing. Other cells above the cleavage are severely damaged. Epidermal regeneration is seen as areas of increased cellular mitosis.

In the **EBS with mottled skin pigmentation**, the bullae form by cytolysis in the hyperpigmented basal cells. The blister floor is suprabasal, and its roof comprises spinous epidermis. Pigmented epithelial cells above and below the blister cavity can be demonstrated with melanin staining. Undamaged skin from persons affected in this way is is ultrastructurally normal.

The histopathologic changes in **EBS Ogna**, which is associated with a tendency to bruising, are undistinguishable from the more classical subtypes.

Specific findings may be associated with **EB Simplex Herpetiformis Dowling Meara.** Areas of skin without lesions, as in other Simplex forms, have normal epidermis. Cytolysis of basal cells is preceded by tonofilament clumping. The blister floor consists of the connective tissue of the papillary dermis. Numerous inflammatory cells predominantly

eosinophils and mast cells may be associated with the blister floor. The borderline of the blister cavity is an intact dermo-epidermal junction. The basal lamina, anchoring fibrils, basal cells, plasma membranes and hemidesmosomes are unaltered and the split usually occurs above the hemidesmosomes. The whole epidermis forms the roof of the blister. All cell layers of the epidermis are flattened in shape, but the blister cavity is filled with serous fluid and cellular debris.

The undersurface of the basal cell layer, which forms part of the blister cavity, may be altered; cytoplasm, plasma membrane, basal lamina and hemidesmosomes are destroyed, possibly on a basis of inflammatory reaction. It is from this involvement that scarring may result.

The other non-scarring form of EB, type **Mendes da Costa**, which is associated with dyspigmentation and microcephaly, has similar intra-epidermal blister formation. There is cytolysis of basal cells and the plasma membrane is disrupted, while basal lamina, hemidesmosomes and anchoring fibrils are unaltered.

COMMENT

Those persons in this study with **EB Simplex Koebner** and **Weber Cockayne** showed the histological changes appropriate for their clinical subtype. None had inconsistent features or newly recognised changes.

In the family with **EBS with mottled pigmentation** the basal cells were highly pigmented as a racial variant - it was at this level that blistering occured. Neither of the affected persons with **EBS Dowling Meara** had fresh bullae present at the time of examination. The skin samples were taken from an area freshly traumatised so that it is not surprising that electron microscopy did not show the tonofilament clumping or inflammatory infiltrate that has been described in this subtype. The clinical features are however classical of this subtype and the diagnosis was confidently made on these grounds.

10.4 EPIDERMOLYSIS BULLOSA ATROPHICANS

The characteristic ultrastructural changes in the atrophicans forms of EB have led to the alternative nomenclature, **Junctional EB.** The plane of cleavage of blistering in these forms is in the lamina lucida, above the basal lamina. By contrast, in the simplex forms blister formation is intra-epidermal, while the plane of cleavage is below the basal lamina in the dystrophic types.

The blister roof is formed by the basal cells, in which perinuclear oedema and numerous vacuoles occur. Tonofilaments are disrupted by the large vacuoles in the basal keratinocytes. Desmosomes are unaltered. The blister floor consists of the basal lamina. During cleavage, portions of basal cell plasma membrane and cytoplasm may remain attached, by hemidesmosomes, to the basal lamina. Anchoring fibrils as well as dermal collagen are unaltered.

In accordance with the great degree of heterogeneity in EB there is marked variability in the ultrastructural changes in junctional EB. A significant number of patients have been reported where, despite poor dermo-epidermal adhesion, the number and structure of hemidesmosomes are within normal limits.

In addition to these changes, microcytic vesicles may be seen between the hemidesmosomes, in association with the plasma membrane of basal keratinocytes. The hemidesmosomes are the key structures in junctional bulla formation. Both hypoplasia and decrease in the number of hemidesmosomes have been reported.

Despite the variability observed in the electron microscopic findings in EBA, there are no significant differences between the subtypes, notably the gravis, or so-called lethal form, or the more benign mitis subtype.

Characteristic changes are seen in the skin of foetuses with EBA, and prenatal diagnosis has been performed successfully in foetal skin samples from the 16th gestational week. Embryology of the skin, and the histopathology of foetal EB will be discussed more fully in Chapter 14 in which prenatal diagnosis is described.

Comment

All skin samples examined from persons with EBA in this study showed the identical changes. Bullae were observed in the lamina lucida, with the intact basal lamina as the blister floor. The hemidesmosomes were hypoplastic in all cases.

These findings were constant in the infants who had died as a result of **EBA Generalisata Gravis** as well as the child who had survived this subtype, and the girl with **EBA Generalisata Mitis.** In addition the foetus who was aborted on grounds of Turner Syndrome, who had coincidental EBA had similar histologic changes.

10.5 EPIDERMOLYSIS BULLOSA DYSTROPHICA

The scarring or dystrophic forms of EB are divided into those which are dominantly and recessively inherited, and the ultrastructural changes of each subtype within the broader groups will be described. Light microscopy in all forms of EBD shows subepidermal blister formation - no detailed information about the dermoepidermal junction can be obtained without electron microscopy.

10.5.1 Autosomal Dominant EB Dystrophica (DDEB)

The dominantly inherited form of Epidermolysis Bullosa Dystrophica has been described as 'anchoring fibril disease'. These fibrils are the key structural component in the pathogenesis of DDEB. Their structure has been described earlier in this section, in the description of normal skin.

Electron microscopic examination demonstrates that the level of cleavage is in the dermis, deep to the basal lamina. There is a decrease or absence of normal anchoring fibrils in the junctional area below the basal lamina. Those present are rudimentary fibrils which are small, thin and lack the typical banding pattern. Other junctional structures are unaltered, including the plasma membrane, hemidesmosomes, anchoring filaments and the basal lamina.

The blister floor is composed of dermis, with no structural alterations in collagen or elastic tissue, or the cellular components of the dermis. The roof of the blister is the basal lamina. Unblistered skin and skin from non-predilection sites are ultrastructurally normal.

The commonest types of DDEB are the **Cockayne Touraine** type, where lesions are confined to the dorsum of extremities, and the **Pasini** or **albopapuloidea** subtype. The latter is characterised by so-called albopapuloid patches and areas affected are mainly the limbs, but the face and buttocks are often affected in infancy.

Bart Syndrome is a autosomal dominant form of EB Dystrophica associated with congenital absence of the skin. The histopathologic findings in areas with bullous lesions are absence of normal anchoring fibrils. The level of dermo-epidermal separation is immediately below the basal lamina. Other skin structures are unaffected.

The fourth, very rare type of DDEB is **pretibial.** The ultrastructural changes of this form parallel those of the other subtypes.

Comment: The histopathological changes of bullous lesions were identical in all persons studied with DDEB, whether **EBD Cockayne Touraine** or **EBD Pasini**. Bullous lesions were subepidermal and anchoring fibrils were decreased in number and structurally abnormal. Depigmented scars examined in this way had the classical histological changes predicted.

Undamaged skin in **EBD Cockayne Touraine** and **EBD Pasini** was ultrastructurally normal.

10.5.2 Autosomal Recessive EB Dystrophica (RDEB)

In the autosomal recessive dystrophic forms, as with the dominantly inherited forms, the level of cleavage is in the dermis below the basal lamina (Anton Lamprecht 1973, 1978). Where DDEB has been named, for simplification, the anchoring fibril disease, RDEB has been labelled as collagenolytic disease. There is, however, considerable overlap between DDEB and RDEB, where the level of cleavage in the formation of bullae is subepidermal.

In an area of blistering, the separation occurs in the dermis, just below the basal lamina. Electron microscopists in the past have suggested that the level of separation may vary, and reports of a split in the intermembranous space have been made

(Vogel and Schyder 1967; Kobayasi 1979). The alterations in skin structure in fresh blisters, as well as experimentally induced bullae, are identical. Separation deep to the basal lamina results in a blister cavity in the area normally occupied by anchoring fibrils. The roof of the blister is basal lamina and attached dermal material, while its floor is composed of intact collagen fibres and other dermal structures.

There is an absence or diminution of normal anchoring fibrils. Pearson (1962, 1971) has long published reports of dissolution of collagen fibres, while Hashimoto (1976) described collagenolysis in blister formation. Collagen degradation may be extensive; however, dermo-epidermal separation may occur irrespective of collagen degradation.

Similar changes are seen in the non-traumatised skin of persons with RDEB. The most significant abnormality is an absence of anchoring fibrils; those present appear abnormal, with a lack of their characteristic cross-banding pattern. The dermo-epidermal junction is intact in these undisturbed areas, and dermal collagen is unaffected. In unaffected and blistered skin, all structures superficial to the basal lamina are normal.

These changes are common to all forms of **RDEB Hallopeau Siemens**, of the localised, generalised or mutilans type. The ultrastructure in **EBD Inversa**, which varies clinically from the **Hallopeau Siemens** subtype, is indistinguishable on electron microscopy from the latter type.

Autosomal recessive **Epidermolysis Bullosa Progressiva**, a very rare subtype with late onset and possible association with deafness, has subepidermal blister formation, consistent with its dystrophic nature. Changes of papillary collagen and loss of superficial elastic fibres are noted. The lamina rara is widened, and electron microscopy reveals deposits of amorphous material within the lamina rara.

Comment: The ultrastructural changes of those persons with RDEB whether of the **Hallopeau Siemens** or the newly described **RDEB Generalisata Gravis**, were consistent with the abnormalities described above. The level of change was subepidermal with consequent rearrangement of collagen below the blister cavity. The most striking abnormalities were those of the anchoring fibrils and these were irrespective of bullae. These structures were markedly reduced in number and where present were slender and peg like. By contrast to normal anchoring fibrils there was no tendency of these to turn back toward the basal lamina. In addition, alterations of the cross banding pattern were visible at high magnification.

10.6 DISCUSSION

The histopathological findings in this study conform to the well-established changes as described in the preceding literature review. Since the resources for electron microscopy are limited, accurate ultrastructural diagnosis can only be offered at the larger referral centres.

Electron microscopic examination of the skin is essential to differentiate the subtype especially where the precise clinical diagnosis is unclear. This is of overwhelming importance in the neonatal period, where scarring and signs of dystrophy have not yet developed and only histology can differentiate between the major subtypes, Simplex, Atrophicans and Dystrophic. Such a specific diagnosis is necessary, in order to establish, in the first instance, prognosis for the infant. Furthermore, treatment in the newborn period may alter depending on the subtype. (These points will be discussed more fully in the section designated 'Management'.)

Precise delineation of the type of EB is important to predict the pattern of inheritance and consequent risk factors for recurrence of EB in any family. It is where the presentation of EB is sporadic that a histological diagnosis can be of great assistance. Where the mode of inheritance is known in a subgroup, most notably in EB Simplex or EB Atrophicans, the chances of recurrence can be calculated according to the usual risk ratios.

BIOPSY TECHNIQUES

In order to achieve good ultrastructural differentiation, a representative skin specimen is essential. Non-traumatised skin, except in RDEB, will not consistently show the changes of EB, and the interpretation of results may be difficult. The optimal study is of an area at the edge of a freshly formed bullous lesion, comprising the junction of normal and blistered skin.

Immediate fixation, after washing the skin sample in phosphate buffered saline, yields the most accurate results. For clear distinction of ultrastructure, an ideal time is two hours in fixative for sections not greater than one-and-a-half millimetres in size; penetration of the specimen by fixative media is a distance of one millimetre.

The technique outlined in 3.3.1 (Methodology) for the removal of skin samples is easy and prevents trauma to the specimen. In this way artefactural histological changes may be minimised.

ULTRASTRUCTURAL CHANGES

The correlation of clinico-pathologic data confirmed the diagnoses in the difficient subtypes of EB. Only intra-epidermal blistering was seen in the Simplex forms. When the skin of patients with EBS was intact, no abnormalities were noted. The biopsy of the edge of a fresh blister demonstrated basal cell cytolysis and a cleavage plane within the basal layer of the epidermis in all persons examined in this way.

Electromicroscopic examination of patients with junctional EB Atrophicans revealed the pathognomonic changes. A split between the plasma membrane of the basal layer and the basal lamina, in the lamina lucida was apparent at the edge of a bullous lesion. The hemidesmosomes were hypoplastic and reduced in number. As predicted, the ultrastructural changes in the child who had survived the so-called 'lethal' form of EBA, were identical to those of the infants similarly affected, who had died. The single prenatal examination of skin, in the form of an autopsy of a foetus aborted for unrelated causes, demonstrated the typical findings of EBA, at 16 weeks gestation.

All persons studied with EBD had subepidermal blistering. The basal lamina formed the roof of the blister while its floor comprised dermal collagen. No defects in this dermal collagen were observed in RDEB nor DDEB. The anchoring fibrils were the most consistently abnormal structures. These were diminished in number, ranging from minimal lessening to almost complete absence. Anchoring fibrils, when present, were structurally deficient. They were smaller and shorter and in most instances were straight and peglike, not turning back towards the basal lamina as in normal skin. A lack of the usual crossbanding pattern of these structures was a non-constant finding.

EB Dystrophica remains something of an enigma to the electron microscopist. Skin sampled from the blistered skin of a person with EBD will yield similar results irrespective of the subtype of EBD or the pattern of inheritance. It is widely agreed that it is impossible therefore to differentiate on skin biopsy between recessive and dominant dystrophic forms of EB. In those persons with a mild dystrophic EB in the absence of a positive family history of EB, one is not able to discern whether this is indeed recessive inheritance, or a fresh mutation for DDEB. This differentiation has great significance in terms of prognosis and genetic counselling.

A skin sample of unblistered tissue may yield some information. Anchoring fibril abnormalities may be seen in the so-called normal skin in RDEB and some forms of DDEB. In the dominantly inherited **EBD Cockayne Touraine**, i.e. that form localised to the distal extremities, ultrastructure of skin in non-predilection areas is within normal limits. This finding may be of assistance in confirming the clinical diagnosis of **EBD Cockayne Touraine** in the sporadic dystrophic person.

CHAPTER 11 BIOCHEMISTRY

A spectrum of biochemical investigations has been carried out in the past in the various forms of EB. In the chapter concerning the 'historical' aspects of EB, earlier work in the field of biochemistry is discussed. Recently many areas have been explored and the salient biochemical findings in the different subtypes will be discussed according to type, as in the previous sections.

11.1 EB SIMPLEX

A deficiency of galactosyl hydroxylysyl glucosyl transferase (GGT) has been found in certain families with EB Simplex (Savoleinen 1981). Accompanying this deficiency is a marked decrease in the urinary excretion of glucosyl galactosyl - hydroxylysine. GGT is an enzyme which catalyses the synthesis of glucosyl galactosyl hydroxylysine in collagen by transferring the monosaccharide unit from uridine diphosphate glucose (UDP) to galactosyl hydroxylysyl residues. This reaction which occurs in rough endoplasmic reticulum requires a bivalent cation, preferably manganese. GGT is also present in the serum and enzyme activity can be measured in skin and cultured skin fibroblasts, as well as in serum.

11.2 EB ATROPHICANS

Collagenase, which has been proven to have increased activity in REBD, has been implicated as a part of the basic defect in EB Atrophicans or functional EB. However, evidence for this is tenuous, with many workers reporting normal collagenase levels in skin fibroblasts of such patients. Suggestion has been made that this increased activity is in fact a secondary response to chronic wound healing.

It has been demonstrated that Phenytoin, a drug used primarily in the treatment of epilepsy, decreases the activity of collagenase. The use of this drug under experimental conditions has therefore been fairly widespread, mainly in RDEB, but several workers have attempted treatment of EBA in this way. This approach has been largely unsuccessful, as would be anticipated in the absence of increased collagenase activity. The subject of Phenytoin therapy will be dealt with more fully in the sections concerning the management of EB.

11.3 EB DYSTROPHICA

For the purpose of discussion, EB Dystrophica will be divided, as in previous chapters, into the autosomal dominant and autosomal recessive subgroups.

11.3.1 Dominant EBD

In **EBD Albopapuloidea (or Pasini)**, glycosaminoglycan metabolism of cultured fibroblasts is deranged. A two-to-threefold increase in sulphated glycosaminoglycans is the consequence of accelerated synthesis (Bauer 1979). There is no evidence for defective degradation of the material. It has been postulated that accumulation of excessive amounts of glycosaminoglycans may alter collagen fibril deposition; this may render the epidermo-dermal junction structurally defective and predispose to bulla formation.

Increased excretion of partially degraded chondroitin sulphate B in the skin and urine has been reported in EBD Pasini (Endo 1974). There was a decrease in urinary excretion of chondroitin sulphates C and A. This led to the suggestion that the basic defect in this form of EB might be an abnormality of the catabolism of acid mucopolysaccharide. The accumulation of this substance is specific to the albopapuloid variety of EB, and does not occur in RDEB or the other form of DDEB.

The finding of increased glycosaminoglycans has important prognostic implications especially in sporadic cases in infancy or early childhood, because the classical albopapuloid lesions only occur in late childhood or early adolescence.

No specific biochemical abnormalities have been found in DDEB in the Cockayne-Touraine type, or in Bart syndrome.

11.3.2 Recessive Dystrophic EB

The most significant biochemical marker is present in RDEB. Cultured fibroblasts from the skin of subjects with RDEB have been found, in vitro, to have a fourfold greater concentration of collagenase than normal controls.

At the ultrastructural level, an association between the blistering process with the destruction of collagen in the papillary dermis has been suggested possibly on the basis of a protease mediated process. (Eisen 1969; Lazarus 1972; Takamori 1983). When the skin from a healthy subject was cultured with the fluid derived from the blister of a person with RDEB, a blister was formed with similar ultrastructural changes to those found in RDEB. The specific factor inducing such blister formation was inactivated by heat (60 °C) for the duration of 30 minutes and by trypsin digestion. When treated with EDTA, EGTA, α_2 macroglobulin, soybean trypsin inhibitor (SBTI) and N-ethyl maleimide (NEM), this factor was inactivated. Dialysis did not have this effect. The

conclusion drawn from this information was that the possible factors responsible for bullous formation in RDEB blister fluid included collagenase, neutral thiol protease and trypsin-like protease. (Takamori 1983)

Collagenase is the enzyme which initiates degradation of collagen in the skin, in its fibrillar form or intact tissue collagen. Collagenase was first detected in skin by Fullmer (1966) and later characterised by Eisen (1968,1969). Human skin collagenase has a pH optimum between 6 and 7,5. It is inhibited by EDTA and cysteine. It is produced largely by the papillary dermis, with minimal quantities in the epidermis and no activity in the deep dermis. Woolley (1973) suggested that the molecular weight of collagenase was 63 000.

Collagenase has been found to be released in large amounts in culture from the margins of healing wounds (Grillo & Gross 1967). The enzyme was originally thought to be produced by epithelial and mesenchymal cells; however, it was later demonstrated that different properties are exhibited by the enzymes from the two sources. It was on this basis, however, that it was suggested that collagenase played a role in wound healing.

There is a two-to-fourfold increase in the capacity of RDEB skin fibroblasts to synthesise collagenase, as compared with normal controls. This is reflected in such an increase in

accumulated immunoreactive collagenase in the culture medium of the fibroblasts in a 24 hour period. There is also an increase in activation of the enzyme by trypsin. Neither total protein synthesis nor the growth kinetics of the cultured cells are altered, and it is postulated that increased collagenase synthesis is a specific property of the RDEB skin fibroblasts (Stricklin 1982; Ehrlich 1984).Other enzymes, notably lactic dehydrogenase and B-glucuronidase are unaltered.

Procollagenases from RDEB fibroblasts show a significant reduction in thermal stability and an increase in the apparent K_M for Ca^{2+}, the thermal stabilising metal cofactor for collagenase. These factors are highly suggestive of a mutant form of collagenase in persons with RDEB.

Scanning electron microscopy and phase contrast optics have demonstrated that RDEB fibroblasts within a collagen matrix have a dendritic appearance (Ehrlich 1983). These fibroblasts can be grown and cultured in collagen lattices. By contrast, normal fibroblasts grown similarly have an elongated and bipolar 'spread-out' appearance. Fibroblasts when populating a collagen matrix induce lattice contraction, thus reducing the size of that matrix. Fibroblasts from a patient with RDEB are unable to spread out and therefore lack the ability to contract the collagen lattice.

Synovial fibroblasts have dendritic morphology; these dendritic cells produce increased amounts of collagenase and prostaglandin E_2 (PGE_2). It is possible, therefore, that RDEB fibroblasts which have increased collagenase synthesis may have parallel increases in PGE_2. On the basis of excess collagenase and prostaglandin E_2 production in RDEB fibroblasts, intracellular cyclic Adenosine Monophosphate (cAMP) synthesis is enhanced. RDEB fibroblasts in collagen lattices have been shown to have an eightfold increase in intracellular cAMP concentration, as compared with their normal counterparts.

Cholera toxin or dibutyryl cAMP, when added to normal fibroblasts, increase the cAMP levels; this inhibits cell elongation and spreading, and the resultant lattice contraction. RDEB fibroblasts have an identical pattern of action to that of unaffected fibroblasts which have been cultured with cholera toxin or dibutyryl cAMP. Elongation and spreading of cells is induced by the same forces. In RDEB cells, intracellular cAMP levels can be reduced by indomethacin, a non-steroidal anti-inflammatory drug, resulting in normal fibroblast-populated collagen matrices, and consequent normal lattice contraction; the RDEB fibroblasts which cannot elongate and spread do not otherwise have this ability.

An additional biochemical finding in RDEB has been increased plasma carcino-embryonic antigen (CEA) levels. CEA is a high molecular weight glycoprotein produced by tumours of endodermal origin, as well as foetal gastro-intestinal tissue. This so-called oncofoetal antigen may be used as a marker for intestinal malignancy, but has also been noted in chronic inflammatory processes. This increase in CEA is specific to RDEB. Normal levels of the antigen are found in the simplex, atrophic and dominant dystrophic forms of EB.

11.4 COMMENT

In this study, collagenolytic activity of the cultured fibroblasts of persons with RDEB was assayed. No significant results were obtained, largely due to technical and laboratory problems. Initially, plates were constructed with too little collagen which decreased the sensitivity of the assay. There was therefore a decrease in the release of collagenase and no accurate measure of collagenolytic activity. The disintegrations per minute (DPM) of the normal controls were comparable to those of the patients. This assay was repeated with an increased collagen content with some improvement in sensitivity.

Collagen is released by a latent enzyme and this process must be activated by a proteolytic enzyme. Trypsin and plasmin serve this function in vivo, and in vitro, trypsinisation activiates collagen release.

The overriding complication in attempting this assay was a recurrence of infection, both bacterial and fungal, to tissue culture. This constantly destroyed cells and made the assay impossible to complete.

SECTION V

NOSOLOGY OF EPIDERMOLYSIS BULLOSA

CHAPTER 12 CLASSIFICATION OF EPIDERMOLYSIS BULLOSA

CHAPTER 12 CLASSIFICATION OF EPIDERMOLYSIS BULLOSA

12.1 CLASSIFICATION OF EB

A major objective of this study is to clarify the classification of EB and to simplify the diagnosis of this uncommon disorder. To date, 26 different subtypes of EB have been reported. These fall into the major subgroups, namely, EB Simplex, EB Atrophicans and EB Dystrophica, and are subdivided according to varying degrees of clinical, genetic, histopathological and biochemical differences.

Listed below (Table 12-I) are the recognised entities. Their names and distinguishing features are listed, together with the author and date of description. Tabulation of the subtypes will be according to the major groups, in accordance with other sections of this thesis.

This table lists 26 subtypes of EB. There is considerable overlap between several of the minor subtypes and the possibility exists that each of these forms may not in fact be an autonomous entity.

TABLE 12-I Recognised entities in EB

EB Subtype

Epidermolysis Bullosa Simplex (EBS)	Distinguishing Features	Author	Date
1. EBS Koebner	Generalised lesions	Koebner	1886
2. EBS Weber Cockayne	Lesions localised to extremities only	Weber & Cockayne	1926; 1938
3. EBS with GGT deficiency	Deficiency of the enzyme galactosyl hydroxylysyl glucosyl transferase	Gedde Dahl	1970
4. EBS Ogna	Bruising associated with blistering	Gedde Dahl	1971
5. EB Herpetiformis Dowling Meara	Herpetiformis grouping of lesions	Dowling & Meara	1954
6. EB with mottled pigmentation	Patchy dyspigmentation of skin	Fischer & Gedde Dahl	1979
7. EB Mendes da Costa	Macular lesions; X-linked recessive inheritancy	Woerdemann	1958
8. EBS Niemi	Intra-epidermal cytolysis and tonofilament clumping	Niemi, Kero et al.	1983
9. EBS, lethal type	Death in infancy; recessive inheritance	Salin	1985

EB Subtype Epidermolysis Bullosa Atrophicans (EBA)	Distinguishing Features	Author	Date
10. EBA Generalisata Gravis	Lethal in infancy	Herlitz; Pearson	1935; 1962
11. EBA with pyloric atresia	As 9., but with pyloric atresia	Bull	1980
12. EBA Generalisata Mitis	Benign form of 9.	Schnyder & Anton Lamprecht	1979
13. EBA Inversa	Distribution of lesions in 'inversa' areas	Gedde Dahl	1981
14. EBA Localisata	Lesions on shins and soles only	Schnyder & Anton Lamprecht	1979
15. Generalised atrophic benign (GABEB)	Atrophic alopecia	Hintner & Wolff	1982
16. EBA with muscular atrophy	Muscular atrophy	de Weerdt	1972
17. EB Junctionalis, Disentis type	Adult form of 9.	Hashimoto	1976

EB Subtype Epidermolysis Bullosa Dystrophica (EBD)	Distinguishing Features	Author	Date
Dominant EBD			
18. EBD Cockayne Touraine	Lesions localised to extremities; malignancy in scars	Cockayne; Touraine	1933; 1942
19. EBD Pasini	Albopapuloid patches	Pasini	1928
20. EBD Pretibial	Late onset; pretibial lesions	Kuske	1946
21. EBD Bart	Bullae with cutis aplasia	Bart	1966
Recessive EBD			
22. EBD Inversa	Lesions in 'inversa' distribution ‡	Gedde Dahl	1971
23. EBD Progressiva	Late onset; hypoacussis	Gedde Dahl	1970
24. EBD Hallopeau Siemens (Localised, Generalised, Mutilans)	Classical scarring EB	Hallopeau; Siemens	1904; 1921
25. EBD Fine	Progressive symmetrical centripetal bullae	Fine	1985
26. EBD Generalisata Gravis	Alopecia; growth retardation	Winship	1985

‡ Inversa areas are groins, axilla, trunk, neck, peri-anal and perivulvar.

12.1.1 Traditional classification

Traditionally, EB has been divided into non-scarring and scarring epidermolyses (Gedde Dahl 1971) and below is the classification proposed by Schnyder and Anton Lamprecht in 1977:

Classification of inherited epidermolyses

NON-SCARRING EPIDERMOLYSES

AUTOSOMAL DOMINANT TYPES

Epidermolysis bullosa simplex Kobner

Epidermolysis bullosa simplex Weber and Cockayne

Epidermolysis bullosa simplex 'Ogna' Gedde-Dahl

Epidermolysis bullosa simplex with mottled pigmentation Fischer and Gedde-Dahl

Epidermolysis bullosa simplex type Bart

Epidermolysis bullosa herpetiformis Dowling and Meara

AUTOSOMAL RECESSIVE TYPES

Epidermolysis bullosa atrophicans generalisata gravis Herlitz

Epidermolysis bullosa atrophicans generalisata mitis Hashimoto, Schnyder and Anton-Lamprecht

Epidermolysis bullosa atrophicans localisata Schnyder and Anton-Lamprecht

Epidermolysis bullosa atrophicans inversa Anton-Lamprecht and Gedde-Dahl

Epidermolysis bullosa progressiva (sive neurotrophica) Gedde-Dahl

X-LINKED RECESSIVE TYPE

Dystrophia bullosa hereditaria typus maculatus Mendes da Costa, van der Valk and Woerdemann

SCARRING EPIDERMOLYSES

AUTOSOMAL DOMINANT TYPES

Epidermolysis bullosa dystrophica Pasini

Epidermolysis bullosa dystrophica Cockayne and Touraine

AUTOSOMAL RECESSIVE TYPES

Epidermolysis bullosa dystrophica Hallopeau and Siemens

Epidermolysis bullosa dystrophica inversa Gedde-Dahl

Gedde Dahl (1981) classified those 16 types of EB which had been delineated diagrammatically according to the cleavage planes in relation to the dermo-epidermal junction. This histologic grouping paralleled the major clinical subgroups.

12.1.2 Proposed Classification

The concept of **major** and **minor** subtypes is proposed. Additional information may be added to the basic framework of the major subgroups to expand this classification to incorporate the details of the minor subgroups.

MAJOR SUBTYPES

1. Non-scarring EB : **EB Simplex**
2. Non-scarring EB with skin atrophy : **EB Atrophicans**
3. Scarring EB : **EB Dystrophica** < autosomal dominant / autosomal recessive

This subcategorisation is of great importance, particularly in terms of prognosis and genetic counselling, where long-term survival and recurrence risks of the disorder are fairly well established. The clinical signs, however, are not easily differentiated in the neonatal period, at a time when accurate prognostication is required. It is not until later that the scarring and dystrophies associated with EBD occur. For this reason, the histopathological confirmation of the subtype is necessary at the outset. The basic ultrastructural changes of the major subgroups are therefore added to the "skeleton" classification.

1.	Non-scarring	**EB Simplex**	Intra-epidermal cleavage
2.	Non-scarring	**EB Atrophicans**	Junctional cleavage
3.	Scarring	**EB Dystrophica**	AD Subepidermal blistering
			AR Subepidermal blistering

MINOR SUBTYPES

Additional clinical, genetic, biochemical and histological details specific to certain forms of EB differentiate the major subgroups into minor subtypes.

Presently, 26 minor subtypes have been delineated as specific entities (Table 12-II)

A stepwise set of classifying tables has been constructed in order to outline the subtypes of EB. These tables range from the basic subdivisions and culminate in a comprehensive tabulation of the salient details of each form of the disorder.

Table 12-II differentiates the subtypes according to the presence of scarring, nail dystrophy and the mode of inheritance.

TABLE 12-II SUBTYPES OF EB OUTLINING CLINICAL AND GENETIC DATA

Subtype	Scarring	Nails	Inheritance
A. SIMPLEX			
1. EBS Koebner (EBSK)	Nil	-	AD
2. EBS Weber Cockayne (EBSWC)	Nil	-	AD
3. EBS with GGT deficiency	Nil	-	AD
4. EBS Ogna	Nil	-	AD
5. EBS with mottled pigmentation	Nil	-	AD
6. EBS Herpetiformis Dowling Meara	Nil	Subungal blisters with normal regeneration of nails	AD
7. EBS Niemi	Nil	-	AD
8. EBS, lethal type	Nil	-	AR
9. EB Mendes da Costa	Nil	-	XR

Subtype	Scarring	Nails	Inheritance
B. ATROPHIC (Junctional)			
10. EBA Generalisata Gravis	Atrophy rare	Clubbing	AR
11. EB Letalis with pyloric atresia	Atrophy rare	-	AR
12. EBA Generalisata Mitis	Skin atrophy	Clubbing	AR
13. EBA Inversa	Skin atrophy	-	AR
14. EBA Localisata	Atrophy	-	AR
15. Generalised atrophic benign EB (GABEB)	Atrophy	-	AR
16. EBA with muscular atrophy	Atrophy	-	AR
17. EB Junctionalis, Disentis type	Atrophy	-	AR
C. DYSTROPHIC			
18. EB Dystrophica Cockayne Touraine	Atrophy, scarring and milia (A,S,M)	Dystrophic	AD
19. EBD Pasini	A,S,M	Dystrophic	AD
20. EBD Pretibial	A,S,M	-	AD
21. EBD Bart	A,S,M	Thumb and big toe	AD
22. EBD Inversa	A,S,M	Dystrophic	AR
23. EBD Progressiva	A,S	-	AR
24. EBD Hallopeau Siemens	A,S,M	Dystrophic	AR
25. EBD Fine	A,S,M	Dystrophic	AR
26. EBD Generalisata Gravis (Winship)	A,S,M	Early shedding	AR

Additional clinical concomitants specific to the different subtypes are listed in Table 12-III. It is believed that EBA with muscular atrophy (16) may be a variant of EBA Inversa (13). Types 12 - EBA Generalisata Mitis, 15 - Generalised Atrophic Benign EB, and 17 - EB Junctionalis Disentis type are all atrophic types where survival into adulthood is usual. However, in the original report of the Disentis type, the proband had two siblings who had died in infancy as a result of EB, and it is more likely that EB Disentis is allelic with Type 10, EBA Generalisata Gravis. There are fewer areas of overlap in the Simplex and Dystrophic forms of EB in which each have specific distinguishing clinical features.

In addition to the clinical observations, the salient histopathological features are tabulated in each subtype. The patterns of inheritance as well as the prognosis for a particular subtype are listed. A comprehensive table of data relating to every aspect of EB is thus presented as Table 12-III.

TABLE 12-III COMPREHENSIVE TABLE OF DATA IN EB

	SUBTYPE	AGE OF ONSET	SCARRING	NAILS	TEETH	INHER-ITANCE	LIFE EXPECTANCY	DISTRIBUTION OF LESIONS	CLINICAL CONCOMITANTS	ELECTRON MICROSCOPY
A.	NON-SCARRING (SIMPLEX) FORMS									
1.	EB Simplex Koebner (EBSK)	Birth	Nil	-	-	AD	Good	Generalised	Worse in Summer	Intra-epidermal blistering
2.	EBS Weber Cockayne (EBSWC)	Walking	Nil	-	-	AD	Good	Hands and feet	Worse in Summer	Basal cell vacuolisation
3.	EBS with GGT* deficiency	Birth	Nil	-	-	AD	Good	Generalised	-	Dissolution of tonofibrils
4.	EBS Ogna	Birth	Nil	-	-	AD	Good	Generalised	Onygrophosis big toes; bruising with blisters	Blistering with cytolysis of basal cells above hemidesmosomes
5.	EBS with mottled pigmentation	Birth	Nil	-	-	AD	Good	Mainly extremities	Speckled hyperpigmentation	Blisters caused by cytolysis in hyper-pigmented basal cells
6.	Herpetiformis Dowling Meara	Birth	Nil	Subungal blisters with normal regeneration of nails	Defective enamel rare	AD	Good	Generalised Herpetiform grouping of lesions, Conjunctiva	Lesions clear in association with fever	Cleft above hemidesmo-somes, 2o inflammatory changes.
7.	EBS Niemi	Birth	Nil	-	-	AD	Good	Generalised	-	Cytolysis of basal cells with clumping of tonofibrils
8.	EB Mendes da Costa	0-3 years	Nil	-	-	XR	Good	Mainly extremities	Depigmentation; microcephaly	Intra-epidermal blistering
9.	EBS, lethal type	Birth	Nil	-	-	AR	Lethal	Generalised	Death in infancy,	

TABLE 12-III COMPREHENSIVE TABLE OF DATA IN EB

	SUBTYPE	AGE OF ONSET	SCARRING	NAILS	TEETH	INHERITANCE	LIFE EXPECTANCY	DISTRIBUTION OF LESIONS	CLINICAL CONCOMITANTS	ELECTRON MICROSCOPY
B.	ATROPHIC (JUNCTIONAL) FORMS									
10.	EBA Generalisata Gravis	Intra-uterine	Atrophy rare	Clubbing	Defective dental enamel	AR	Lethal	Hands and feet spared	Generalised Mucosal involvement	Junctional Blistering
11.	EB Letalis with pyloric atresia	Intra-uterine	Atrophy rare	-	Defective enamel	AR	Lethal	Generalised	Pyloric atresia	Hypoplasia and number
12.	EBA Generalisata Mitis	Birth	Skin atrophy	Clubbing	Punctated enamel	AR	Good	Generalised,	Alopecia; oral, genital and oesophageal	
13.	EBA Inversa	Birth	Skin atrophy	-	Dental hypoplasia	AR	Females improve after menarche	Groins, genitals, axillae, trunk, shins	Albostriate skin changes; corneal erosion.	Junctional Hypoplasia and blisters; number of hemidesmosomes; lack of sub-basal dense plate
14.	EBA Localisata	± 6 years	Atrophy	Dystrophic	Enamel hypoplasia	AR	-	Shins and soles only	Private syndrome	
15.	Generalised Atrophic Benign EB (GABEB)	Birth	Atrophy	-	Carious	AR	Good	Generalised	Scalp alopecia; patchy-diffuse Shagreen naevocytic naevi	
16.	EBA with muscular atrophy	Birth	Atrophy	-	-	AR	Good	Generalised		
17.	EB Junctionalis (Disentis type)	Birth	Atrophy	Atrophic, small	-	AR	Good	Minimal on hands and feet	Absence of body hair; naevoid and palmar hyperkeratosis	

TABLE 12-III COMPREHENSIVE TABLE OF DATA IN EB

	SUBTYPE	AGE OF ONSET	SCARRING	NAILS	TEETH	INHERITANCE	LIFE EXPECTANCY	DISTRIBUTION OF LESIONS	CLINICAL CONCOMITANTS	ELECTRON MICROSCOPY
C.	SCARRING (DYSTROPHIC) FORMS									
18.	EB Dystrophica Cockayne Touraine	0-5 years	Atrophy, scarring & milia (A,S,M)	Dystrophic	-	AD	Good	Dorsum of extremities	Malignant change in scars	Dermolytic below basal lamina; blisters anchoring fibrils; changes only in predilection sites
19.	EBD-Pasini	First week	A,S,M	Dystrophic	-	AD	Good	Mainly limbs; oral mucosa	Albopapuloid patches on trunk present in adolescence	As 13, but changes in all sites
20.	EBD Pretibial	11-24 years	A,S,M	-	-	AD	Good	Pretibial	Occasional albopapular lesions	
21.	EBD Bart	Intra-uterine	A,S,M	Thumb and big toe	-	AD	Moderate	Generalised; corneal	Congenital absence of skin and skin ulceration	Dermolytic blistering
22.	EBD Inversa	Birth	A,S,M	Dystrophic	-	AR	Good	Intertriginous distribution; trunk	Anal and oesophageal strictures; sidero-poenic anaemia	
23.	EBD Progressiva	Late childhood/ adolescence	A,S	-	-	AR	Good	Hands, feet, knees, elbows; oral mucosa	Progressive perceptive deafness	Widened lamina vara with amorphous deposits; normal hemidesmosomes
24.	EBD Hallopeau Siemens local general mutilans	Birth/ infancy	A,S,M	Dystrophic	Hypoplastic enamel	AR	Variable	Localised Generalised	Syndactyly, contractures strictures	Dermolytic blisters with dissolution of collagen fibrils; secondary absence of anchoring fibrils
25.	EBD Fine	Birth	A,S,M	Dystrophic	-	AR	Good	Proximal arms and legs	Slow centripetal progression of symmetrical blister formation	Dermolytic blisters; virtual absence of anchoring fibrils
26.	EBD Generalisata Gravis (Winship)	Birth	A,S,M	Early shedding	Hypoplastic enamel	AR	Fair	Very widespread distribution	Alopecia, short stature, contractures	Dermolytic blisters; anchoring fibrils absent

Using all these data. a new comprehensive, yet uncomplicated classification chart can thus be constructed:

CLASSIFICATION OF EPIDERMOLYSIS BULLOSA

EPIDERMOLYSIS BULLOSA SIMPLEX

NON-SCARRING EPIDERMOLYSIS BULLOSA
WITH AUTOSOMAL DOMINANT INHERITANCE:

EBS Koebner

EBS Weber Cockayne

EBS Ogna

EBS with GGT deficiency

EB Herpetiformis Dowling Meara

EBS with mottled pigmentation

EBS Niemi

NON-SCARRING EPIDERMOLYSIS BULLOSA
WITH AUTOSOMAL RECESSIVE INHERITANCE:

EBS, lethal type

NON-SCARRING EPIDERMOLYSIS BULLOSA
WITH X-LINKED RECESSIVE INHERITANCE:

EB Mendes da Costa

EPIDERMOLYSIS BYLLOSA ATROPHICANS

NON-SCARRING ATROPHIC EPIDERMOLYSIS BULLOSA
WITH AUTOSOMAL RECESSIVE INHERITANCE:

EBA Generalisata Gravis (Herlitz, Pearson)

EBA with pyloric atresia

EBA Inversa

EBA Generalisata Mitis

EBA Localisata

EBA with muscular atrophy

Generalised Atrophic Benign Epidermolysis Bullosa

Epidermolysis Bullosa Junctionalis, Disentis type

EPIDERMOLYSIS BULLOSA

SCARRING ATROPHIC EPIDERMOLYSIS BULLOSA

WITH AUTOSOMAL DOMINANT INHERITANCE:

EBD Cockayne Touraine

EBD Pasini

EBD Pretibial

EBD Bart

SCARRING EPIDERMOLYSIS BULLOSA

WITH AUTOSOMAL RECESSIVE INHERITANCE:

EBD Inversa

EBD Progressiva

EBD Hallopeau Siemens — Localised / Generalised / Mutilans

EBD Fine

EBD Generalisata Gravis (Winship)

12.2 AN EFFECTIVE DIAGNOSTIC PROCESS

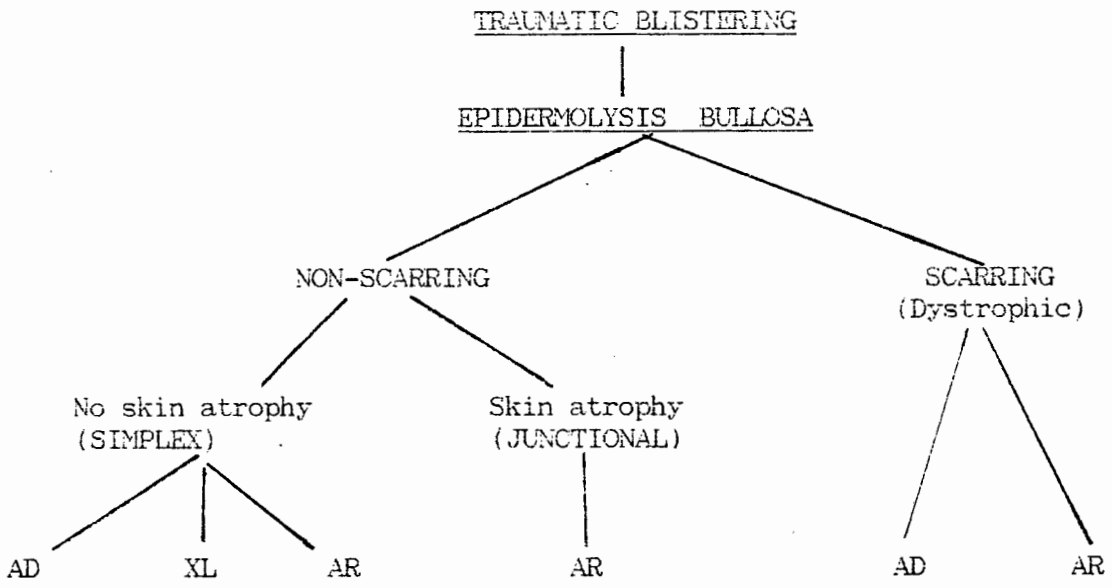
A major objective of this study is to simplify the diagnosis of this uncommon disorder, which is infrequently encountered even by the specialist dermatologist or paediatrician.

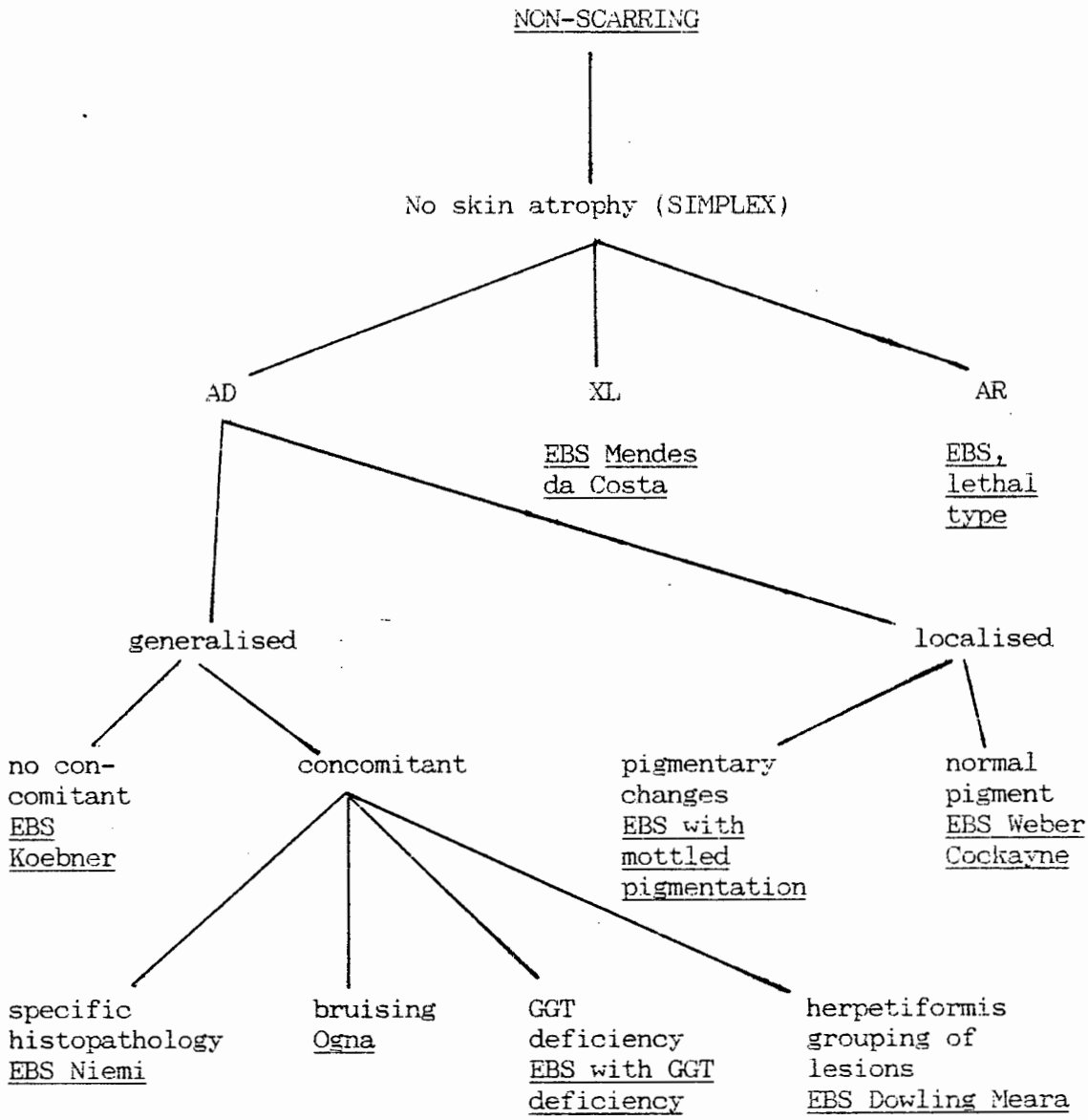
The diagnosis of EB is established initially according to the conventional criteria, as outlined earlier in the thesis, namely:-

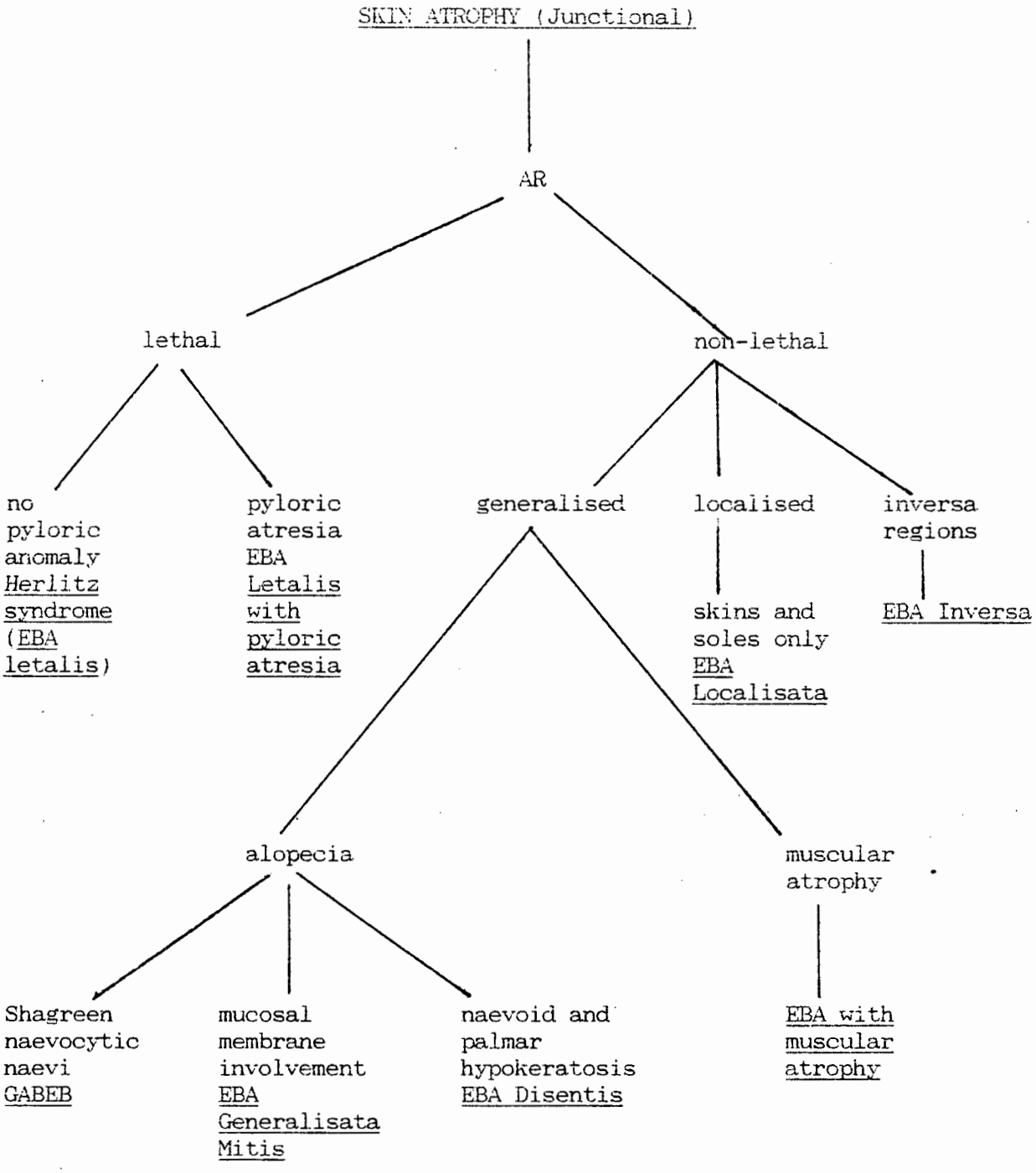
1. Traumatically induced or spontaneous bulla formation
2. Increased skin fragility as a concomitant to chronic blister formation
3. The absence of a preceding systemic illness
4. The absence of intake of drugs or toxic agents
5. The exclusion of photosensitisation of the skin
6. Exclusion of other conditions entering into the differential diagnosis of EB, aplasia cutis congenita, cutaneous porphyria, bullous pemphigoid
7. A positive family history for EB.

Once these criteria have been met and the diagnosis of the Eb 'group of diseases' is made, the patient can be assigned to a major group, EBS, EBA or EBD. In adults or older children, the diagnosis of the major subtype is usually made on clinical grounds. In the neonate, however, clinical differentiation between EBS, EBA and EBD is not easily achieved and electron microscopic examination of the skin is essential in the diagnosis of a major subtype. Thereafter using additional clinico pathologic information the diagnosis of a specific subtype can then be made.

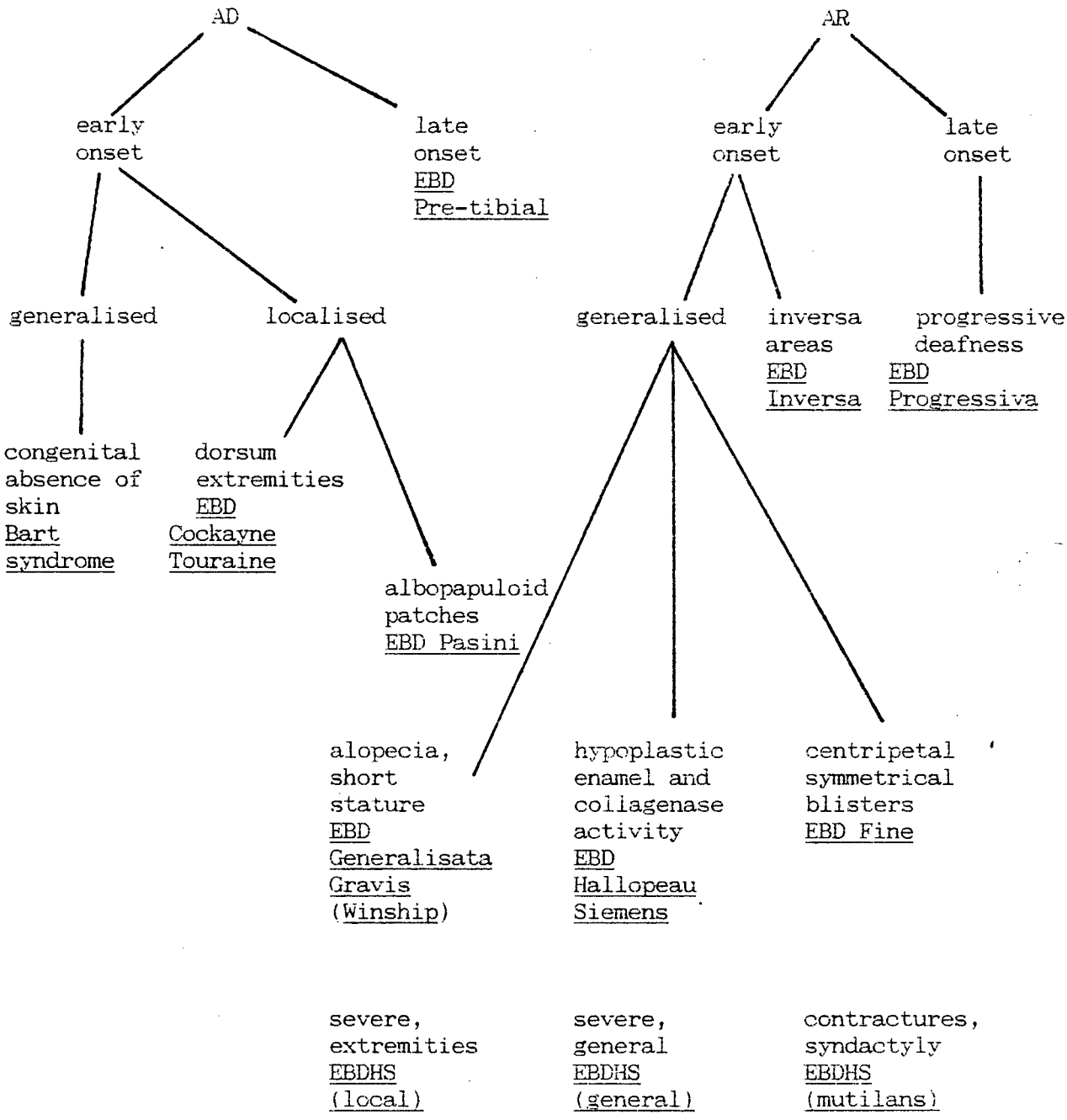
In order to facilitate this diagnostic process a dichotomous key has been constructed; this algorithm comprises a series of flow charts. When used with a correlation of all available positive data and the elimination of negative factors, these charts assist in the assigning of a specific subtype of EB to an affected person.


TRAUMATIC BLISTERING
EPIDERMOLYSIS BULLOSA
NON-SCARRING
SCARRING
(Dystrophic)
No skin atrophy
(SIMPLEX)
Skin atrophy
(JUNCTIONAL)
AD
XL
AR
AR
AD
AR


NON-SCARRING
No skin atrophy (SIMPLEX)
AD
XL
AR
EBS Mendes da Costa
EBS, lethal type
generalised
localised
no concomitant EBS Koebner
concomitant
pigmentary changes EBS with mottled pigmentation
normal pigment EBS Weber Cockayne
specific histopathology EBS Niemi
bruising Ogna
GGT deficiency EBS with GGT deficiency
herpetiformis grouping of lesions EBS Dowling Meara


SKIN ATROPHY (Junctional)
AR
lethal
non-lethal
no pyloric anomaly Herlitz syndrome (EBA letalis)
pyloric atresia EBA Letalis with pyloric atresia
generalised
localised
inversa regions
skins and soles only EBA Localisata
EBA Inversa
alopecia
muscular atrophy
Shagreen naevocytic naevi GABEB
mucosal membrane involvement EBA Generalisata Mitis
naevoid and palmar hypokeratosis EBA Disentis
EBA with muscular atrophy

SCARRING (Dystrophic)



SECTION VI

MANAGEMENT OF EPIDERMOLYSIS BULLOSA

13.4 SURGICAL INTERVENTION

Rarely, reconstructive surgery is necessary in the treatment of EB. In RDEB Hallopeau Siemens Mutilans, severe deformities may result from scarring. Secondary soft tissue fusion leads to mitten deformities of the hands and feet.

These severe complications are best managed surgically by means of release and repair of syndactyly and contractures.

Anaesthesia is potentially hazardous in EB as the forces exerted during intubation may cause traumatic blistering of the trachea. For this reason it is imperative that the anaesthetist should be aware that the patient has EB, with its complications, before embarking on a general anaesthetic.

13.5 HOME MANAGEMENT OF EB

The practical problems facing parents of a child with EB are immense, and the 'home management' of this condition is by far the most significant factor.

Trauma is the major precipitating factor and steps to avoid frictional forces are a primary strategy. The avoidance of rough clothing and bedclothes in the infant period will help to prevent blistering. In the hot South African climate, perspiration will predispose to more friction and the infant should not be allowed to wear damp clothes or wet nappies for any length of time.

Once the infant becomes ambulant, blistering often worsens, and in many instances it is at this time that EB first presents. Well-fitting footwear is essential to avoid severe bullae of the feet. Loose clothing of soft fabrics with a minimum of seams are optimal.

The draining of blisters is a contraversial issue. The bullae of EB, especially those on the digits, spread rapidly within the cleavage plane, and digital blisters tend to become circumferential. It is my belief that to drain the blister contains its growth. This can easily be done by the patient or parent; the skin is cleaned thoroughly and then, using a thin sterile needly, two holes are made at opposite sides of the blister. The blister fluid is then 'milked' out in a controlled manner, leaving the blister roof almost intact to prevent sepsis. This manouvre is performed by almost all the persons in this study without the introduction of infection. The symptomatic relief afforded by draining the blisters is immense.

The home care of a person with EB is time-consuming and demanding. The problems facing all those affected will be discussed further in Section VII, entitled 'Social Implications of Epidermolysis Bullosa'.

CHAPTER 14 GENETIC MANAGEMENT

The genetic management of Epidermolysis Bullosa has four aspects:

1) genetic counselling
2) prenatal diagnosis
3) carrier detection
4) termination of pregnancy.

These facets are reviewed in the light of published data and experience of this study.

14.1 GENETIC COUNSELLING

In the South African context, in the absence of objective techniques for prenatal diagnosis, genetic counselling is restricted to the statistical recurrence risks, depending on the pattern of inheritance established.

In EB Simplex, conventional counselling for an autosomal dominant disorder is offered. Families are educated as regards the nature of the disease and are reassured by the fact that variability of expression within a kindred is unlikely.

In similar vein, EB Atrophicans is an autosomal recessive trait, and confident counselling can be take place on this basis. The prognosis in EBA is generally very poor, and during the neonatal period, support is essential for the parents.

In EB Dystrophica, where the pattern of inheritance is clear from the family history, counselling according to the usual recurrence risks can be undertaken. Penetrance should be complete and expressivity does not vary within a minor subtype or a family so affected.

It is in the moderate or mildly affected person with EBD that a major counselling dilemma arises. It may be impossible to differentiate, in the sporadic presentation of such a person, between RDEB in its milder forms, and DDEB. Genetic counselling is therefore extremely difficult and both autosomal dominant and autosomal recessive risk factors must be explained. In the situation where there is confusion, the family has to be aware that future offspring may be at risk for EB. If the patient is a new AD mutation, however, there would be at a 50:50 recurrence risk for his children.

14.2 PRENATAL DIAGNOSIS

An important factor in any severe chronic inherited disorder is the feasibility of prenatal diagnosis. Epidermolysis Bullosa is an incurable debilitating disorder, and even in its mildest forms, poses significant limitations on the quality of life of the affected person. Without objective means of carrier detection and prenatal diagnosis, the genetic counsellor can merely quote statistical recurrence risks of this disorder.

Methods for the antenatal diagnosis of many inherited disorders have recently been established and, prenatal testing for numerous inborn skin diseases has been performed since 1980 (Anton Lamprecht 1981).

14.2.1 Foetal skin biopsy

Foetal skin sampling can be undertaken from the middle of the second trimester of pregnancy, and specimens obtained may be examined histopathologically. The ultrastructure of the developing skin must be compared with the normal for that gestational age. Holbrook (1981) has undertaken very elegant research into the differentiation and development of skin at all stages of gestation of foetuses.

Before describing techniques for skin sampling, the embryogenesis of the skin will be described.

14.2.1.1 The structure of foetal skin

The epidermis

By the end of the second month of gestation, the epidermis comprises only the basal layer. This is associated with the basal lamina. Periderm and epidermal cells are filled with glycogen and rudimentary organelles are present. Filament systems begin to develop at about the 36th day after conception and late in the second month, they associate with desmosomes. Hemidesmosomes are formed at the end of the second month, at the time when cells of the stratum intermedium are developing. Hemidesmosomes are structurally complete and have reached a frequency approximating mature skin by the fourth month.

The stratum intermedium of the foetal skin corresponds with the adult stratum spinosum. By the fourth month this consists of two or three layers of glycogen-filled cells. Filaments are dense in this region and a large number of desmosomes are present. Lamellar granules appear at about 20 weeks gestation, in superficial cells, but may be seen earlier in association with intra-epidermal sweat ducts.

Intermediate cells undergo terminal differentiation to become granular cells. These have some of the characteristics of a cornified cell, together with features of an intermediate cell. A true granular layer develops at 20 weeks. The amount of glycogen decreases and keratohyalin granules are present. Once the granular layer is established, keratinisation of cornified cells is completed. By the end of the second trimester, there is a thin stratum corneum which persists as a few layers before birth.

All definitive layers of the epidermis derived from the surface ectoderm are developed by the end of the second trimester. Cells of other origins, such as melanocytes which migrate from the neural crest, appear in the second month as the precursory stage of melanin production. Likewise Langerhans cells appear in the third month. The basal lamina begins as focal thickenings below the hemidesmosomes and by the 19th week it is identified as a continuous layer.

The dermis

The formation of the dermis is a complex and ill-understood process. The cellular component diminishes with gestational age, as the tissue becomes thicker and more fibrous. At the second month, the dermis is a watery cellular mesh of mesenchymal cells joined to form a syncytium. A ground substance matrix is secreted by these cells, consisting of abundant sugar and a sparse fibrillar component. Collagen fibrils develop below the basal lamina at the dermo-epidermal junction. At the third month the connective tissue ripens as the dermal cells separate and the dermis becomes richer in fibrous tissue. Collagen bundles are large and dense.

At 60 days, anchoring filaments are observed in association with the hemidesmosomes, and by the 72nd day of gestation, the anchoring fibrils have developed. The collagen layer, into which anchoring fibrils extend, thickens and consists of swirls of fibrils. The deep and proximal collagen migrate together, and in the fourth and fifth months the patterns of papillary and reticular dermis are formed. Hair follicles and subcutaneous adipose tissue also develop at this time.

At mid-gestation, there is a greater amount of type III collagen than type I in the dermis; this ratio alters with an increased amount of type I collagen as the foetus grows Elastic fibres appear by 22 weeks, initially around the vasculature, but later within the collagen matrix.

Most of the dermal cells can be seen from approximately 14 weeks; Schwann cells, perineural cells, histiocytes and mast cells are comparable to these components of adult dermis. Fibroblasts are seen in abundant numbers, while plasma cells may only be identified at a later stage.

14.2.1.2 Techniques for foetal skin biopsy

Foetal skin biopsy is conventionally undertaken via foetoscopy. The introduction of the instrument, transabdominally into the amniotic cavity, facilitates direct visualisation of the foetus as well as the sampling of foetal tissues including skin. Foetoscopy is performed under ultrasonic guidance.

Elias (1980) reported the first successful removal of a sample of foetal skin. The technique employed at that time was the so-called 'blind' biopsy. Using this method a sharp trocar and cannula were introduced percutaneously into the uterus, under local anaesthetic. A foetoscope, within a 1,7 mm 'Needlescope', was then inserted through the cannula. Once the cannula had been placed on the skin at the appropriate site, the foetoscope was withdrawn and replaced by a biopsy forceps. The skin sample was then removed without direct vision.

More recently, Gustavii (1983) has described a method for biopsy under direct vision, using the 'two-cannula' biopsy technique. This procedure is performed under general anaesthetic and the uterus is exposed by means of a low abdominal incision. The foetoscope is then inserted into the

amniotic cavity in the same way as a 'blind' biopsy, and the sample site is chosen. At this point a second trocar and cannula are inserted at a distance of 3-5 cm from the initial foetoscope. Using this method a second foetoscope is introduced; it is then replaced by the biopsy forceps and the entire procedure is visualised through the first foetoscope.

Rodeck (1980) has constructed a biopsy forceps that is small enough to pass through the cannula simultaneously with the foetoscope. This instrument offers a great advantage over the two-cannula direct vision approach, as only one puncture into the uterus and membranes need be made.

This technique of direct vision of the procedure has obvious advantages over the conventional 'blind' biopsy. Sampling of the wrong tissue may occur and there have been reports of the removal of foetal membranes, myometrium and even trophoblast tissue during blind biopsy. There is increased risk of injury to the foetus, placenta or uterine wall as there is less accurate control of the biopsy. Using direct vision, less biological material need be taken, as the operator is able to confidently assess the origin of the tissue.

A disadvantage of the two-cannula approach is the fact that two entries into the uterus are necessary, which may result in a slightly higher risk of miscarriage. In addition, this procedure is undertaken under general anaesthesia.

Foetoscopy is an invasive procedure with a risk of morbidity and mortality. In skilled hands, the current incidence of induced abortion range between 5 and 10% of pregnancies examined. Apart from abortion, other complications of foetoscopy are injury to the maternal, foetal or placental tissues or chronic leakage of amniotic fluid. Preterm labour and perinatal death may ensue. as may a small percentage of perinatal deaths.

Skin samples removed from the foetus are processed for electron microscopy using standard procedures, and the ultrastructure of the skin is studied.

A new method of foetal skin biopsy is the use of an ultrasound guided fine needle. This procedure lacks the advantage of direct visualisation; however under good ultrasonic imaging the introduction of a very tiny instrument may help to minimise morbidity to the foetus and mother.

Using foetoscopy, antenatal diagnosis has successfully been performed in **EBA Generalisata Gravis (Herlitz)**, as well as **EBA Inversa** from the 16th week of pregnancy (Anton Lamprecht 1981, 1984; Löfburg 1986). The hemidesmosomes are of utmost importance in this analysis and a foetus with **Herlitz syndrome** will have a reduction in the number of hemidesmosomes together with abnormalities or rudimentary forms of these structures. Junctional cleavage within the lamina lucida may be observed. In addition to the lethal subtypes, the typical changes of EB Dystrophica have been observed in skin samples from foetuses with **EBD Hallopeau Siemens**.

14.2.2 Other Methods of Antenatal Diagnosis

Foetoscopy is invasive and has recognised risks of foetal and maternal morbidity and mortality. In South Africa, foetoscopy is currently unavailable. For both of these reasons, alternative modes of prenatal diagnosis are very important, especially in the South African context where it is difficult for an 'at risk' mother to travel abroad to a centre where foetoscopy is practised.

In **EB Simplex Ogna**, genetic linkage to the GPT polymorphism has been demonstrated (Gedde Dahl 1973). Prenatal diagnosis was achieved with 97% accuracy by subjecting foetal blood to GPT typing. This procedure is however invasive, as the foetal blood is withdrawn via a foetoscope.

Other suggested methods of prenatal diagnosis are discussed in the following subsections.

14.2.2.1 Linkage studies

If tight linkage is proven between a marker and a disease process, this may constitute a means of prenatal diagnosis. In this study HLA groups red cell enzymes and blood groups, as well as a polymorphic probe, L7, were investigated for linkage to EB.

a) Restriction fragment length polymorphisms (RFLP):
There was no positive linkage to the probe L7 in RDEB and this RFLP cannot be used in antenatal diagnosis or carrier detection.

b) HLA typing; red cell enzymes, blood groups:
Linkage analysis was undertaken in search of a marker for EB. No significant linkage relationships were observed to any subtype of EB in this study.

14.2.2.2 Biochemical assays

a) Collagenase assay

In EBD Hallopeau Siemens, in vitro experiments have shown a significant increase in collagenase activity (see Chapter 11). This knowledge may be extrapolated to the antenatal setting. Collagenase activity in the amniocytes of normal and 'at risk' pregnancies may be compared with possible useful results.

The biochemical assay for collagenase in this study was unsuccessful and it was therefore not possible to gauge the quantity of this enzyme in the amniotic fluid of unaffected pregnancies, in order to establish a normal range. Similarly, 'at risk' foetuses could not be subjected to this testing. Indeed, during the two year period of the study, no 'at risk' mothers known to the investigator became pregnant.

The application of this theory, should it be feasible biochemically, would be a relatively simple process. Amniotic fluid would be extracted by amniocentesis and the cells cultured for experimentation (see Methodology, Chapter 3). Amniocentesis is a routine procedure in most major centres, and when performed by a skilled operator under ultrasonic guidance, has a foetal mortality risk of less that 1%, with even lower chance of foetal or maternal morbidity.

Confirmation or negation of this theory will be attempted again biochemically in the near future.

b) EBD Pasini

Biochemical defects have been found in **EBD Albopapuloidea Pasini**: there is deranged glycosaminoglycan metabolism (Bauer 1979) and an increase of chondroitin sulphate is excreted in the urine (Endo 1974). It is possible that this information may be extrapolated similarly to the outline above relating to collagenase, in an attempt to find a means of prenatal diagnosis for this subtype.

14.3 CARRIER DETECTION

There are no objective means of carrier detection in the recessive forms of EB, viz. EBA and RDEB. No significant markers have been discovered in the parents of affected children and electron-microscopic examination of the skin of parents yields normal results.

14.4 TERMINATION OF PREGNANCY

The option of termination of pregnancy is available to affected families, whether the foetus has the disorder confirmed by prenatal testing, or on empiric risk factors. The South African law makes allowances for termination on grounds of foetal malformation and disease.

The Abortion and Sterilisation Act of 1975 reads:

"3(1) Abortion may be procured by a medical practitioner only, and then only .....

(e) where there exists a serious risk that the child to be born will suffer from a physical or mental defect of such a nature that he will be irreparably seriously handicapped, and two other medical practitioners have certified in writing that, in their opinion, there exists, on scientific grounds, such a risk; .....".

Within these terms it may be legal and ethical to terminate a pregnancy at risk of Epidermolysis Bullosa. There is some debate as to whether the milder forms, for example EB Simplex, constitute a serious enough threat to well-being to 'qualify' for abortion. This decision ultimately rests in the hands of the parents, one of whom would usually be affected in the case of the dominantly inherited EB Simplex. Most colleagues would agree that there is little doubt about the acceptibility of termination of a foetus with lethal EBA, or the debilitating

dystrophic forms of the disease.

In the South African context, without objective prenatal testing, termination can only be offered to persons at risk and according to statistics. Because of this, the procedure would be early, optimally at less than 12 weeks gestation, and simple curettage would suffice. Prenatal diagnosis by foetal skin sampling is performed at approximately 20 weeks gestation. A positive diagnosis at this later stage would necessitate prostaglandin induction of labour and the delivery of the immature foetus.

The termination of a pregnancy cannot be entered into lightly and parents considering this decision require counselling before and after this event. Sterilisation of one or both parents is an option to be considered, though with the likelihood of prenatal diagnosis becoming available in South Africa in the foreseeable future, such final measures are inappropriate.

SECTION VII

SOCIAL IMPLICATIONS OF EPIDERMOLYSIS BULLOSA

THE QUALITY OF LIFE

Epidermolysis Bullosa is a severe chronic disorder which markedly impairs the quality of life of the affected person. Severe blistering results from the most minute trauma, and in the severe RDEB subgroup, bullae may appear spontaneously.

During infancy the lesions are more frequent, and constant blistering with resultant loss of skin requires regular dressings and meticulous care. This is very demanding of the time and resources of the parents, who may be ill-equipped to deal with long-term problems of this nature. The beginnings of ambulation yield a worsening of the blistering tendency; with resultant pain. Many parents are unable to cope with an affected infant, and long periods of hospitalisation are not unusual. Some individuals are institutionalised throughout their youth.

In the growing child, huge restrictions are placed on all activities. Any form of physical exertion will cause the formation of bullae. Participation in games and sport apart froom swimming is virtually impossible in all types of EB.

Clothing must be specially chosen, especially footwear, and the child may be stigmatised on these grounds, as well as for the underlying skin disorder. Hot weather worsens the blistering tendencies of many affected persons, most notably in EB Simplex, where the winter months usually herald a marked improvement.

Career choices, too, are limited to an extent. Manual labour is virtually impossible, and to the unskilled worker with EB, the prospect of finding employment is a very real problem. In certain forms, even the pressure exerted by a pen held for any length of time will result in much discomfort. Deformities of the hands may markedly impair dexterity.

In any chronic disorder, personal relationships are often formed with difficulty. In EB of the dominant type, there is the added fear of transmitting the gene responsible to the offspring.

EB MISINTERPRETED AS CHILD ABUSE

The clinical manifestations of EB may closely resemble the lesions seen in certain patterns of child abuse, notably cigarette burns and neglected impetigo. Confusion between EB and inflicted lesions has important medical, social and legal implications. During this study, the the parents of three of the children with EB, were accused of child abuse or neglect, resulting in considerable anguish. Details are given below.

The family of the eight year old child with **EB Atrophicans Generalisata Gravis** were visited by representatives of the Department of Social Welfare, after they had been alerted by neighbours who suspected non-accidental injury to the child.

An infant boy with the classical manifestations of **EB Dystrophica Hallopeau Siemens (Generalised)** was noted by members of the Police Force to have 'open sores' and blisters. On the basis of these lesions which were mistaken for cigarette burns and excessive sun exposure, a charge of child abuse was laid against his father, who was detained in custody. These charges were later dropped after the nature of this chronic skin disorder was explained to the police authorities by the investigator of this study.

The young boy with **EB Simplex Dowling Meara**, on entering school, was brought to the attention of the Multi-disciplinary Child Abuse Unit in a major hospital by a social worker who was convinced that these injuries had been inflicted and that he was neglected by his unmarried mother.

While an awareness in the community of the pernicious social disease of child abuse is necessary, the consequences of criminal charges against a family who have to cope with a severe chronic condition are potentially injurious. From the legal point of view, the parents of these children might be entitled to take legal action against those persons making such unjustified accusations. Many chronic skin disorders including EB are considered in the differential diagnosis of the multiple skin lesions of child abuse, and can be excluded clinically so that unnecessary social investigation can be avoided.

These parents will be saved the embarrassment of having well-intentioned but unfortunate accusations made against them if their children wear Medic-Alert discs. These identifying discs will make their recognition easier especially for paramedical workers, school nurses and doctors who meet them for the first time in the absence of their parents.

COMMENT

A South African affiliate of DEBRA (Dystrophic Epidermolysis Bullosa Research Association) has been formed. This aims to serve as a support group for affected individuals and their families as well as a forum for the dissemination of information on and ongoing research into EB. This will not be restricted to EB Dystrophica, but will encompass all forms of EB.

CONCLUSION

EB to date has been an unexplored subject in South Africa. Neither the frequency of the genodermatosis as a whole, nor the relative prevalence of its 26 subtypes has previously been established. The existence of the genes responsible for EB in the different ethnic groups had not previously been documented.

Data concerning 82 persons in 40 families is presented in this thesis. The frequency of EB in South Africa has by far exceeded the predictions made at the outset. All the major subtypes of EB are represented in South Africa in varying frequencies in the peoples of different ethnic origin. Within the broad groups of Simplex, Atrophicans and Dystrophica, 11 of the 26 minor subtypes were encountered.

A new unique entity within the broad category of EB has emerged in South Africa. **Epidermolysis Bullosa Dystrophica Generalisata Gravis (Winship)** is a hitherto undelineated subtype of the condition.

Heterogeneity at the genetic and clinical level is well documented in the world literature and the large variety of forms of EB encountered in South Africa, along with the new subtype described, further confirms heterogeneity. Indeed, the survival of a child with **EB Atrophicans Generalisata Gravis**, which is lethal in infancy, may be a manifestation of still greater heterogeneity; conversely, she may be the product of a genetic compound of two allelic recessive genes.

Despite the marked phenotypic variations in the different subtypes, expression within a family is invariable, and homogeneity of a subtype in a kindred is predictable. This observation is important, especially in terms of genetic counselling.

The classification of EB has always been complex and in some respects over-complicated. A new approach to diagnosis using a system of flow charts in a dichotomous arrangement has been suggested in this thesis. In the classification of the disorder, the concept of 'major' and 'minor' subtypes of EB is proposed. The three major types, i.e. Simplex, Atrophicans and Dystrophica, AR or AD may be further broken down into the minor subtypes. However, for practical purposes of predictions of prognosis, recurrence risks to future pregnancies and, indeed, management, no further distinction need be made. For this reason, two classifications which are complementary have been constructed.

Special investigations in this study have confirmed existing information. The ultrastructural changes of the different subtypes are well delineated and studies of this nature yielded results as predicted. The electron microscopic investigation of the teeth in EB demonstrated significant changes in the structure of the dentition.

Ultrastructural histopathological studies are essential in the neonatal period, when the acute lesions in an infant are similar in the different subtypes and complications such as scarring and atrophy have not yet developed. The ultrastructural changes of EB are not age dependent and are useful to differentiate between the major subtypes. The is of particular relevance to the prognosis of the infant, and the counselling of the parents. Indeed, the management of the specific subtype will depend on an accurate diagnosis. With advancing age, the clinical manifestations become more specific, and the importance of histology diminishes to some extent.

A technique for the removal of skin samples in EB is outlined in Chapter 3. This method is specifically designed method to minimise trauma to the specimen, so reducing artifactual alterations of the architecture of the skin.

Studies of genetic linkage failed to demonstrate significant linkage in any of the EB subtypes to HLA, red cell enzymes or blood groups. Similarly L ^{7}, the polymorphic probe which recognises a site close to Q23 on Chromosome 11 was not specifically linked to EBD Hallopeau Siemens, in this study.

The procedures facilitating prenatal diagnosis are as yet unavailable in South Africa. Methods less invasive than foetoscopy have been sought without success and foetoscopy remains the only method of antenatal diagnosis worldwide. No effective means of carrier detection have been developed.

An aspect which cannot be ignored in EB is the social implication of the disease, and this aspect of the genodermatosis has been explored. An affiliate of the British Society DEBRA (Dystrophic Epidermolysis Bullosa Research Association) has been established in South Africa to form a support group for those affected and their families.

Suggestions for ongoing studies

Epidermolysis Bullosa is a condition that occurs in all the peoples of South Africa and ongoing research into its causes and management is important. The recent development of a monoclonal antibody to type VII collagen, a major structural component of the anchoring fibrils, has laid the groundwork for a study of this antibody with the dermo-epidermal junction in EB Dystrophica.

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APPENDIX

UNIVERSITY OF CAPE TOWN

(WITH WHICH IS INCORPORATED THE SOUTH AFRICAN COLLEGE)

PROFESSOR PETER BEIGHTON,
M.D., Ph.D., F.R.C.P., D.C.H.
TELEPHONE 47-1250
Ext 297

DEPARTMENT OF HUMAN GENETICS
MEDICAL SCHOOL
OBSERVATORY, 7925
SOUTH AFRICA

Dear Doctor

I am presently Research Associate in the Department of Human Genetics at the University of Cape Town, and plan to embark on a course in Dermatology in the near future.

I am planning to do a Doctoral thesis on the subject of Epidermolysis Bullosa and need to know whether a sufficient number of cases are accessible to me for investigation, before I start. I have circularised all the Dermatologists and Paediatricians on the Medical Register in order to establish these numbers, with good success. I apologise for the duplication if you have already received my earlier letter.

I am now contacting the heads of the academic centres for Dermatology in an attempt to ascertain further patient numbers. I would be grateful if you could let me know this information, and if you would allow me access to details and past records of these patients.

I promise to keep all records confidential, and naturally any costs incurred by patients participating in this study would be met by my research grant.

Thank you for your assistance.

Yours faithfully,

I M WINSHIP (MB, ChB)

UNIVERSITY OF CAPE TOWN

(WITH WHICH IS INCORPORATED THE SOUTH AFRICAN COLLEGE)

PROFESSOR PETER BEIGHTON,
M.D., Ph.D., F.R.C.P., D.C.H.
TELEPHONE 47-1250
Ext 297

DEPARTMENT OF HUMAN GENETICS
MEDICAL SCHOOL
OBSERVATORY, 7925
SOUTH AFRICA

28th October 1983

Dear Doctor,

I am presently a Senior House Officer in the Department of Human Genetics, University of Cape Town, and plan to embark on an academic career in Dermatology in the near future.

I intend to do a doctoral thesis during 1984 and my proposed subject is a study of the Epidermolysis Bullosa group of inherited disorders. Before beginning such a study, I need to know whether a sufficient number of patients would be accessible to me for investigation. For this reason, I would be most grateful if you would let me have an indication of the number of patients with Epidermolysis Bullosa known to your practice.

Should the affected persons be willing to participate in this study, I would be able to travel throughout the Republic in order to see them. All expenses would be met from my resources and there would be no cost of any kind to the patients. In addition, no invasive tests would be undertaken.

I enclose a reply slip and would be most grateful if you would be kind enough to return this to me.

Thank you for your help.

Yours faithfully,

I M WINSHIP (M.B., ChB.)

REPLY SLIP

NAME : _____

ADDRESS : _____

_____

TELEPHONE NUMBER : _____

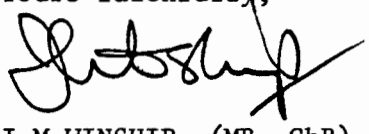
I have _____ known cases of Epidermolysis Bullosa in my practice.

Dear Dr

Thank you so much for your reply to my Epidermolysis Bullosa search.

I am having quite good success with my numbers, and am most grateful for the consideration being shown to me.

Yours faithfully,



I M WINSHIP (MB, ChB)

PROFORMA : **EPIDERMOLYSIS BULLOSA**

NAME:

DATE OF EXAMINATION:

DATE OF BIRTH:

RACE:

TRIBAL GROUP:

ADDRESS:

PHONE:

REFERRING DOCTOR AND ADDRESS:

PEDIGREE:

POSTULATED MODE OF INHERITANCE:

SPECIFIC HISTORY

AGE:

AGE OF ONSET:

BLISTERING IN UTERO:

DISTRIBUTION OF LESIONS (AT VARIOUS STAGES):

SCARRING:

NAILS - ever present
- when lost
- if regained

TEETH:

PROGRESSION/RECOVERY:

WAS DIAGNOSIS CLEAR CUT?

ANY OTHER ASSOCIATED FEATURES:

VACCINATION:

GENERAL HISTORY:

ANY OTHER UNASSOCIATED FEATURES

EXAMINATION:

CLINICAL SUBTYPE:

DISTRIBUTION OF LESIONS:

SCARRING:

NAILS:

EYES:

TEETH:

GENERAL EXAMINATION:

DIFFERENTIAL DIAGNOSIS:

POSSIBLE HETEROGENEITY WITHIN FAMILY:

DATE	TREATMENT GIVEN	EFFECT OF TREATMENT

LABORATORY

1 HISTOPATHOLOGY: - Light microscopy

- E/M

2 BIOCHEMISTRY - COLLAGENASE

3 LINKAGE STUDIES - HLA

- L_7

4 PHOTOGRAPHS

5 PERMISSION TO PUBLISH

6 ANY OTHER SPECIAL INVESTIGATIONS INDICATED

PROTOCOL FOR FIXATION AND EMBEDDING OF SKIN.

1. Fix in Karnovsky's fixative for 120 minutes at 4° C.
2. Rinse in 0.1M phosphate buffer, 3 times in 10 minutes.
3. Post fix in 50:50 solution of 2% OsO_4 and 0.2M phosphate buffer for 60 minutes.
4. Rinse in buffer, 3 times in 10 minutes.
5. Dehydrate in alcohol:

70% Ethanol	3 times in 10 minutes
80% Ethanol	3 times in 10 minutes
90% Ethanol	3 times in 10 minutes
100% Ethanol	3 times in 30 minutes

6. Acetone for 60 minutes.
7. Overnight in 50:50 Acetone:ERL
8. Fresh ERL for 120 minutes.
9. Fresh ERL for 120 minutes under vacuum.
10. Fresh ERL for 120 minutes under vacuum.
11. Embed in fresh ERL.
12. Polymerise at 60° C for 16 hours.

KARNOVSKY'S FIXATIVE.

Dissolve 2g paraformaldehyde in 25 ml of H_2O, with heating to steam but not to boil. (70°C)

Add 1N NaOH dropwise with shaking, until clear(1-3 drops required)

Cool and add 5 ml of 50% gluteraldehyde

Add 20 ml 0.2M(4.28g/100 ml) cacodylate buffer: pH 7.4-7.5

Add 25 mg $CaCl_2$ anhydrous. Final pH is 7.2.

EPIDERMOLYSIS BULLOSA IN SOUTHERN AFRICA - A NATIONWIDE SURVEY.

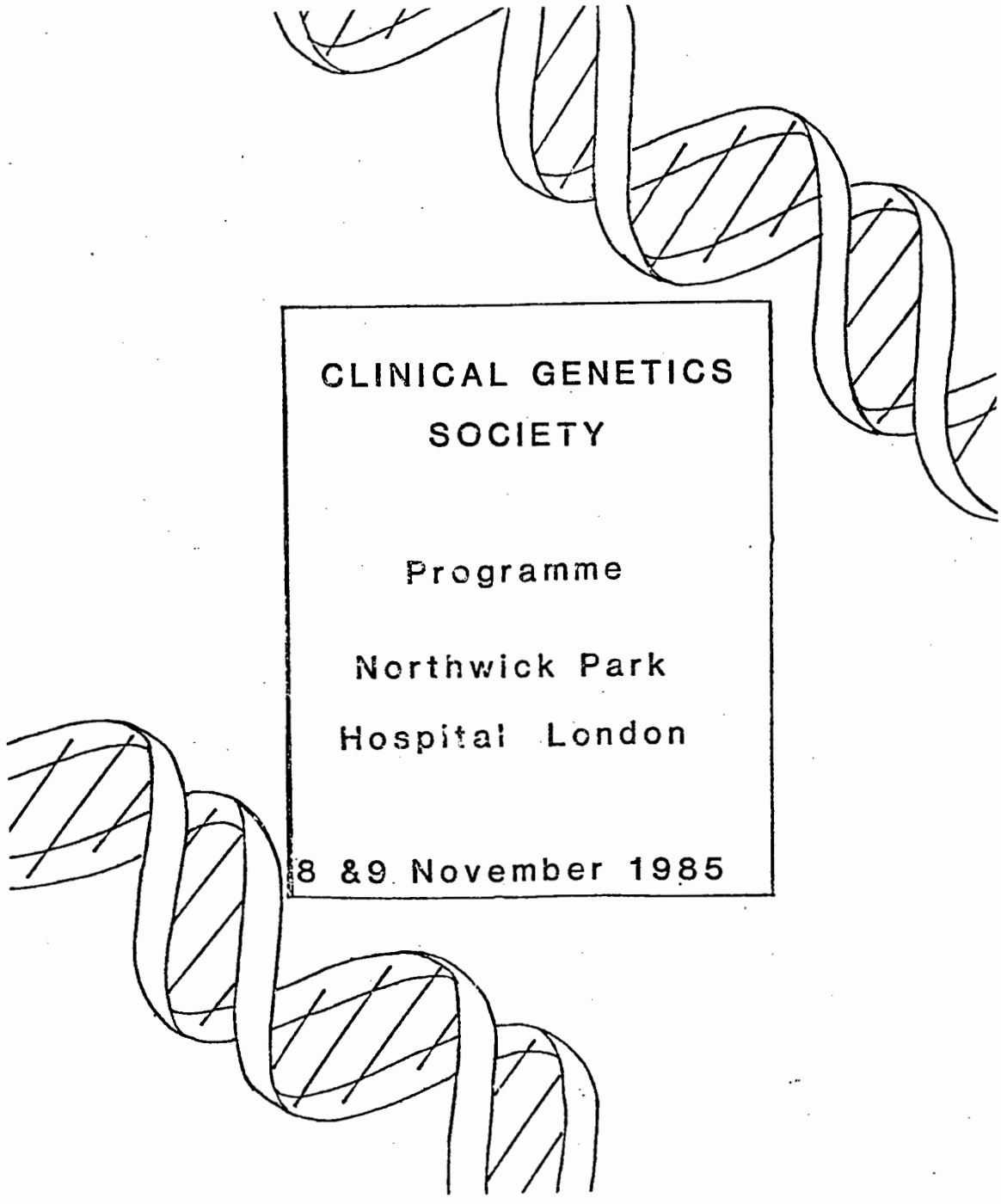
I.M. WINSHIP.

M.R.C. Unit for Inherited Skeletal Disorders, University of Cape Town, South Africa.

Epidermolysis Bullosa (EB) is a rare but important genodermatosis. The condition is heterogenous and several different subtypes have been delineated, some of which are potentially lethal.

A nationwide study of EB in South Africa has been undertaken, in which 50 affected persons had been studied. The clinical, genetic, epidemiological, histopathological, biochemical and molecular facets of the disorder have been investigated in these persons.

It has become apparent that, although uncommon, almost all the established subtypes of EB are present in South Africa. Despite heterogeneity, there is consistency in the manifestations of EB within any specific family. Precise diagnosis of the various forms of EB and recognition of their presence has considerable clinical and genetical implications.

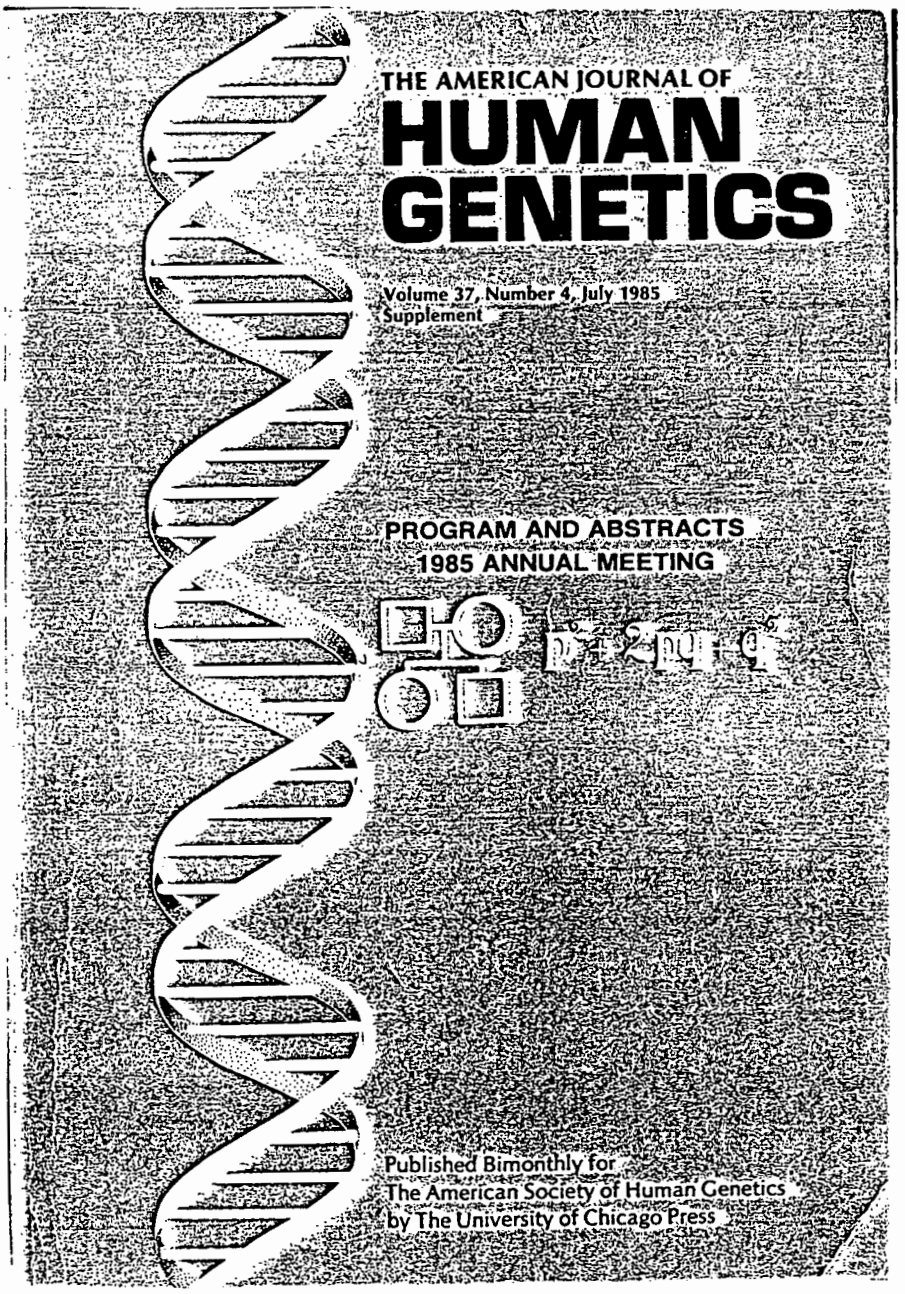


(241)

HETEROGENEITY IN EPIDERMOLYSIS BULLOSA IN SOUTH AFRICA. L.M. Winship, MRC Unit for inherited skeletal disorders, University of Cape Town, South Africa. (Intro. by Dr Jack Goldblatt).

Epidermolysis Bullosa Hereditaria (EB) is the descriptive term used for the mechanobullous genodermatoses. The condition is heterogeneous and many different subtypes have been delineated. There is confusion in the classification of these subtypes, and in order to elucidate this problem, 50 affected persons have been studied using clinical, genetic, histopathological, biochemical and molecular parameters, in a nationwide investigation of EB in South Africa.

It has become apparent that although uncommon, the gene for EB exists in South Africans of all ethnic origins, and almost all the subtypes of EB are present in South Africa. There are, however, considerable discrepancies in the relative prevalence of the various forms of EB. The observations of disparate clinical presentation and frequencies have important implications for genetic management particularly counselling and antenatal diagnosis. These findings and a classification of EB will be presented and discussed.



Reprinted from "SA Medical Journal", Vol. 69, 7 June 1986, pp. 743 - 746.

Epidermolysis bullosa in South Africa

INGRID WINSHIP

Summary

The term 'epidermolysis bullosa hereditaria' is applied to a heterogeneous group of mechanobullous diseases of which at least 19 different forms have been reported. Classification of these separate genetic entities into various subtypes is possible on clinical, genetic, biochemical and histopathological bases. Some forms are potentially lethal, while others are benign. Many of the subgroups are represented in South Africa, although little has been published locally on these conditions. On the basis of a wide-scale study in the RSA, the condition is reviewed in order to present current concepts and future developments of this rare but significant genodermatosis.

S Afr Med J 1986; 69: 743-746.

Epidermolysis bullosa hereditaria is a rare group of inherited disorders of the skin characterized by blistering induced by minimal trauma. The phenomenon was first reported in 1876 by von Hebra,[1] who described *'erblichen Pemphigus'* in a family where members of three generations complained of blistering on minor trauma. Köbner,[2] in 1886, proposed the name 'epidermolysis bullosa hereditaria', which became generally accepted. However, this has latterly been shown to be a misnomer since the ultrastructural defect is not in fact epidermal lysis.

In recent years, this unusual condition has been the focus of much international attention, although the South African experience of epidermolysis bullosa has not been comprehensively documented. In the only publication in the RSA, Menter and Patz[3] described it in blacks in the Transvaal in 1971.

An extensive investigation of the clinical, genetic and epidemiological features of epidermolysis bullosa in the RSA was conducted, employing biochemical, histopathological and molecular techniques, in search of the basic defect. A review to aid understanding of this interesting and important condition is presented.

Clinical features

The clinical features vary depending on the subtype. Initial presentation in most forms is at birth or in infancy, and manifests as blistering after trauma. The late sequelae are skin atrophy, scarring, milia and nail dystrophy; these depend on the level of blister formation in relation to the dermo-epidermal junction (Fig. 1). There is considerable overlap of the clinical features of the different subtypes; clinical assessment alone is inadequate in establishing a definitive diagnosis of a specific form.

The degree of severity of epidermolysis bullosa ranges from the innocuous to the more severe and even lethal types. One form in which the diagnosis may easily be missed is the very mild type which presents in late childhood or adolescence; blistering occurs only with participation in sport or when the affected individual is subjected to the physical stresses experienced on entering the army. Apart from this moderate increase in skin fragility and a tendency to blister with pressure, the person remains healthy.

The severe forms present at birth with a range of appearances from absence of skin to blistering confined to pressure areas. The mouth, oesophagus, teeth and nails may be affected. Generalized blistering of the whole body may ensue.

Specific clinical concomitants of the various subtypes are tabulated in Table I.

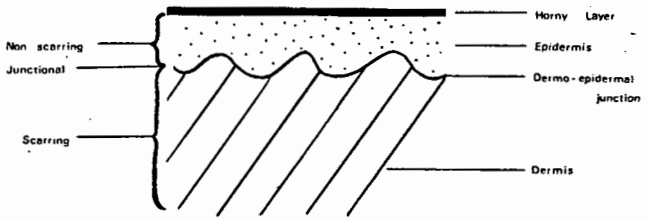


Fig. 1. Diagrammatic representation of the dermo-epidermal junction.

Classification

There are three main categories of epidermolysis bullosa: (*i*) non-scarring (simplex); (*ii*) non-scarring with skin atrophy (junctional); and (*iii*) scarring (dystrophic).

Specific clinical features such as the anatomical distribution of lesions and the presence of nail dystrophies, as well as ultrastructural changes, differentiate these three main groups, which can be further subdivided in terms of the mode of inheritance. The features of the different subtypes are listed in Table I.

Genetics

The condition is uncommon and there is marked geographical variation in the frequency of the gene in its different forms. Patterns of inheritance vary according to the subtype, and both autosomal dominant and autosomal recessive forms are known. Furthermore, an X-linked form has been recognized in a single Dutch family.[4]

In general, the non-scarring forms appear to be dominantly inherited, while the junctional types have a recessive pattern. Both dominant and recessive inheritance have been noted in the scarring forms.

Despite great heterogeneity, with at least 19 distinct genetic forms documented,[5,6] the manifestations within a family appear to be consistent in terms of clinical and ultrastructural features and severity.

Menter and Patz[3] recorded relatively few cases in whites, with only 1 case in their own series. By contrast, at least 50% of the affected people in this study are white, although it has been noted in all the population groups of the RSA.

The specific genetic linkage relations in the various subtypes serve both to differentiate between clinically similar types and to offer a means for prenatal diagnosis.

Histopathology

Ultrastructural and clinical features depend essentially on the level of blister formation in relation to the dermo-epidermal

Department of Human Genetics and MRC Unit for Inherited Disorders of the Skeleton, University of Cape Town
INGRID WINSHIP, M.B. CH.B., *Research Associate*

TABLE I. COMPARABLE TABLE OF SUBTYPES OF EPIDERMOLYSIS BULLOSA

Subtype	Age of onset	Scarring	Nails	Teeth	Inheritance	Life expectancy§	Distribution of lesions	Clinical concomitants	Electron microscopy
Non-scarring (simplex forms)									
EBS Köbner (EBSK)	Birth	Nil	—	—	AD	Good	Generalized	—	Intra-epidermal blistering, basal cell vacuolization
EBS Weber Cockayne (EBSWC)	Walking	Nil	—	—	AD	Good	Hands and feet only	—	Dissolution of tonofibrils
EBS with GHGT* deficiency	Birth	Nil	—	—	AD	Good	General	—	
EBS Ogna	Birth	Nil	—	—	AD	Good	General	Onygrophosis big toes	Blistering with cytolysis of basal cells above hemidesmosomes
EBS with mottled pigmentation	Birth	Nil	—	—	AD	Good	Mainly limbs	Bruising with blisters Speckled hyperpigmentation	Blisters caused by cytolysis in hyperpigmented basal cells
Herpetiformis Dowling Meara	Birth	Nil	Subungal blisters with normal regeneration of nails	Defective enamel, rare	AD	Good	Herpetiform grouping of lesions, generalized; conjunctiva	Lesions clear in association with fever	Cytolysis of basal cells with clumping of tonofibrils. Cleft above hemidesmosomes, 2° inflammatory changes
EB Mendes da Costa	0-3 yrs	Nil	—	—	XR	Good	Mainly extremities	Depigmentation; microcephaly	Intra-epidermal blistering
Atrophic (junctional) forms									
EBA generalisata† gravis	Intra-uterine	Atrophy rare	Clubbing	Defective dental enamel	AR	Poor	Hands and feet spared	Laryngeal involvement	Junctional blisters. Hypoplasia and ↓ number of hemidesmosomes. Lack of subbasal dense plate
EB letalis with pyloric atresia	Intra-uterine	Atrophy rare	—	Defective enamel	AR	Poor	Generalized	Pyloric atresia	
EBA generalisata mitis	Birth	Skin atrophy	Clubbing	Punctated enamel	AR	Good	Generalized, hands and feet not spared	—	
EBA inversa	Birth	Skin atrophy	—	Dental hypoplasia	AR	Females improve after menarche	Groins, genitalia, axillae, trunk, shins	Albostriate skin changes	
Scarring (dystrophic) forms									
EBD Cockayne Touraine	0-5 yrs	Atrophy, scarring and milia	Dystrophic	—	AD	Good	Dorsum of extremities	Malignancy noted in scars	Dermolytic blisters below basal lamina. ↓ anchoring fibrils. Changes only in predilection sites
EBD Pasini	1st wk	Atrophy, scarring and milia	Dystrophic	—	AD	Good	Mainly limbs. Oral mucosa	Albopapuloid‡ patches present in adolescence	As EBD Cockayne Touraine, but changes in all sites

EBD pretibial	11-24 yrs	Atrophy, scarring and milia	—	—	AD	Good	Pretibial	Occasional albopapular lesions	Dermolytic blistering.
EBD Bart	Intra-uterine	Atrophy, scarring and milia	Thumb and big toe	—	AD	Moderate	Generalized. Corneal	Congenital absence of skin and skin ulceration	
EBD inversa	Birth	Atrophy, scarring and milia	Dystrophic	—	AR	Good	Peri-anal, perivulvar	Anal and oesophageal strictures. Sideropoenic anaemia	
EBD progressiva	Late childhood/ adolescence	Atrophy and scarring	—	—	AR	Good	Hands, feet, knees, elbows. Oral mucosa	Progressive perceptive deafness	Widened lamina vara with amorphous deposits. Normal hemidesmosomes
EBD Hallopeau-Siemens	Birth/infancy	Atrophy, scarring and milia	Dystrophic	Hypoplastic enamel	AR	Local general mutilans	Hands, feet, knees, elbows. Oral oesophageal and corneal	Syndactyly, contractures	Dermolytic blisters with dissolution of collagen fibrils. Secondary absence of anchoring fibrils

AD = autosomal dominant; AR = autosomal recessive; XR = X-linked recessive; EB = epidermolysis bullosa; EBS = epidermolysis bullosa simplex; EBA = epidermolysis bullosa atrophica; EBD = epidermolysis bullosa dystrophica.
*Glucosylgalactosyl-hydroxylysyl glucosyl transferase (GHGT) deficiency: the deficiency of an enzyme that catalyses glucosylation of galactosyl hydroxylysyl residues in collagen biosynthesis.
†Also known as EB letalis or the Herlitz syndrome.
‡Albopapuloid lesions are slightly raised pale areas of variable size, clearly demarcated from the surrounding skin.
§Life expectancy is not equated with quality of life.

junction (Fig. 1). Intra-epidermal blisters heal without scarring; junctional blisters, i.e. those between the plasma membrane of basal cells and the sub-basal dense plate, will cause skin atrophy but no scarring. Dermolytic blistering, deep to the basal lamina, results in scarring milia and atrophy.

Light microscopy will detect intra-epidermal blistering but is of little value in the accurate differentiation of all the subtypes. Electron microscopy has reached a level of great sophistication and accuracy, and the ultrastructural defects have been well established in many forms.

Biochemistry

Important biochemical investigation into the condition is being undertaken, the most significant finding being increased collagenase activity in the recessive dystrophic type.[7] Furthermore a deficiency of galactosyl-hydroxylysyl glucosyl transferase[8] in a dominant simplex subtype has led to its recognition as a separate entity.

Fig. 2. Large denuded areas of de-roofed blisters on the legs and feet of a neonate with a junctional form of epidermolysis bullosa.

Fig. 3. Scarring and nail dystrophy seen in a child with recessive dystrophic epidermolysis bullosa.

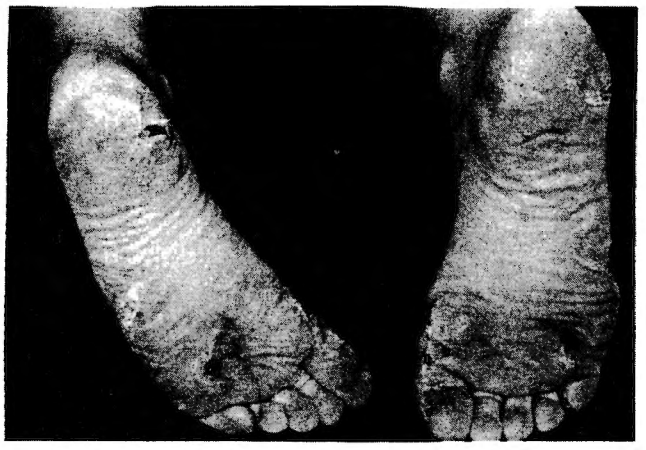


Fig. 4. Incapacitating blistering of the feet in a dystrophic form of epidermolysis bullosa.

Management

To date, no really effective treatment has been found for any of the types. Palliation has been achieved with varying success in some cases with the use of systemic and topical preparations of vitamin E and corticosteroids. Recently, successful topical treatment of localized forms has been reported by using aluminium chloride hexahydrate;[9] to date we have no experience of this therapy.

A recent approach to treatment in the recessive dystrophic form with increased collagenase activity is the use of phenytoin (Epanutin; Parke-Davis). This is known to decrease collagenase activity *in vitro,* and extrapolating this knowledge to this clinical setting has led to fairly successful treatment of recessive epidermolysis bullosa dystrophica.[10] In addition, good results have been achieved in a number of cases of the lethal junctional type known as the Herlitz syndrome.[11]

In the more severe forms where scarring has resulted in contracture formation, surgical intervention is an important therapeutic option.

Differential diagnosis

The general diagnosis on clinical grounds is not difficult, though ultrastructural examination is necessary to classify the subtype. Bart's syndrome, i.e. epidermolysis bullosa with congenital localized absence of the skin, is well recognized as a part of the disease spectrum.[12] The coexistence with aplasia cutis congenita has posed a diagnostic question; blistering does not occur with the areas of aplasia and the usual scalp defect of aplasia cutis congenita is not associated with epidermolysis bullosa. Although the two are recognized as distinct entities, Carmi *et al.*[13] suggested linkage between these genes and pyloric atresia.

Epidermolysis bullosa acquisita, though not an inherited disease, is uncommon and warrants mention. Roenigk *et al.*[14] set out distinct criteria, stipulating that this diagnosis could be made where the clinical lesions commenced in adulthood, without a family history and after the exclusion of other bullous diseases. More recently, additional criteria have been delineated.[15] The presence of IgG at the basement membrane zone on direct immunofluorescent microscopy as well as IgG deposition beneath the basal lamina with specific electron microscopic findings confirm the diagnosis of the acquired form.

The latter, while rare, may be associated with systemic disease, generally of the auto-immune type. Inflammatory bowel disease and, less commonly, systemic lupus erythematosus, amyloidosis, tuberculosis, and the result of renal dialysis are some of the associations.

Comment

Epidermolysis bullosa hereditaria, though rare, is devastating for affected individuals and their families. The mildest forms impose immense limitations, while patients with the severe forms are totally incapacitated. This spectrum extends to the lethal form, where death ensues in infancy. Although the ultrastructural abnormalities have been well illustrated by electron microscopy and immunofluorescence, the nature of the defect responsible for blistering is as yet ill understood. Newer lines of investigation include biochemical studies of collagenase activity[7] and monoclonal antibody reactions.[16] Genetic linkage studies have been undertaken in the various types and it is anticipated that in the near future gene localization may be possible using restriction fragment polymorphisms.

In some forms, prenatal diagnosis can be undertaken by electron microscopic examination of fetal skin biopsy specimens.[17] At present this test is not available in the RSA, but biochemical parameters for prenatal diagnosis are being investigated.

Treatment is largely unsuccessful, but a variety of methods are continually being evaluated.

Epidermolysis bullosa in the RSA ranges in presentation from the mildest to most severe form of the disease. For this reason a nation-wide study of this genodermatosis is under way, and the results will be published later elsewhere.

I would like to thank Professor Peter Beighton and Professor Norma Saxe for their guidance, Sister Gail Christy for help with illustrations and Barbara Hindle for typing the manuscript. Thanks also to Dr W. Murray and the staff of the South African Medical Research Council's Institute for Electron Microscopy.

This project was supported by the South African Medical Research Council, the University of Cape Town Staff Research Fund, and the Mauerberger Foundation.

The author would be grateful for any further information regarding patients or clinical experiences with epidermolysis bullosa from colleagues in the RSA.

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