THE PROTEINS OF NORMAL AND PSORIATIC HUMAN EPIDERMIS

by

IRENE HUNTER

A Thesis presented
for the degree
of
Master of Science

December, 1977

Department of Biochemistry
Glasgow University

"The human machine is not no different from the animal machine or the fish machine or the bird machine or the reptile machine or the insect machine! It's just a whole God damn lot more complicated and consequently more trouble to keep together".

Tennessee Williams
"Cat on a Hot Tin Roof"

ACKNOWLEDGEMENTS

I would like to thank Dr. David Skerrow for his interest and encouragement throughout this project, the late Professor J.A. Milne for the provision of facilities in the Department of Dermatology and Professor R.M.S. Smellie for the use of facilities in the Department of Biochemistry.

I am also indebted to Mr. Robert Blackie for expert technical assistance and to Mr. Ian McKie for all photographic work.

CONTENTS

SUMMARY

The epidermal fibrous protein has been successfully extracted from the cornified cells of human plantar callus and stratum corneum with buffered urea solutions. The proteins of these two horny cell layers are structurally dissimilar and differ also from the fibrous protein, prekeratin, of the living cell layers. Human prekeratin is a multichain protein, and on polyacrylamide gels, three different polypeptide chains of molecular weights 70,000, 63,000 and 55,000 can be detected. All three of these chains are present in plantar callus, but there is a much higher proportion of the 70,000 molecular weight chain than in purified prekeratin. The 55,000 molecular weight chain is not present in stratum corneum.

The polypeptide chains of the fibrous protein are the major constituents of both living and horny cell layers, but in extracts of foreskin epidermis a prominent pale-blue staining polypeptide has been detected. It has a molecular weight of 45,000 and is apparently unrelated to any previously extracted epidermal protein.

The 55,000 and 63,000 molecular weight chains of the fibrous protein are prominent in psoriatic scales, but the 70,000 molecular weight chain is deficient and occasionally absent. The psoriatic fibrous protein is structurally abnormal resembling neither the protein of callus nor stratum corneum. Effective treatment of

psoriasis causes changes in the fibrous protein, finally resulting in a normal structure. The uninvolved callus and stratum corneum of psoriatics have fibrous protein structures indistinguishable from those of the normal tissues.

There ia an overall similarity between the amino acid analyses of callus, stratum corneum, psoriatic scale and human prekeratin, apart from differences in their contents of glycine and serine. Differences in the tissue contents of these two amino acids appear to be related to changes in the intensity of the 70,000 molecular weight chain of the fibrous protein.

Varying the conditions of extraction of the horny cell layers alters the amount but not the polypeptide composition of the isolated material. In the absence of a reducing agent, a large amount of fibrous protein polypeptides are released from psoriatic scale but only a very small amount from plantar callus.

These results are discussed in relation to normal keratinisation and to the defects in this process in psoriatic epidermis.

CHEMICALS

Acrylamide, bovine serum albumin, carbonic anhydrase (bovine erythrocytes), catalase (beef liver), glycine, ovalbumin, phosphorylase a and trypsin (type III) were obtained from the Sigma Chemical Co., London.

All other chemicals were Analar reagents supplied by B.D.H. Chemicals Ltd., Poole, Dorset.

GENERAL INTRODUCTION

NORMAL EPIDERMIS

Adult mammalian skin consits of two distinct layers of different embryological origin (Balinsky, 1970). The mesodermally derived dermis is composed of a fibrillar network secreted by fibroblastic cells embedded in a matrix of gelatinous mucopolysaccharide. The outer layer, the epidermis, is a stratified squamous epithelium of ectodermal origin. It is separated from the underlying dermis by the basement membrane, an electron-dense fibrillar matrix, anchored by dermal fibrils (Heaphy & Winkelmann, 1977). The epidermis is avascular and nutrients pass by diffusion across the basement membrane from dermal blood capillaries.

The epidermal cell population is produced and maintained by the proliferation of cells adjacent to the basement membrane. Daughter cells produced by mitosis in the basal layer move up through the spinous and granular layers finally reaching the outer stratum corneum, a tough, water resistant barrier between the organism and its environment. The upward movement of cells is accompanied by the synthesis of characteristic differentiation products and distinct morphological changes.

Ultrastructurally, the horizontal stratification of the epidermis is clearly seen (Brody, 1960). Basal cells contain all the usual subcellular organelles, the unique presence of centrioles confirming that proliferation is restricted to this layer. The basal cell cytoplasm

is rich in the primary epidermal differentiation product, the tonofibrils, comprising bundles of individual filaments about 7nm in diameter (Odland & Reed, 1967).

The spinous layer is characterised by the presence of a distinct intercellular space into which project protrusions of the plasma membranes giving rise to the so called 'prickle cell' appearance. Small oval particles (membrane coating granules) appear for the first time and have a highly ordered internal structure of alternating dense and less dense lamellae, 2-3 nm thick (Lavker, 1975; Matoltsy & Bednarz, 1975). The tonofibrils have a more amorphous appearance and the individual filaments are less distinct.

The granular layer contains numerous keratohyalin granules which may be round, oval or irregular in shape and of variable size. The tonofibrils are less opaque than those of the lower layers and tend to parallel the surface of the epidermis. The membrane coating granules first observed in the spinous layer are also present in the granular layer and as keratinisation proceeds they discharge their lamellae into the intercellular space where they apparently remain intact and often contact the plasma membrane (Lavker, 1975). The lamellae are believed to have a lipid content (Breathnach & Wyllie, 1966), which may aid the barrier function of the epidermis.

The onset of cornification is marked by considerable

cell flattening and is accompanied by the release of hydrolytic enzymes from the lysosomes, which bring about degradation of the subcellular organelles. Transition cells (T-cells) in which cell flattening is almost complete, may occasionally be observed above the granular layer. The cytoplasm of horny cells becomes filled with bundles of tonofilaments embedded in a highly opaque interfilamentous matrix. The plasma membrane is thickened by the deposition of electron-dense material on its inner surface, the main component of which appears to be protein (Matoltsy & Matoltsy, 1966; Sun & Green, 1976). Cornified cells are lost from the surface of the epidermis by desquamation, proliferation of the basal cells maintaining a balanced cell population.

Columns of stacked horny cells may occasionally be observed in the epidermis (Mackenzie, 1975) and these interdigitate in a step-like manner (Menton & Eisen, 1971a). Regular cell alignment is most prominent in the thin epidermis of mouse, or guinea pig ear and appears to be related to a low rate of cell proliferation (Christophers et al, 1974).

The cell stacking extends into the living cell layers and basal cells lying beneath a column lead to the formation of the superficial horny cells (Potten, 1974). The proliferation of the basal cells is apparently under the control of a centrally located cell (Allen & Potten, 1974).

Control of growth and differentiation in the epidermis apparently involves both dermal and epidermal influences. In embryogenesis, the dermal influence is particularly apparent and epithelium from one body site transplanted onto mesoderm at another site develops the morphological characteristics specified by that mesoderm (Cairns & Saunders, 1954). In adult tissue the dermis was found to determine the kind of epidermis produced by ear, sole and trunk, but this was not the case for tongue and cheek pouch (Billingham & Silvers, 1967).

It is possible to grow keratinocytes in dispersed cell culture (Constable et al, 1974; Prunières et al, 1976) and stratification and keratinisation may occur, as demonstrated by the presence of keratohyalin granules, tonofibrils, desmosomes and the basement membrane (Mann & Constable,1977). Human keratinocytes grown on collagen gels form multi-layered structures (Karasek & Charlton, 1971) and addition of viable fibroblasts or fibroblast conditioned medium increases the cell survival time. Subculturing of keratinocytes normally results in de-differentiation (Regnier et al, 1973).

Stable cultures of keratinocytes may be established by growing cells on a feeder layer of X-irradiated fibroblasts (Rheinwald & Green, 1975). Such cultures have a lifetime of up to fifty cell generations and undergo keratinisation (Sun & Green, 1976) and desquamation (Green, 1977).

Thus, epidermal growth and differentiation <u>in vitro</u>

is dependent upon fibroblasts or their products, but attempts to isolate an epidermal stimulation factor have been of limited success. A fraction isolated from rabbit fibroblast conditioned medium, which stimulates epidermal growth *in vitro*, has been partially characterised (Melbye & Karasek, 1973). The substance was dialysable, heat-stable, had a molecular weight of less than 1,000 and was protease and nuclease resistant. Epidermal growth factor (EGF), a polypeptide of molecular weight about 7,000, isolated from the submaxillary gland of male mice, was shown to stimulate epidermal growth both *in vivo* and *in vitro* (Cohen, 1972), but its role in normal growth and differentiation is not clear.

In addition to these exogenous compounds, a number of intra-epidermal factors have been implicated in growth control. The epidermis contains a tissue specific, species non-specific mitotic inhibitor, the "epidermal chalone" (Bullough, 1962). It was characterised as a protein or glycoprotein of molecular weight 30,000 - 40,000 and was trypsin sensitive (Hondius Boldingh & Laurence, 1968). The epidermal chalone apparently inhibits the cell cycle in both G_1 and G_2 phases. It is the intracellular chalone level which determines the fate of a cell. Below a critical chalone level, the cell undergoes mitosis, while above this level, cell maturation occurs. If there is a sudden decrease in the chalone level due to cell damage, then the newer post-mitotic cells revert to mitosis (Bullough, 1975). Chalone systems have been proposed to exist in a number of tissues, but to date no chalone has been obtained in

a purified form from any source.

The nucleotide, cyclic AMP is apparently an important factor in the control of growth and differentiation in a number of systems (Froelich & Rachmeler, 1972; Pastan, 1972; Judewicz et al, 1973). Low cellular cyclic AMP levels stimulate proliferation, while at high levels proliferation is inhibited and differentiation is stimulated (Sheppard, 1971). In vitro inhibition of mitosis has been demonstrated by addition of dibutyryl cyclic AMP (Voorhees et al, 1972) and cyclic AMP elevating drugs (Delescluse et al, 1974) to cultured epidermal cells. Cyclic GMP apparently has an opposite effect to cyclic AMP, and it is thought to be the ratio of these two cyclic nucleotides which exerts a controlling influence over growth and differentiation in a tissue (Voorhees et al, 1976).

Biochemical studies have indicated that all the major pathways of intermediary metabolism are qualitatively functional in the epidermis. Many of the enzymes of the Embden-Meyerhof pathway have been detected (Weber, 1964) and regional variations in enzyme activity may occur within the epidermis (Adachi & Yamasawa, 1966). Glucose metabolism by the pentose phosphate pathway is also present within the epidermis (Fréinkel, 1960; Jarrett, 1971) being particularly active in the upper cell layers (Im & Adachi, 1966). Although the epidermis is capable of synthesising glycogen (Halprin & Ohkawara, 1966), its

utilisation is confined to times of increased metabolic activity.

The human skin is an active site of lipid biosynthesis (Rheinertson & Wheatley, 1959), but quantitively the epidermis is much more active than the dermis (Hsia, 1971), especially in the synthesis of sterols (Nicolaides & Rothman, 1955) and polar lipids. Within the epidermis the phospholipid content of the stratum corneum is considerably lower than that of the non-keratinised layers (Rheinertson & Wheatley, 1959).

Normal human skin contains a number of proteases, some of which have been isolated and characterised (Hopsu-Havu & Jansen, 1969; Fräki & Hopsu-Havu, 1976) and a number of protease inhibitors. Proteolytic enzymes are found predominantly in the basal layer and their activity decreases towards the skin surface.

The epidermis has also a large number of acid hydrolases. These enzymes which include proteases, lipases, nucleases and carbohydrate degrading enzymes are located in small, membrane bound organelles, the lysosomes, first detected in the epidermis by Rowden (1967, 1968). Histochemically, the strongest reaction for the acid hydrolases occurs in the transitional zone of the epidermis, where they have been implicated in the degradation of the subcellular organelles during normal keratinisation. The hydrolytic enzymes are believed to be synthesised in the basal cells and carried up through the epidermis in the lysosomes. Acid hydrolases have been detected in the

stratum corneum, but only about 10% of their activity appears to survive terminal differentiation (Roelfzema et al, 1976).

The specific incorporation of glycine and histidine into the granular layer of the epidermis (Fukuyama et al, 1965) and the histochemical localisation of histidine in the keratohyalin granules (Reaven & Cox, 1965; Fukuyama & Epstein, 1966, 1967) was attributed to protein synthesis in the upper layers (Bernstein, 1964) and a histidine rich, cystine poor protein was isolated (Hoober & Bernstein, 1966; Gumucio et al, 1967). Extraction of keratohyalin granules from newborn rat epidermis (Matoltsy & Matoltsy, 1970) and subsequent amino acid analysis, showed that cystine and proline were present in large amounts whereas only a small amount of histidine was present. Labelling studies (Tezuka & Freedberg, 1972) failed to show histidine incorporation into keratolyalin fractions, while keratohyalin extracted by Ugel (1969, 1971) was found to be a ribonucleoprotein in which the protein component was rich in serine, arginine, glycine and histidine (Ugel & Idler, 1972).

The great variety of 'keratohyalins' so far detected undoubtedly reflects to a large extent differences in the extraction procedures, and it is possible that keratohyalin granules contain a number of proteins. Although the exact function of keratohyalin remains unknown, it is believed to form the interfilamentous matrix of the horny cells (Lavker & Matoltsy, 1970).

The α-type X-ray diffraction pattern characteristic of keratinising epithelia is due to the fibrous protein of the tonofilaments. Rudall (1952) using 6M urea solutions performed the first successful extraction of mammalian epidermis, isolating both fibrous and non-fibrous protein components. The fibrous protein fraction 'epidermin' retained the ability to give an α-keratin diffraction pattern, but its heterogeneous nature prevented further structural characterisation.

It was not until the work of Matoltsy (1964, 1965) that the fibrous protein was obtained in a homogeneous form. Using 0.1M citric acid-sodium citrate (CASC) buffer, pH2.6 he was able to extract 'prekeratin', which was purified by serial precipitation, from the non-keratinised layers of cows' nose epidermis. Physicochemical analysis of the fibrous protein (Skerrow, 1972, 1974) showed that it was a dimer of three chain subunits, with a total molecular weight of 375,000. The individual polypeptide chains of the protein could be separated on SDS polyacrylamide gels, each subunit consisting of one chain of molecular weight 72,000 and two chains of molecular weight 60,000. This chain composition was observed whether the protein was treated with a reducing agent prior to electrophoresis or not, and indicated a lack of interchain disulphide bonds. The 40% α-helix content of each subunit was present as two discrete coiled coil regions of the molecule, each about 20nm in length and separated by non-helical regions of undetermined dimensions. The ability of the isolated prekeratin to aggregate into long filaments resembling those <u>in vivo</u>,

and to give an α-keratin X-ray diffraction pattern (Matoltsy, 1965) suggests that these molecules are undenatured structural units of the tonofilaments.

A subsequent analysis of the bovine fibrous protein (Steinert, 1975) found that it consisted of six different polypeptide chains on polyacrylamide gels, and this was attributed to the increased resolution of the electrophoretic system used. The chain molecular weights ranged from 47,000 - 58,000 and were therefore, somewhat lower than those of Skerrow (1974), although the reason for this was not clear.

In yet another study of cows' nose prekeratin, Baden et al (1973a) originally characterised the protein as consisting of two major chains of molecular weights 47,000 and 58,000 and a constantly occurring minor band of molecular weight 98,000, while in later studies (Lee et al, 1975; Baden et al, 1976) prekeratin had a four chain structure in which bands A and A' had molecular weights close to 45,000 and bands B and B' had molecular weights of 67,000 and 56,000 respectively. Both A and B type chains were required to give an α-type diffraction pattern and subunit structures of AA'B and AA'B' were proposed, which now closely resemble the three chain structures identified by Skerrow (1974). The studies of Steinert (1975) and Baden et al (1973a) confirmed the original observation that prekeratin lacked interchain disulphide bonds.

More recently (Skerrow, 1977a) human prekeratin was isolated and purified by a modifed CASC extraction

procedure. It was similar in composition to the bovine fibrous protein, lacked interchain disulphide bonds and consisted of three polypeptide chains on polyacrylamide gels of molecular weights 70,000, 63,000 and 55,000.

Fibrous protein fractions extracted from human epidermis with 0.02M NaOH, were found to consist of two fast moving bands, A and B and a large amount of material which did not enter the gel (Bauer, 1972). It was proposed that A was a dimer and B a monomer of the fibrous protein and that the fibrous protein macromolecule was built up by a process of polymerisation. Identical electrophoretic patterns were also obtained for rat and rabbit fibrous proteins and for cows' nose prekeratin.

CASC buffer is ineffective in the extraction of proteins from 'keratinised' tissues, and this insolubility was originally attributed to disulphide bonding of the fibrous protein (Rudall, 1968, Fraser et al, 1972). Extraction of the horny layer generally requires the use of denaturing solvents and although Baden et al (1976) found that the epidermal fibrous protein from cows' nose and human stratum corneum could be isolated with urea solutions, separation of the individual polypeptide chains required pretreatment of the protein with a reducing agent. Thus, during normal keratinisation, it was proposed that the fibrous protein which lacked disulphide bonds in the living cells became stabilised by interchain disulphide bonds in the horny cell layers.

Steinert (1975), however, disagreed with this observation, and found that the same amount of fibrous protein polypeptides could be isolated from bovine stratum corneum in the absence of a reducing agent as in its presence, but that mechanical disruption of the horny layer was required in the former case. He concluded that the cell membrane was the site of disulphide bond stabilisation in the horny layer and this was in agreement with the chemical study of Matoltsy & Matoltsy (1966).

In following the fate of the fibrous protein during normal keratinisation, no differences in polypeptide composition have been detected between the living and horny cells of bovine (Steinert, 1975; Baden et al, 1976) or human epidermis (Bauer, 1972).

The α-keratin X-ray diffraction pattern of keratinised tissues (Astbury & Street, 1931) was originally explained as resulting from a right handed α-helix (Pauling et al, 1951), but this did not fit the observed reflections exactly. Both Crick (1953) and Pauling & Corey (1953) then independently suggested that a tilted helix with a coiled coil structure would provide a better fit. The diffraction pattern of mammalian α-keratin was found to deviate from that expected for a coiled coil and thus there are probably a number of departures from this idealised conformation (Fraser et al, 1971).

Psoriatic Epidermis

Psoriasis is an extremely common skin disease affecting 1 - 2% of the population of the United Kingdom (Ingram, 1954).

Evidence of a familial tendency suggests that psoriasis is genetically determined and an autosomal dominant mode of inheritance has been proposed (Kimberling & Dobson, 1973). Lomholt (1963) found that the elbows, knees and scalp were the most commonly affected sites, and Caucasians are apparently more predisposed towards psoriasis than Asiatics or Negros.

In the psoriatic epidermis the keratinocytes are considerably enlarged, as are the nuclei, nucleoli and mitochondria (Brody, 1962) and there is a reduction in the amount and the extent of aggregation of the tonofilaments (Lagerholm, 1965). The basement membrane is highly convoluted and breaks in it have been detected (Cox, 1969). Psoriatic epidermis lacks keratohyalin granules and T-cells have not been found. The cytoplasm of the keratinocytes increases in opacity towards the stratum corneum but the normal plasma membrane thickening is absent (Brody, 1962). A lack of organelle degradation and defective desquamation of the parakeratotic stratum corneum results in the formation of the fine silvery scales associated with the disease.

A distinct intercellular space, normally confined to the lower layers of the epidermis extends as far as the granular layer in psoriatic tissue and is thought to be the result of a defective glycocalyx on the psoriatic keratinocytes (Mercer & Maibach, 1968). Villous projections are detected on the cell surfaces in psoriasis and a number of other scaling dermatoses. (Griffiths & Marks, 1973) and these appear to be closely related to increased

mitotic activity and parakeratotic scaling. Barrière et al (1974) believe that the presence of these protrusions is insufficient to characterise a particular pathological epidermis, but that their shape may be a diagnostic feature. The even distribution of villi on psoriatic horny cells and the presence of depressions of a similar size may allow intimate cell contact and increase cell adhesion (Dawber et al, 1972). A large number of granules or vacuoles (occurring either singly or in aggregates) is randomly distibuted within the cytoplasm and intercellular space of psoriatic stratum corneum (Brody et al, 1974) and the excavations frequently found on cell surfaces may be the result of granule or vacuole excretion. The columnar organisation which appears to be an intrinsic property of the epidermis below a particular rate of proliferation (Christophers et al, 1974) is completely lacking in psoriasis (Menton & Eisen, 1971b).

Attempts to detect the initial morphological change in psoriasis have implicated dermal blood vessels (Telner & Fekete, 1961) and extended, oedematous dermal papillae containing dilated and tortuous capillary loops have been detected underlying psoriatic lesions (Braverman & Yen, 1977). The dermal changes are however, apparently preceded by epidermally located disturbances of the spinous layer (Pedace et al, 1969).

In vivo and in vitro autoradiographic studies of DNA synthesis have shown the number of labelled cells to be at least four times higher in psoriatic than in normal epidermis. The duration of mitosis in psoriasis has been

reported as lengthened (Fisher & Wells, 1968) or shortened (Goodwin et al, 1974) while Weinstein & Frost (1968) found that the whole cell cycle was compressed into 37 hours as opposed to 152 hours normally (Epstein & Maibach, 1965).

Bauer & De Grood (1975) used impulse cytophotometry, a rapid method for analysis of cell kinetic data where light intensities are measured as a function of cell characteristics and have obtained a cell cycle time of about 50 hours for both normal and psoriatic epidermis. There is some evidence of a resting cell population in the epidermis (Gelfant & Candelas, 1972) and it is believed that the increased cell numbers in psoriasis are due to the release of non-cycling (G_0) and blocked (G_1 or G_2) cells. The normal transit time of 3 - 4 weeks for keratinocytes through the epidermis, is reduced in psoriasis to about 3 - 4 days (Rothberg et al, 1961; Van Scott & Ekel, 1963).

The balance between the nucleotides cyclic AMP and cyclic GMP implicated in the control of growth and differentiation in normal epidermis (Voorhees & Mier, 1974) is profoundly altered in psoriatic lesions. Decreased cyclic AMP levels are detected in psoriatic epidermis (Voorhees et al, 1976) which may be due to defective adenyl cyclase activity (Mahrle & Orfanos, 1977) while increased levels of cyclic GMP are found. Cyclic GMP stimulates the breakdown of cyclic AMP in vitro (Beavo et al, 1971) and this may be the cause of the low levels in psoriasis. Cyclic AMP inhibits the cell cycle in both G_1 and G_2 phases (Abell & Monahan, 1973) and the increased proliferation of

basal cells in psoriasis may be explained by a lack of inhibition of mitosis in the pathological epidermis.

As early as 1923, Gans reported increased glucose and oxygen consumption by psoriatic epidermis and subsequent metabolic studies have been both diverse and extensive. Individual metabolites have been measured (Halprin & Okawara, 1966), enzyme activities have been quantified (Hammar, 1970) and whole pathway studies have been undertaken (Jarrett, 1971).

Psoriatic scale has a low free amino acid content (Wheatley & Farber, 1961) and increased polypeptide levels (Flesch et al, 1962) thought to be the result of impaired enzymic degradation of protein. The lipid content of psoriatic scale is apparently three times higher than that of normal horny cells (Wheatley & Farber, 1961) and increased sterol and fatty acid biosynthesis have been reported in psoriatic lesions (Cooper et al, 1976). Qualitative abnormalities of the phospholipid fraction are found in psoriatic epidermis (Tsamboas et al, 1977) with increased levels of sphingomyelin and phosphatidyl inositol and a significant decrease in the lysophosphatidylcholine and diphosphatidyl glycerol levels. Anionic lipids such as phosphatidyl inositol have been found to modulate the activity of membrane bound adenyl cyclase (Michell, 1975) and the altered phospholipid levels in psoriasis may have caused the reduced cyclic AMP levels found in lesions.

Fräki & Hopsu-Havu (1976) have extracted a number of proteases from psoriatic scales and these have been

characterised with respect to preferential substrate and pH optima. They differ from the enzymes of healthy skin and include a histone splitting protease which may cause de-repression of psoriatic cells, leading to increased DNA synthesis and cell division. Normal epidermal proteases are predominantly located in the basal layer with decreasing activity towards the skin surface while a strong proteolytic activity has been detected in the parakeratotic stratum corneum of psoriatics (Herrmann,1976).

Increased levels of acid hydrolases are found in psoriatic lesions, with arylsulphatase and β-glucosidase activities being particularly elevated (Mier & Van den Hurk, 1976a, b). Some 30% of the original activity of the hydrolases is retained in psoriatic scales, about three times as much as survives normal keratinisation (Roelfzema et al, 1976). It is not clear why enhanced hydrolase activity fails to complete organelle digestion in psoriatic epidermis, although it has been proposed that enzyme release from the lysosomes may be defective (Reid & Jarrett, 1967).

The lack of labelled histidine incorporation into the 'histidine-rich' protein in psoriatic epidermis is correlated with the absence of keratohyalin granules (Voorhees et al, 1968) although high tissue histidase activity has been reported (Reaven & Cox, 1965). It was suggested that failure to de-repress keratohyalin cistrons could cause the observed synthetic defect.

Little information is available on the molecular structure of the fibrous protein of psoriatic epidermis. Roe (1959) isolated a fibrous component (protein A) from

the parakeratotic stratum corneum of psoriatics which appeared to be identical to the normal fibrous protein. An α-fibrous protein extracted from psoriatic stratum corneum (Baden & Bonar, 1968) was compositionally similar to the protein from normal epidermis. Preliminary structural studies have shown that the psoriatic fibrous protein is abnormal (Skerrow, 1977b).

Most of the disturbances detected in psoriatic scale are believed to be the result of, rather than the cause of the disease. It has been suggested, however, that the increase in epidermopoiesis found in psoriatic epidermis may cause the defective cellular architecture, characterised by the absent granular layer and parakeratotic stratum corneum, and that the controlling factors are either the cyclic nucleotides (Voorhees & Mier, 1974) or the epidermal chalone (Elgjo, 1972) but as yet there is no evidence to support this being the primary fault.

During treatment of psoriasis, the granular layer apparently re-appears prior to a decrease in mitotic activity (Fry & McMinn, 1968) and in experimentally induced hyperplasia of guinea pig epidermis where the keratinocyte transit time and epidermal thickness are the same as those in psoriasis, no parakeratosis is detected (Christophers & Braun-Falco, 1970). These results tend to favour a defect of the keratinisation as the initial cause of psoriasis.

CHAPTER 1: NORMAL KERATINISATION

1.1 INTRODUCTION

During normal keratinisation, the epidermal tonofilaments undergo a process of aggregation, and in the horny layer the cells become filled with filament bundles embedded in an opaque interfilamentous matrix (Brody, 1960). Ultrastructurally, there is apparently no difference between the individual filaments of the living and horny cell layers.

Using CASC buffer, it is possible to solubilise the living layers of the epidermis, and the fibrous protein of the tonofilaments 'prekeratin' has been isolated (Matoltsy, 1965; Skerrow, 1977a). Epidermal prekeratin is a multichain protein which has the ability to aggregate into long filaments similar to those observed *in vivo* (Matoltsy, 1965), and apparently represents non-denatured units of the tonofilaments.

Although the tonofilaments of the living and horny cell layers are indistinguishable, the cornified cells may be identified by their flattened appearance, a lack of sub-cellular organelles and by a thickened cell envelope. The major component of the thickened cell envelope of horny cells is protein (Matoltsy & Matoltsy, 1966; Sun & Green, 1976) which is apparently stabilised by disulphide bonds (Matoltsy & Matoltsy, 1966).

The thickness of the horny layer varies at different body sites, being about 15μm thick on most surfaces, but increasing to 600μm in palmar and plantar callus. The cells of the normal stratum corneum have a highly organised

spatial arrangement forming stacked columns, which interdigitate at step-like depressions (Menton & Eisen, 1971a). Corneocytes are normally hexagonal in shape, have surface folds and creases and low villous projections. Palmar and plantar callus lack a regular cellular arrangement (Mackenzie & Linder, 1973) and the cells interdigitate via distinct surface microvilli (Spearman & Hardy, 1976).

The horny cell layer is resistant to the action of CASC buffer, and it has been suggested that the fibrous protein is stabilised by disulphide bonds (Rudall, 1968; Baden & Bonar, 1968). Denaturing solvents in the presence of a reducing agent are generally used to extract cornified cell proteins (Baden & Bonar, 1968; Tezuka & Freedberg, 1972; Huang et al, 1975). The individual components of these heterogeneous extracts have been separated on SLS polyacrylamide gels, a technique widely used in the study of epidermal proteins.

SDS polyacrylamide gel electrophoresis (Weber & Osborn, 1969) has been shown to be a reliable method of molecular weight determination, with results generally in close agreement with those obtained by more conventional physicochemical procedures (Weber et al, 1972). In the presence of a reducing agent, the detergent SDS binds strongly to proteins dissociating them into their constituent polypeptide chains, and leaving them with an overall negative charge. Movement of the peptides through the polyacrylamide gels is then a function of their molecular

weight which may be determined by reference to standard protein markers. Gel electrophoresis is rapid, requires only microgram quantities of material and provides reliable, reproducible results. Glycoproteins, proteins with a high intrinsic charge and peptides below about 15,000 molecular weight may however, migrate abnormally on SDS gels, and this is not a suitable method of molecular weight determination for such molecules.

Previous work has failed to demonstrate any difference in the polypeptide composition of the fibrous protein from the living and horny cell layers of either bovine (Steinert, 1975; Baden et al, 1976) or human epidermis (Bauer, 1972). It has been concluded that there is no change in the structure of the fibrous protein during normal keratinisation.

The aim of the present study was to extract epidermal proteins from both normal stratum corneum and plantar callus and to compare the chains of the fibrous protein with those of prekeratin from the living cells. By this procedure it was intended to identify any changes in the molecular weights or relative amounts of the fibrous protein chains which might accompany normal keratinisation in human epidermis.

1.2. MATERIALS AND METHODS

1.2.1. MATERIALS

Tissue Samples

Breast tissue was obtained from plastic surgery operations and foreskins from circumscision of children under ten years of age. Tissue samples were immersed in distilled water and transported to the laboratory on ice. Epidermis was removed from stretched skin by gentle scraping with a scalpel blade and either used immediately or stored in distilled water at $-30^{o}C$ for up to three months.

Stratum corneum and plantar callus were obtained by scraping of forearms and soles respectively and stored desiccated at $-30^{o}C$.

1.2.2. METHODS

Preparation of Human Prekeratin

Human prekeratin was extracted from the living layers of the epidermis by the method of Skerrow (1977a), a modification of the original extraction procedure of Matoltsy (1965).

Extraction of Tissue with Urea Solutions, pH7.2

Tissue samples (20 mg callus or stratum corneum; 100 mg wet weight epidermis) were immersed in 2 ml 50 mM Tris-HCl pH7.2 containing 6 M urea, 2% ($^{v}/_{v}$) 2-mercaptoethanol. After extraction at 4°C for 24 hr., the samples were homogenised for 2 min. with an Ultra Turrax homogeniser, sonicated for 30 sec. and left to stand for 2 hr. at 4°C. The extract was centrifuged at 50,000 x g for 10 min., the supernatant decanted and the pellet discarded. After addition of excess solid SDS (about 100 mg), the supernatant was boiled for 2 min. and then dialysed overnight into 0.01 M phosphate buffer, pH7, containing 0.1% SDS, 0.1% 2-mercaptoethanol, prior to SDS gel electrophoresis.

Preparation of Human "Stratum Corneum" Residue from Whole Epidermis

Due to the difficulty in obtaining stratum corneum in large quantities, horny layer residues may be prepared from whole epidermis, by the prior extraction of living cell proteins with CASC buffer. This method has been used to prepare both bovine and human stratum corneum (Baden et al,

1973b, 1976) from which fibrous proteins have been extracted. A human "stratum corneum" residue was prepared in this way, and the structure of the fibrous protein compared with that of normal stratum corneum derived directly.

Breast epidermis (100 mg wet weight) underwent an initial human prekeratin extraction in 2 ml CASC buffer, pH 2.6 (Skerrow, 1977a). The resulting residue was soaked in 2 ml CASC buffer for 2 days at 4°C, homogenised for 2 min. with an Ultra Turrax homogeniser, sonicated for 30 sec., centrifuged at 50,000 x g for 10 min. and the supernatant discarded. The pellet was immersed in 2 ml 50mM Tris-HCl, pH 7.2 containing 6M urea and extracted for a further two days. The sample was then homogenised for 2 min., sonicated for 30 sec., centrifuged at 50,000x g and the supernatant discarded. The final pellet was immersed in 2 ml 50mM Tris-HCl, pH 7.2 containing 6M urea, 2% 2-mercaptoethanol and extracted overnight at 4°C. The sample was then homogenised for 2 min., sonicated for 30 sec., centrifuged at 50,000 x g and the pellet discarded. After addition of excess solid SDS, the final supernatant (stratum corneum extract) was boiled for 2 min., before overnight dialysis into 0.01M phosphate buffer containing 0.1% SDS, 0.1% 2-mercaptoethanol.

Electrophoresis

SDS polyacrylamide gel electrophoresis was performed by the method of Weber & Osborn (1969) using 7.5% gels with half the standard amount of cross-linker. The current was

8mA / tube and the running time was $3\frac{1}{2}$ - 4 hr. Gels for densitometry were electrophoresed for 6 hr.

Standard molecular weight markers used were: phosphorylase a (94,000), bovine serum albumin (68,000), catalase (58,000), ovalbumin (43,000) and carbonic anhydrase (29,000).

Densitometry

Gels stained for protein with Coomassie Brilliant Blue were scanned at 540 nm using a Gilford 250 spectrophotometer, fitted with a linear transport accessory.

Elution of Protein from Gels

Protein was eluted from polyacrylamide gels by a method described by Weber et al (1972). The required protein band was cut from gels and the pieces macerated in 4 volumes 0.01 M NH_4HCO_3, containing 0.1% SDS. Samples were incubated at 37°C for 6 - 12 hr., the eluate removed and the extraction repeated. The combined eluates were lyophilised.

Bound SDS was removed by dissolving the lyophilised material in sufficient water to give a 1% SDS solution. To one part of this solution were added nine parts of acetone. The precipitated protein was collected by centrifugation and washed with 90% acetone.

Amino Acid Analysis

Protein samples were hydrolysed in redistilled, constant boiling HCl, at 110°C under N_2 for 24 hr. and 48 hr.

Analyses were performed on a Joelco JLC 5AH amino acid analyser and corrections made for destruction and slow release of residues. Cysteine and cystine were determined after performic acid oxidation.

1.3 RESULTS

Characterisation of Human Prekeratin

Human prekeratin prepared by the method of Skerrow (1977a) was electrophoretically pure and characterised on polyacrylamide gels as having three chains of molecular weights 70,000, 63,000 and 55,000. Table 1(a) shows the results of quantitative densitometry of prekeratin. The polypeptide chains were not present in a simple 1:1:1 ratio, but the ratio of the 70,000 molecular weight chain to the other two remained fairly constant at approximately 1:2. This was analogous to the chain distribution of the bovine protein (Skerrow, 1974).

Urea Extraction of Human Epidermis

Three polypeptide chains corresponding to those of prekeratin were the major components of the urea extracts of both breast and foreskin epidermis (Fig. 1). There were in addition a number of faint polypeptide bands on the gels of total urea extracts of epidermis. The most prominent of these minor polypeptides was an unusual, pale-blue staining band found in extracts of foreskin epidermis (Fig. 1(c)) but this was absent from extracts of breast tissue (Fig. 1(a)). The fibrous protein polypeptides extracted from the human epidermis with urea solutions were indistinguishable from those extracted with CASC buffer.

Urea Extraction of Stratum Corneum

Only two major polypeptides were detected in extracts

of normal stratum corneum (Fig. 5(b)). These were very similar to chains 1 and 2 of prekeratin, the upper chain having a molecular weight of 70,000, while the lower chain had an estimated molecular weight of 60,000. Chain 3 was not present.

Spreading of the polypeptide chains during prolonged electrophoresis made densitometry difficult and chain separation was not significantly improved on either 5% or 10% acrylamide gels. Table 1(b) shows the results of successful scans and direct comparison of the stratum corneum and living cell proteins (Fig. 2) clearly demonstrated the structural change which accompanied keratinisation.

Urea Extraction of "Stratum Corneum" Residue from Whole Epidermis

Stratum corneum may be derived directly by scraping or sellotape stripping of a suitable body area or by extracting the proteins of the non-keratinised layers of whole epidermis to leave a horny layer residue. Breast "stratum corneum" prepared by the latter method failed to give the two chain structure characteristic of normal stratum corneum and retained instead a three chain structure similar to that of whole breast epidermis (Fig. 3).

Extraction of the living cell layers of whole epidermis with CASC buffer is apparently not a suitable method of preparing human stratum corneum. Extreme caution should be exercised if using this preparative procedure, and it is

advisable to first check the fibrous protein structure obtained with that of normal stratum corneum.

Urea Extraction of Plantar Callus

Analysis of urea extracts of plantar callus, showed that the most prominent components were three polypeptides with molecular weights identical to those of prekeratin (Fig. 6(b)). Band spreading again made densitometry impossible in a number of cases, but the results of successful scans are shown (Table 1(c)). The chain ratios showed considerable variability, but in each case the 70,000 molecular weight polypeptide was always most intense. Fig. 4 shows the structural differences between prekeratin from the living layers of the epidermis and the fibrous protein of plantar callus.

Characterisation of the Pale-Blue Staining Polypeptide of Foreskin Epidermis

The pale-blue staining band detected in extracts of foreskin epidermis (Fig. 1(c)) was eluted from gels and subjected to preliminary characterisation. It had a molecular weight of 45,000 and an amino acid composition as shown in Table 2 (a).

It was more basic than the epidermal fibrous protein (Skerrow, 1977a) and lacked cystine, but the possibility that it may represent a breakdown product of the fibrous protein polypeptides cannot be excluded. However, no polypeptides of this type have been observed in prekeratin solutions kept over a period of several months. The 45,000 molecular weight polypeptide has not been

detected in extracts of callus or stratum corneum, and its composition is unrelated to keratohyalin or actin. Dale (1977) found a protein of similar molecular weight in the epidermis of newborn rat, but it was compositionally dissimilar to the 45,000 molecular weight protein of foreskin epidermis. In particular the protein of rat tissue contained higher levels of arginine, serine and glutamic acid, but lacked lysine, found in large amounts in 45,000 molecular weight protein. In addition, the protein of newborn rat appears to be restricted to the horny cell layers.

Table 1: The Molar Ratio of the Fibrous Protein Chains of Prekeratin, Stratum Corneum and Plantar Callus.

Chain 1:	Molecular weight 70,000
Chain 2:	Molecular weight 63,000
Chain 3:	Molecular weight 55,000

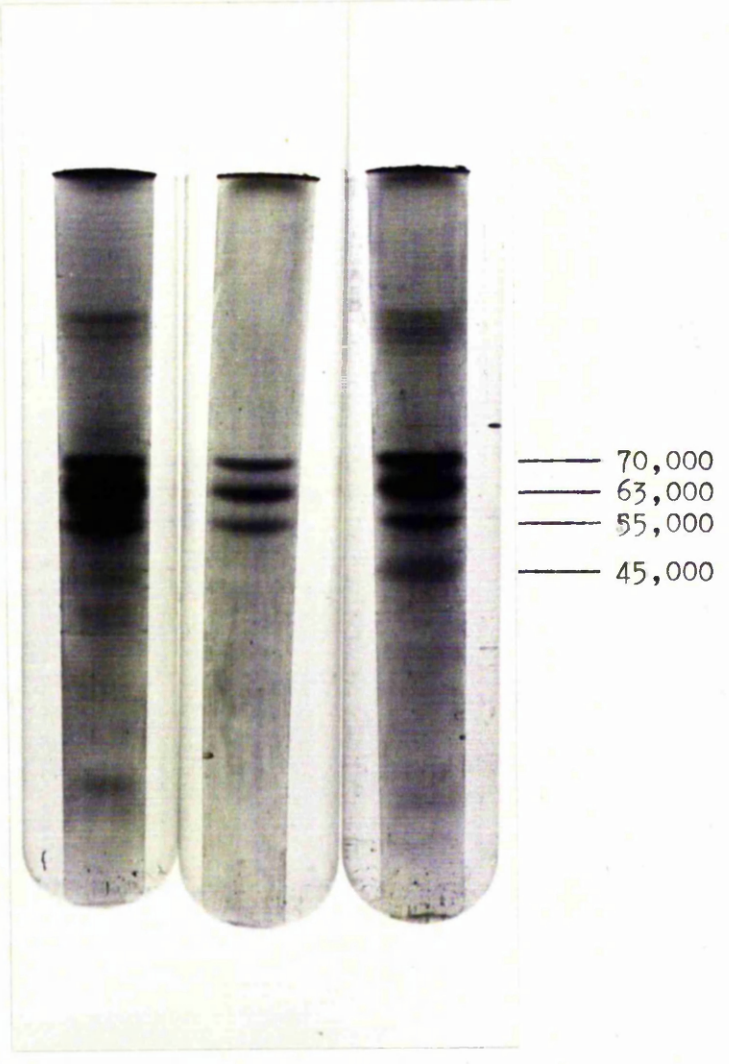
	Sample	Chain 1 : Chain 2 : Chain 3	Chain 1 : Chain 2 + Chain 3
	Prekeratin (foreskin)	1.2 : 2.0 : 1.0	1.0 : 2.5
	"	1.3 : 1.8 : 1.0	1.0 : 2.2
(a)			
	Prekeratin (breast)	1.2 : 1.6 : 1.0	1.0 : 2.2
	"	1.3 : 1.9 : 1.0	1.0 : 2.2
	Stratum corneum	1.0 : 1.8 : 0.0	
(b)	"	1.0 : 1.9 : 0.0	
	"	1.0 : 2.3 : 0.0	
	Callus	2.0 : 1.5 : 1.0	1.0 : 1.2
	"	2.0 : 1.0 : 1.5	1.0 : 1.2
(c)	"	2.0 : 1.0 : 1.5	1.0 : 1.2
	"	1.8 : 1.4 : 1.0	1.0 : 1.3
	"	3.7 : 1.5 : 1.0	1.0 : 0.7
	"	5.0 : 1.7 : 1.0	1.0 : 0.5

Figure 1: Comparison of Urea Extracts of Whole Epidermis and Purified Prekeratin.

(a) Extract of breast epidermis

(b) Purified prekeratin

(c) Extract of foreskin epidermis



(a) (b) (c)

Figure 1: Determination of the Molecular Weights of the Individual Polypeptide Chains of the Fibrous Protein of Stratum Corneum.

The arrows (⟶) indicate the mobility of the polypeptide chains of the fibrous protein of stratum corneum on 7.5% polyacrylamide gels.
The mobility of the polypeptide chains of prekeratin are shown for comparison (⇢).
Prekeratin consists of three different polypeptide chains of molecular weights 70,000, 63,000 and 55,000. Stratum corneum has two polypeptide chains of molecular weights 70,000 and 60,000.
The molecular weight markers are: phosphorylase a (94,000), bovine serum albumin (68,000), catalase (58,000), ovalbumin (43,000) and carbonic anhydrase (29,000).

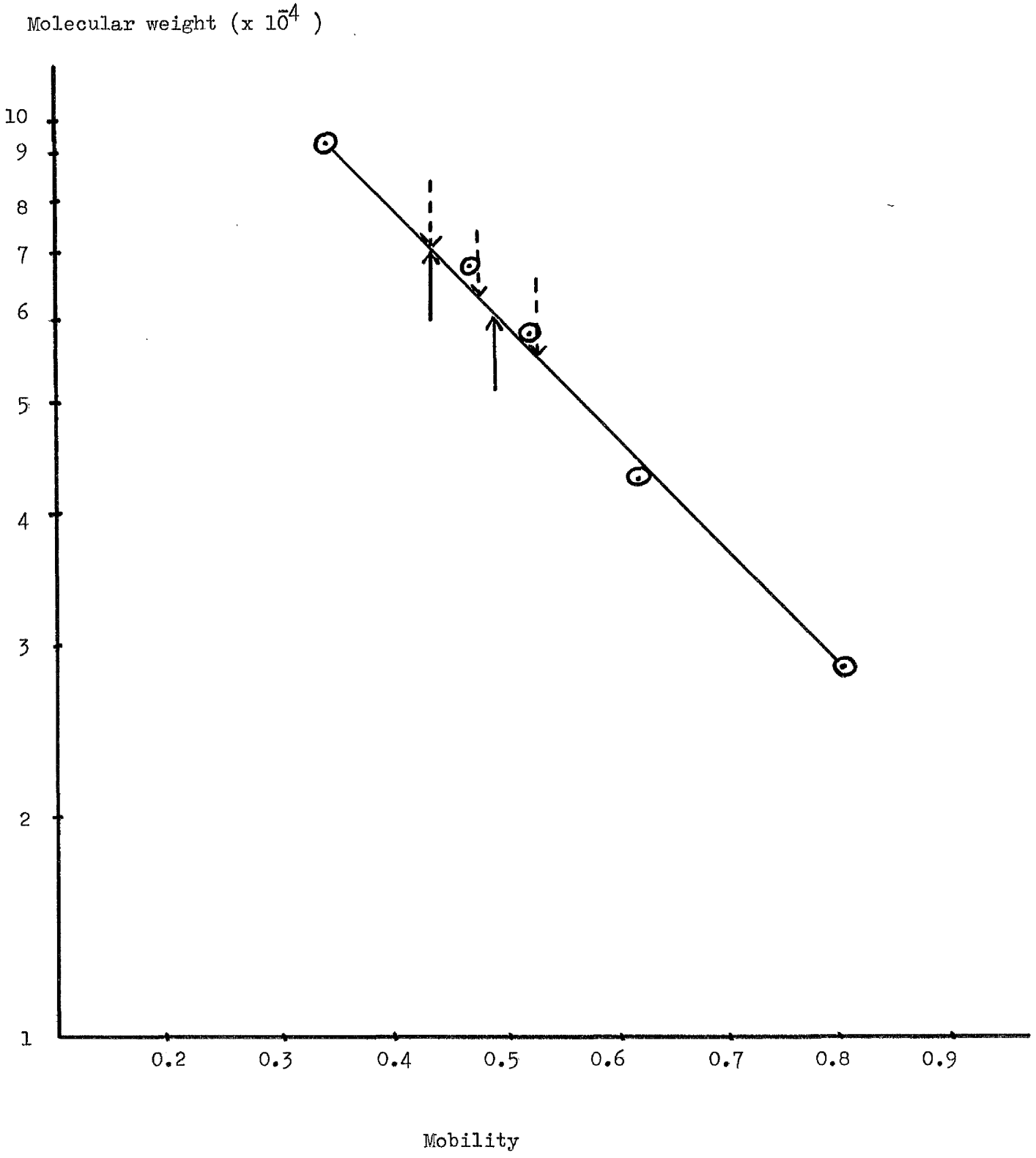

Molecular weight (x 10^4)
10
9
8
7
6
5
4
3
2
1
0.2
0.3
0.4
0.5
0.6
0.7
0.8
0.9
Mobility

Figure 2: Comparison of Densitometer Traces of Purified Prekeratin and the Fibrous Protein of Stratum Corneum

(——) Prekeratin.

(– –) Stratum corneum.

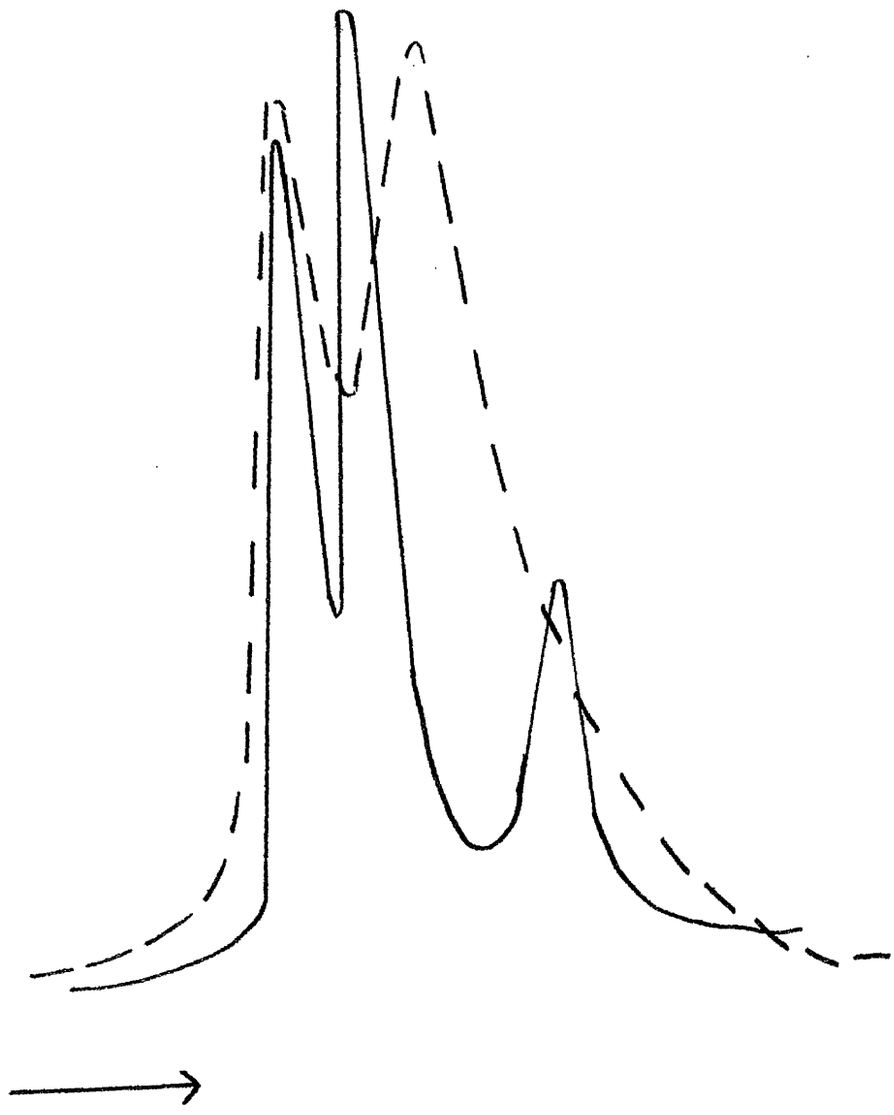
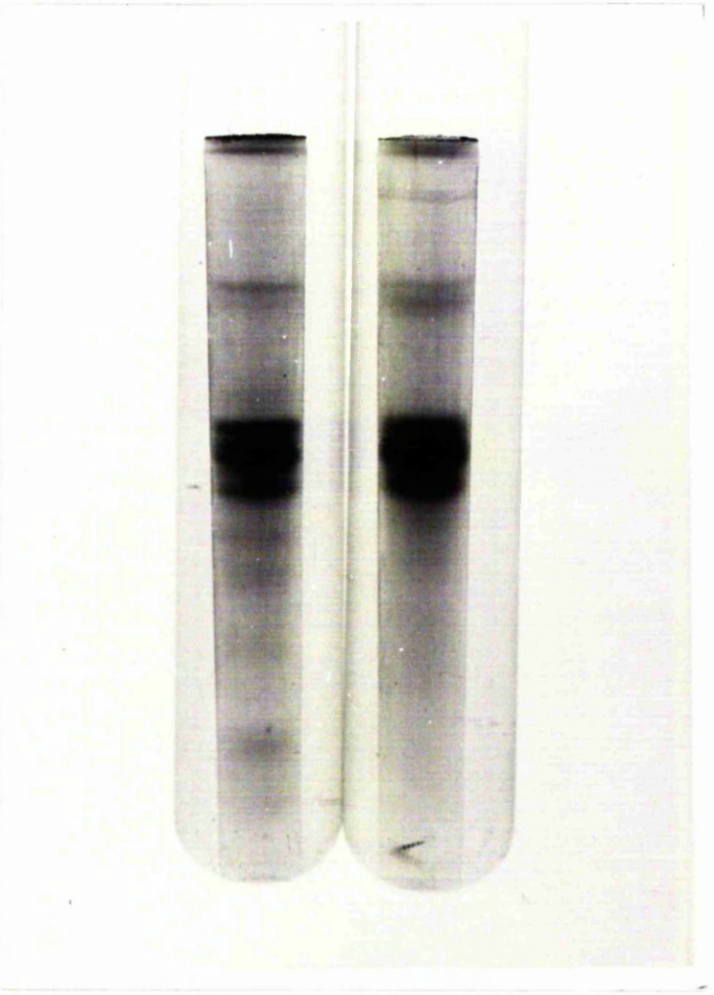


Figure 3: Comparison of the Fibrous Proteins from Breast Epidermis and Breast "Stratum Corneum".

(a) Breast epidermis

(b) Breast "stratum corneum"



(a) (b)

Figure 4: Comparison of Densitometer Traces of Purified Prekeratin and the Fibrous Protein of Plantar Callus.

(——) Prekeratin.

(– –) Callus.

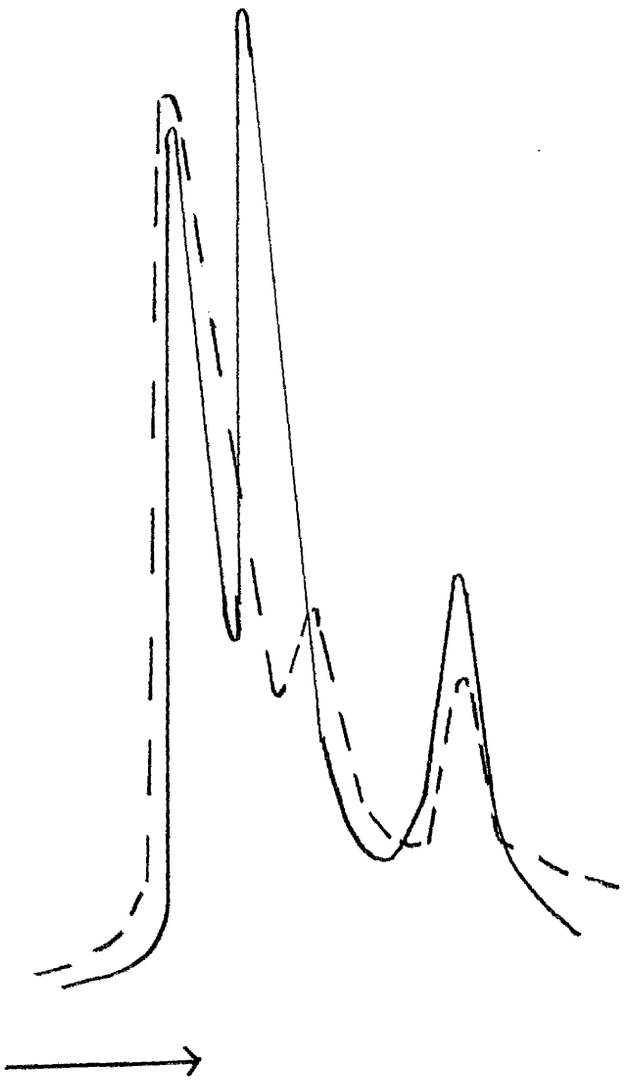


Table 2: Amino Acid Analysis.

The Composition (residues / 1000 residues)of:

(a) 45,000 molecular weight protein of foreskin epidermis

(b) Keratohyalin (Tezuka & Freedberg, 1972)

(c) Keratohyalin (Matoltsy & Matoltsy, 1970)

(d) Actin (Kirschenbaum, 1972)

(e) Stratum corneum basic protein (Dale, 1977)

Amino Acid	(a)	(b)	(c)	(d)	(e)
Lysine	92.0	5.5	26.0	51.7	0
Histidine	32.8	33.0	13.0	19.7	79.0
Arginine	53.2	82.0	62.0	51.7	139.0
Aspartic Acid	76.9	75.0	51.0	95.9	36.0
Threonine	57.8	54.0	41.0	68.9	57.0
Serine	101.1	93.0	90.0	56.6	173.0
Glutamic Acid	111.7	145.0	103.0	109.6	205.0
Proline	49.7	38.0	133.0	51.7	28.0
Glycine	140.0	106.0	130.0	78.7	144.0
Alanine	74.1	101.0	38.0	78.7	118.0
Valine	45.5	46.0	44.0	54.1	6.0
Methionine	0	10.0	0	41.8	0
Isoleucine	50.4	38.0	25.0	71.3	14.0
Leucine	71.7	75.0	92.0	71.3	0
Tyrosine	0	24.0	13.0	41.8	trace
Phenylalanine	43.1	29.0	35.0	34.4	0
Tryptophan	N.E.	N.E.	N.E.	41.8	N.E.
$\frac{1}{2}$ cystine	0	16.0	104.0	12.3	0

1.4 DISCUSSION

Human prekeratin consists of three different polypeptide chains on polyacrylamide gels. The stoichiometry of the polypeptide chains of prekeratin suggests the existance of a number of subunit structures *in situ*, and these may be the result of sequential changes from cell layer to cell layer. Dale & Stern (1975) have also detected alterations in the protein profile from each cell stratum of newborn rat epidermis, and some of these differences may be due to changes in the fibrous protein structure. CASC buffer does not attack all living cells at the same rate, the spinous layer apparently being the initial target. Thus, the incubation time in the buffer will probably be an important factor in determining the composition of the final extract. The ratio of the 70,000 molecular weight chain to the other two remained fairly constant at about 1:2 and was consistent with a three chain subunit structure. The reproducibility of the chain ratios obtained in the present study, no doubt reflects the constant method of preparation, whereas prolonged incubation may yield differing values.

During the transition from the living to the horny cell layers in normal epidermis, the fibrous protein undergoes a dramatic structural change. Only two prominent fibrous protein chains were detected in extracts of normal stratum corneum, the 55,000 molecular weight chain being lost or modified during terminal differentiation.

One possible mechanism for this structural change is that the chain is subject to proteolytic digestion. The onset of cornification is accompanied by the release of a large number of acid hydrolases from the lysosomes in the upper granular layer, which bring about the digestion of the subcellular organelles. Amongst these enzymes are a number of proteases (Jarrett, 1973) which may attack the fibrous protein, and digest the 55,000 molecular weight chain. The loss of only one of the fibrous protein chains would require preferential attack, and this will be examined more closely in Chapter 2. The absence of the 55,000 molecular weight chain may alter the mobility on polyacrylamide gels of the remaining polypeptides (Weber et al, 1972) and this may most readily explain the apparent decrease in the molecular weight of chain 2 in normal stratum corneum. The loss of a 3,000 molecular weight fragment although less likely, cannot be excluded.

Another possible explanation of the fibrous protein structure of normal stratum corneum is that the 55,000 molecular weight chain has been cross-linked during keratinisation in such a way as to prevent extraction, and there is evidence for the existence of cross-linking enzymes in the epidermis. Epidermal transglutaminases have been detected in cows' nose (Buxman & Wuepper, 1975) and human epidermis (Goldsmith & Baden, 1973) and in extracts of cultured keratinocytes (Rice & Green, 1977). They were localised in the granular layer, where they

have been implicated in keratin cross-linking via ε-(γ-glutamyl) lysine bonds (Buxman & Wuepper, 1975). Asquith et al (1970) identified such cross-links in digests of wool keratin and they have been suggested to exist in the stratum corneum proteins of both bovine and human epidermis (Baden & Goldsmith, 1972). However, in studies of human callus and cultured keratinocytes, Rice & Green (1977) found that ε-(γ-glutamyl) lysine dipeptides were almost exclusively localised in the cornified envelope protein.

The fibrous protein of normal stratum corneum is shown here to consist of two polypeptide chains. Comparison of the horny cell fibrous protein with that of the living cell layers indicates that a structural change accompanies normal cornification. Preliminary results suggest that a 1:2 chain ratio is present in human stratum corneum fibrous protein. Two polypeptides have been previously detected in newborn rat stratum corneum (Huang et al, 1975) and in the stratum corneum of cultured mouse keratinocytes (Steinert et al, 1977). The chain molecular weights were almost identical in each case and were present in a 1:1 ratio. However, in neither of these studies, was the structure of the living cell fibrous protein known, and no conclusion could be drawn as to whether the fibrous protein undergoes structural alteration during keratinisation.

The fibrous protein of plantar callus has been

shown here to be structurally dissimilar to that of the normal stratum corneum, retaining three polypeptide chains in the horny layer. The chain molecular weights are identical to those of prekeratin, although their intensities are somewhat altered. The chain ratios are extremely variable, but the 70,000 molecular weight chain is always most intense.

The fibrous protein structure in callus may again be the result of post-synthetic modification by either digestion or cross-linking. In order to account for the observed results, such processes would have to be more extensive than in the stratum corneum attacking both 63,000 and 55,000 molecular weight chains. Modification causes less complete chain loss in plantar callus.

Increased synthesis of the 70,000 molecular weight chain due to transcriptional or translational changes could also account for the observed protein structure in callus. Although it was assumed that the normal prekeratin structure was universally present in the living cell layers of human epidermis, no specific analysis of plantar living cell protein has been undertaken, and an altered fibrous protein may be synthesised in this tissue.

Ultrastructurally, the tonofilaments of the living and horny cell layers are apparently identical, while at the molecular level, significant structural changes in the fibrous protein, as keratinisation proceeds, have been detected. It is possible also, to distinguish

between normal stratum corneum and plantar callus of human epidermis by their fibrous protein structure.

In studies of bovine epidermis, Baden et al (1973a, 1976) and Steinert (1975) failed to detect differences in the polypeptide composition of living and horny cell fibrous protein. Baden et al (1973b,1976) prepared stratum corneum from whole epidermis by first extracting the living cell layers with CASC buffer. Fibrous proteins extracted from human "stratum corneum" prepared in this way were structurally more comparable to the living than the horny cell fibrous protein. This may explain the constant structure of the bovine protein. However, Steinert (1975) having obtained bovine horny layer directly, was still unable to detect any structural differences, and it may be that the thickened stratum corneum of bovine epidermis more closely resembles human callus, which retains a three chain structure similar to that of the living cell layers. Changes in chain intensities were not detected in bovine horny cell protein.

The only other major protein detected in human epidermis is seen as an unusual pale-blue staining band found in foreskin extracts. The protein had a molecular weight of 45,000 and was apparently restricted to the living cell layers. It has been shown to be unrelated to keratohyalin, actin or a basic protein of similar molecular weight found in newborn rat stratum corneum. The composition of the 45,000 molecular weight protein is not similar

to that of prekeratin overall, but it is possible that it may be a breakdown product of one of the fibrous protein chains.

CHAPTER 2: ABNORMAL KERATINISATION - PSORIASIS

2:1 INTRODUCTION

The psoriatic epidermis is characterised by the absence of keratohyalin granules (Brody, 1962), basal cell hyperproliferation (Weinstein & Frost, 1968) and a decreased transit time for keratinocytes through the epidermis (Van Scott & Ekel, 1963). The psoriatic stratum corneum lacks a regular cellular organisation (Menton & Eisen, 1971b) and there is a decrease in both the amount and the extent of aggregation of the epidermal tonofilaments (Brody, 1962; Lagerholm, 1965) which are otherwise indistinguishable from those of the normal epidermis. Psoriatic horny cells lack the plasma membrane thickening found in the normal tissue and a lack of subcellular organelle digestion gives rise to a parakeratotic stratum corneum.

Their have been few studies of the psoriatic tonofilamentaprotein. Roe (1959) isolated a fibrous protein from psoriatic epidermis which was apparently identical to the normal protein, as was the fibrous protein of psoriatic scales isolated by Bauer (1972). Skerrow (1977b) found however, that the fibrous protein of psoriatic scales was not structurally identical to human prekeratin or to the fibrous protein of callus.

Basal cell hyperproliferation has been implicated as the initial cause of psoriasis and both the cyclic nucleotides and chalone are suggested to control proliferation in the epidermis. Voorhees & Mier (1974) found that

the cyclic nucleotide levels in psoriatic epidermis were significantly different from those of the normal tissue, with the cyclic AMP level decreased and the cyclic GMP level elevated (Voorhees et al, 1976). Although this represents yet another abnormality of the psoriatic epidermis, its relationship to the initial cause of the disease remains unknown. Elgjo (1972) suggests that the chalone mechanism in psoriasis may be defective, but there is no evidence to suggest that this is the primary cause of pathogenesis.

It has also been proposed that a defect of keratinisation rather than hyperproliferation may initiate psoriasis. This is supported by the finding that during treatment of psoriatics, the granular layer reappears prior to a decrease in mitotic activity (Fry & McMinn, 1968). Also, Christophers & Braun Falco (1970) found that in experimental epidermal hyperplasia, it was possible to increase both the thickness and the turnover time of the epidermis to values very similar to those found in psoriasis without causing parakeratosis, although parakeratosis did occur if there was a defect in cell differentiation.

The stacking of horny cells into regularly arranged columns has been observed in normal epidermis (Menton & Eisen, 1971a) but is absent in psoriatic tissue (Menton & Eisen, 1971b). It was found that if normal stacked epidermis was wounded by compression, there was an increase in mitotic activity without the loss of cell order, while cell loss or damage caused by wounding led to disruption of

cell stacking (Christophers, 1971). Apparently then, an increased rate of new cell production by itself is not sufficient to produce loss of order or parakeratosis in mammalian epidermis. Nevertheless, it is clear that drugs which decrease cell proliferation are effective anti-psoriatics.

Corticosteroids may clear the disease very quickly, but withdrawal of this treatment often results in severe relapse. Triamcinolone causes a decrease in epidermal thickness (Jarrett & Witham, 1961) and may reduce cytoplasmic RNA levels, assumed to reflect lower levels of protein synthesis. In human skin, triamcinalone decreases the uptake of tritiated thymidine (Fisher & Maibach, 1971).

Methotrexate and hydroxyurea both act by inhibiting DNA synthesis. Methotrexate, a folic acid antagonist, competes as a substrate for dihydrofolate reductase and exerts its effect in the 'S' phase of the cell cycle (Weinstein & Velasco, 1972). Hydroxyurea inhibits pyrimidine biosynthesis and is apparently less effective but less toxic than methotrexate (Leavell et al, 1973). These antimitotic reagents although of proved use in the treatment of psoriasis may have dangerous side effects, are generally only employed in severe cases and require careful patient selection.

The combination of psoralens and long wavelength UV light (PUVA) is apparently a highly effective method of treating psoriasis which does not have many of the side effects of systemic treatment or the inconvenience of

topical therapy. Photoexcitation of the psoralen causes it to form a stable bond with the pyrimidine bases of DNA or interstrand cross-links between opposite bases (Petrozzi et al, 1977) resulting in the inhibition of DNA synthesis and cell multiplication. Wolff et al (1977) found that 90% of patients treated in this way were completely cleared.

Ultrastructurally, large paranuclear vacuoles were detected in PUVA treated psoriatic epidermis, and these grew to the detriment of the nucleus (Vukas et al, 1977). Topical administration of psoralens followed by UV light caused a high incidence of carcinomas (Griffin, 1959) while oral administration of the drug and UV light did not increase the level of carcinogenesis above that for UV light alone (MacDonald et al, 1963). The effect of long term treatment with PUVA remains to be evaluated.

The patients in this present study were being treated with topical applications of either crude coal tar (CCT) or dithranol. CCT contains thousands of compounds most of which remain unidentified, and although this treatment has proved effective, its mode of action remains unclear.

Dithranol is an effective anti-psoriatic structurally similar to acridine which is known to complex with DNA. It has been suggested that by interacting with DNA, dithranol may inhibit protein synthesis (Swanbeck & Thyresson, 1965) while work with bacterial systems suggests that mitochondrial DNA may be the primary target (Zetterberg & Swanbeck, 1971). Dithranol has the ability to decrease the activity of glyceraldehyde-3-phosphate dehydrogenase,

and glucose-6-phosphate dehydrogenase in psoriatic lesions (Hammar, 1970) either by synthetic control or direct regulation of enzyme activity.

The present work was undertaken in order to compare psoriatic scale proteins with those of normal callus and stratum corneum. In addition, the result of effective treatment on the structure of the fibrous protein has been studied.

2.2 MATERIALS AND METHODS

2.2.1 MATERIALS

Tissue Samples

Psoriatic scales were obtained from hospitalised patients being treated with crude coal tar or dithranol. Scales were collected from any affected area by gentle scraping, but preference was given to plaques several cm. in diameter and situated on flat areas of the trunk. When studying a patient over a complete treatment period, psoriatic scales were always collected from the same plaque and at the same time relative to the application of treatment. Psoriatic scales were stored desiccated at -30°C.

2.2.2 METHODS

Extraction of Tissue

Psoriatic scale (20mg/2ml) was extracted in 50 mM Tris-HCl, pH7.2 containing 6 M urea, 2% 2-mercaptoethanol as described previously.

Trypsinisation

Tryptic digestion of epidermis was by the addition of 1 ml trypsin solution (1mg/ml, in 50 mM Tris-HCl, pH 8) to 100 mg wet weight tissue. Samples were incubated at 37°C in a shaking water bath for various time intervals, from a few min. to two hr. The reaction was stopped by the addition of solid urea to give a final concentration of 6 M, and 2% ($^{v}/_{v}$) 2-mercaptoethanol. After stirring for a few min. samples were left to stand at 4°C for 24 hr., then extracted as described in Chapter 1.

Control samples containing 100 mg tissue/ml Tris-HCl, pH8 were extracted for 2 hr. at either 37°C or 4°C before the addition or urea and reducing agent. After stirring for a few min. the samples were left to stand at 4°C for 24 hr. and extracted as described in Chapter 1. A normal extraction of epidermis in 50mM Tris-HCl, pH 7.2 containing 6 M urea, 2% 2-mercaptoetland was also performed, as described in Chapter 1.

Electrophoresis and densitometry were as described previously.

2.3 RESULTS

Characterisation of the Fibrous Protein from Psoriatic Scale

When extracts of psoriatic scale were run on polyacrylamide gels, a major structural difference was noted (Fig. 5(a)). Whereas chains 2 and 3 of prekeratin were clearly present, chain 1 was either absent or greatly diminished in amount. A number of other minor bands were also present, including one of molecular weight 45,000, but this latter polypeptide lacked the pale-blue staining property of the polypeptide of the same molecular weight from foreskin epidermis.

Samples of crude coal tar or dithranol failed to produce any bands on polyacrylamide gels and prior extraction of psoriatic scales with chloroform: methanol (3:1) did not alter the polypeptide composition of psoriatic horny layer extracts. Thus, the abnormal fibrous protein structure detected in psoriatic scales does not appear to be an artefact, due to either the presence of topical treatment material or the increased lipid content of the tissue.

Comparison of the Fibrous Proteins of Psoriatic Scale and Normal Horny Layer

(a) Stratum Corneum

There were distinct structural differences between the fibrous proteins of normal stratum corneum and psoriatic scale (Fig. 5). The retention of chain 3 and the deficient chain 1 of the psoriatic protein characterised

it as abnormal with respect to the control tissue. There was in addition a significant increase in the number of low molecular weight polypeptides in psoriatic scale.

(b) Callus

The same three chains were present in extracts of both callus and psoriatic scale, but the chain intensities were somewhat different in the two tissues (Fig. 6). Thus, the intense chain 1 of callus was much depleted in the pathological horny layer, and again the psoriatic fibrous protein was abnormal when compared with the control. The large number of low molecular weight polypeptides detected in psoriatic scale extracts was not present in similar extracts of plantar callus.

The Effect of Treatment on the Psoriatic Fibrous Protein Structure

Remission of psoriasis during treatment was studied in about 20 patients. In some, treatment was not effective, while others were discharged at a relatively early stage of remission. Patients A and B described below are typical of the majority of in-patients who were treated successfully over a period of one to two weeks and then discharged in an advanced state of remission. In patient C, a particularly advanced stage of remission was achieved in the plaque studied, because more slowly responding plaques on other body sites delayed his discharge.

Patient A was treated over a period of 11 days and at the end of that time scaling had almost ceased. Chain 1 initially absent, gradually increased in intensity as

treatment proceeded (Fig. 7). Densitometer traces of the polypeptides confirmed the increased chain intensity (Fig. 8) and quantitatively this was reflected as a decrease in the ratio of chain 1 : chain 2 + chain 3 (Table 3(a)). As recovery proceeded there was no detectable qualitative or quantitative change in any of the low molecular weight polypeptides.

A subsequent series of extracts from patient B was obtained over a treatment period of 8 days. This patient was less severely affected and chain 1 was already present prior to treatment. The intensity of chain 1 gradually increased and was followed by densitometry (Table 3(b)).

Fig. 9 shows the effect of treatment on the fibrous protein from psoriatic scales of patient C. In this patient treated over a period of 14 days, there was not only a detectable increase in the intensity of chain 1, but this was accompanied by a decrease in the intensity of chain 3. The final product had a structure closely resembling that of normal stratum corneum in which chains 1 and 2 of prekeratin were prominent. The changing protein structure during recovery was followed by densitometry (Fig. 10) but spreading of the polypeptides prevented quantitative analysis. Towards the end of the treatment period, there was an increase in the amount of low molecular weight polypeptides.

The Structure of the Fibrous Protein in Uninvolved Horny Layer of Psoriatics

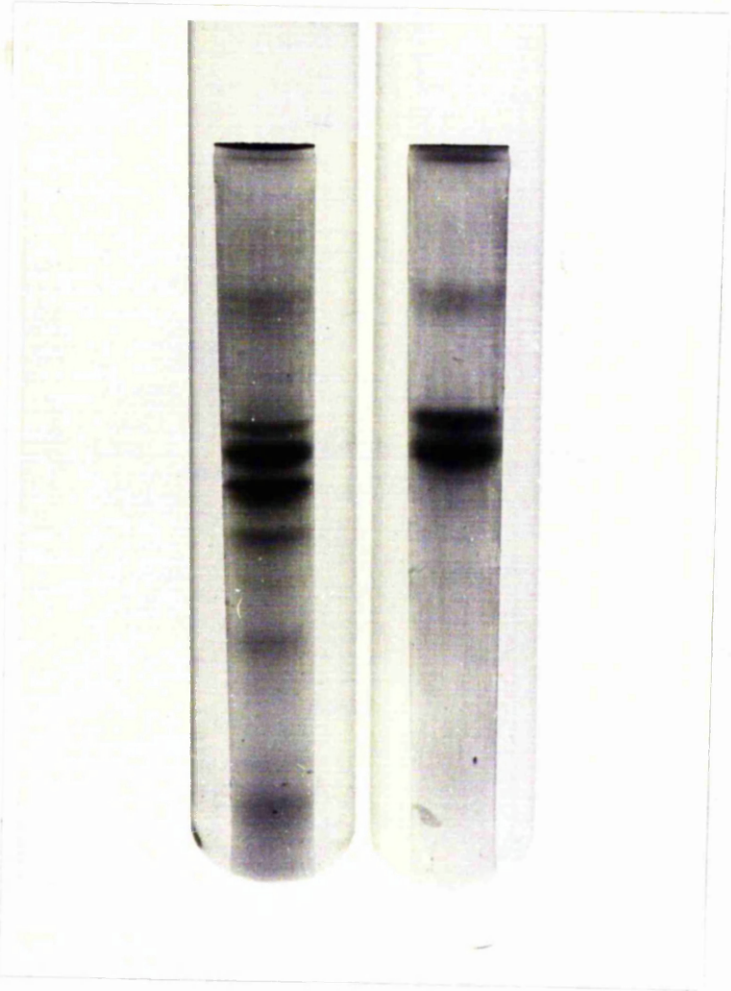
(a) Stratum Corneum

Stratum corneum from an uninvolved area of patient

Figure 5: Comparison of the Fibrous Proteins of Psoriatic Scale and Normal Stratum Corneum.

(a) Psoriatic scale extract

(b) Stratum corneum extract



(a) (b)

Figure 5: Determination of the Molecular Weights of the Individual Polypeptide Chains of the Fibrous Protein of Psoriatic Scale.

The arrows (⟶) indicate the mobility of the polypeptide chains of the fibrous protein of psoriatic scale on 7.5% polyacrylamide gels.
The mobility of the polypeptide chains of prekeratin are shown for comparison (⇢).
Prekeratin consists of three different polypeptide chains of molecular weights 70,000, 63,000 and 55,000. The molecular weights of the psoriatic fibrous protein polypeptides are identical to those of prekeratin, but the 70,000 molecular weight chain is much reduced in amount(⋯).
The molecular weight markers are: phosphorylase a (94,000), bovine serum albumin (68,000), catalase (58,000), ovalbumin (43,000), and carbonic anhydrase (29,000).

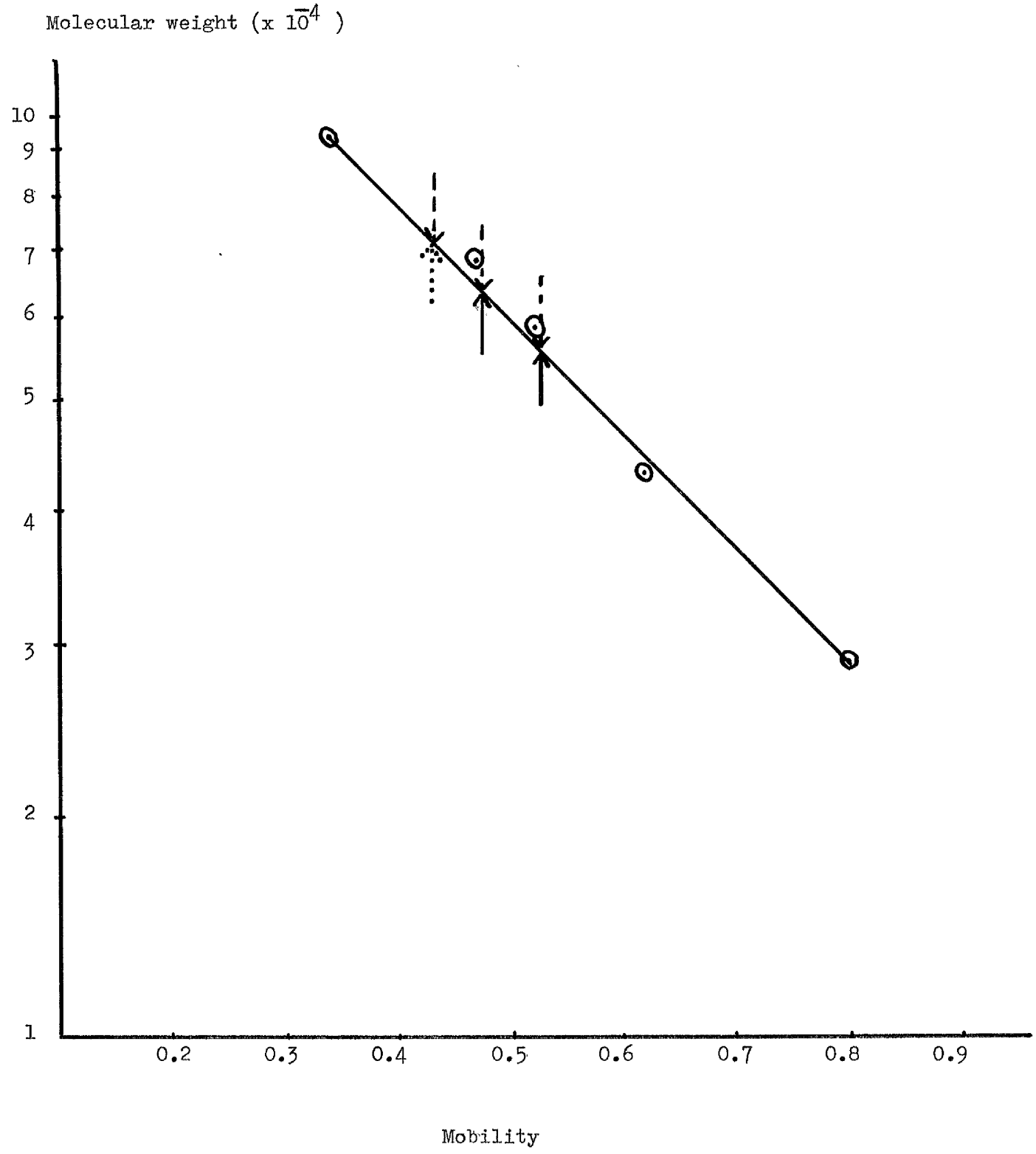
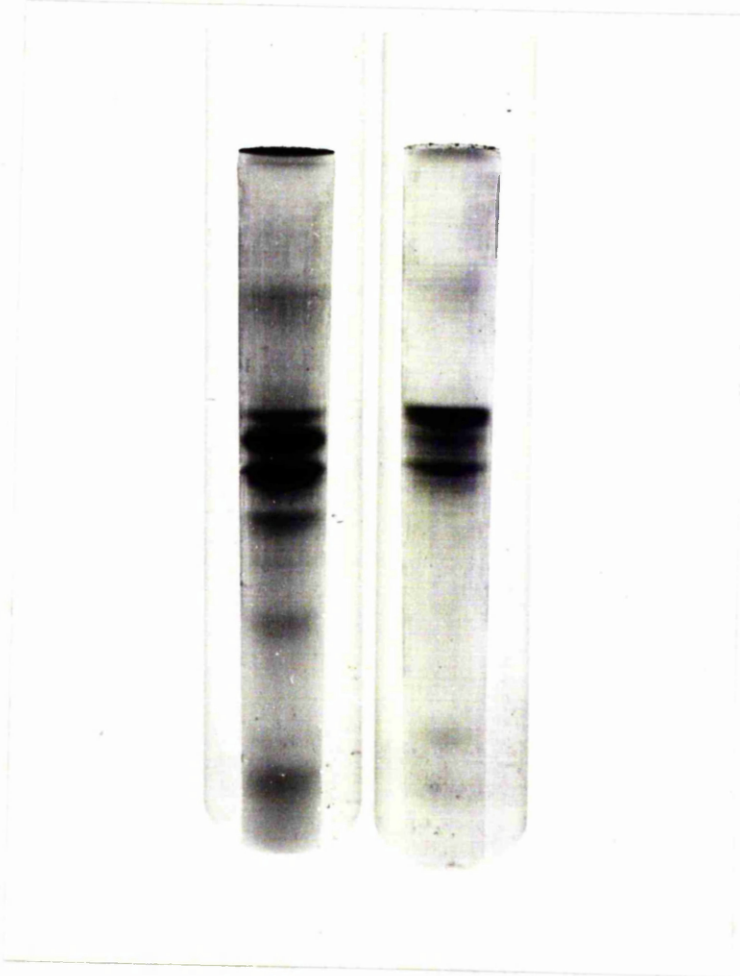

Molecular weight (x 10^{-4})
10
9
8
7
6
5
4
3
2
1
0.2
0.3
0.4
0.5
0.6
0.7
0.8
0.9
Mobility

Figure 6: Comparison of the Fibrous Proteins of Psoriatic Scale and Plantar Callus.

(a) Psoriatic scale extract

(b) Plantar callus extract



(a) (b)

Figure 7: Structural Changes in the Epidermal Fibrous Protein During Recovery of Patient A.

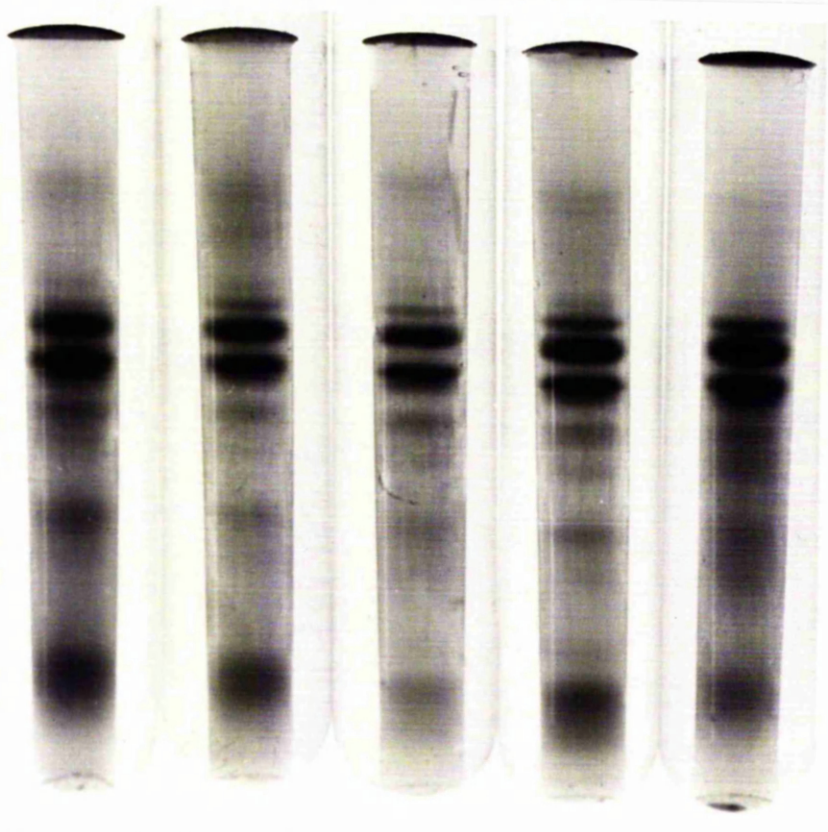
(a) Day 1, prior to treatment

(b) Day 4 of treatment

(c) Day 7 " "

(d) Day 9 " "

(e) Day 11" "



(a) (b) (c) (d) (e)

Figure 8: Densitometer Traces of the Chains of the Epidermal Fibrous Protein During Recovery of Patient A.

(a) Day 1, prior to treatment
(b) Day 4 of treatment
(c) Day 7 " "
(d) Day 9 " "
(e) Day 11 " "

Chain 1: molecular weight 70,000
Chain 2: molecular weight 63,000
Chain 3: molecular weight 55,000

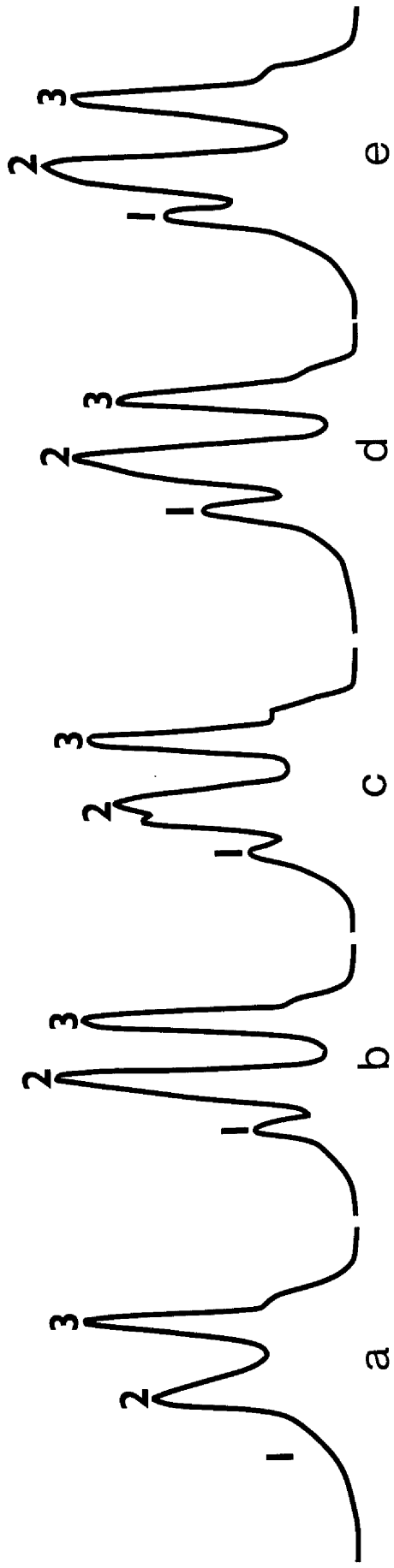

1
2
3
a
1
2
3
b
1
2
3
c
1
2
3
d
1
2
3
e

Table 3: The Molar Ratio of the Fibrous Protein Chains During Psoriatic Recovery

	Sample	Chain 1	Chain 2	Chain 3	Chain 1	Chain 2 + Chain 3
	Patient A					
	Day 1	0.0	1.0	1.0		
	Day 4	1.0	3.7	3.0	1.0	6.8
(a)	Day 7	1.0	3.8	2.6	1.0	6.4
	Day 9	1.0	3.2	2.0	1.0	5.2
	Day 11	1.0	2.4	1.6	1.0	4.0
	Patient B					
	Day 1	1.0	3.0	2.0	1.0	5.0
(b)	Day 5	1.0	3.0	1.3	1.0	4.3
	Day 8	1.7	3.3	1.0	1.0	2.5

Figure 9: Structural Changes in the Epidermal Fibrous Protein During Recovery of Patient C.

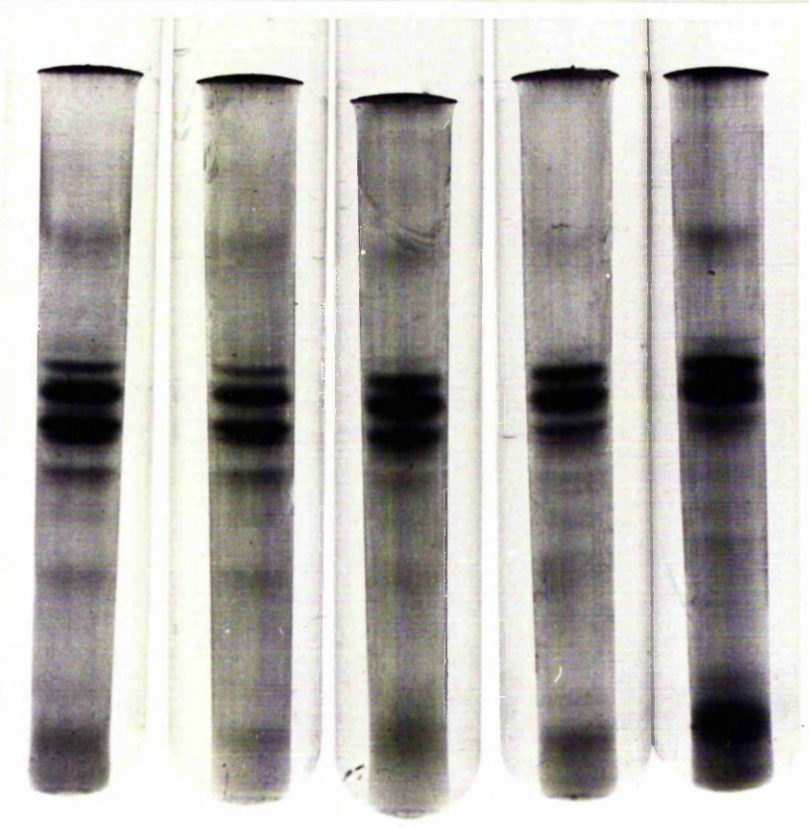
(a) Day 1, prior to treatment

(b) Day 4 of treatment

(c) Day 8 " "

(d) Day 11 " "

(e) Day 14 " "

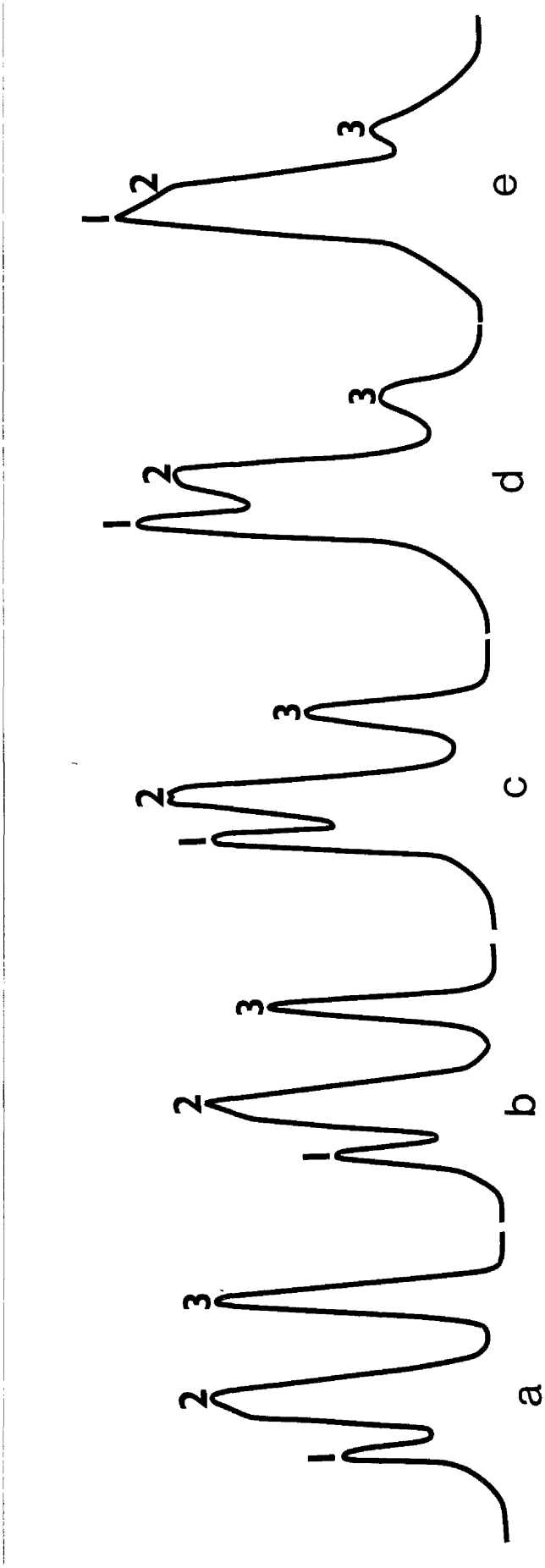


(a) (b) (c) (d) (e)

Figure 10: Densitometer Traces of the Chains of the Epidermal Fibrous Protein During Recovery of Patient C

(a) Day 1, prior to treatment
(b) Day 4 of treatment
(c) Day 8 " "
(d) Day 11 " "
(e) Day 14 " "

Chain 1: molecular weight 70,000
Chain 2: molecular weight 63,000
Chain 3: molecular weight 55,000


1
2
3
a
1
2
3
b
1
2
3
c
1
2
3
d
1
2
3
e

C had a normal fibrous protein structure (Fig. 11(c)), almost identical to that obtained at the end of treatment. The structure in both cases corresponded to chains 1 and 2 of prekeratin and differed only from normal stratum corneum in that chain 2 had a molecular weight of 63,000 rather than 60,000.

(b) Callus

Urea extracts of the uninvolved callus of several psoriatic patients had a three chain fibrous protein structure with an intense chain 1 characteristic of the normal tissue (Fig. 11 (a)), while involved callus showed a typical "psoriatic pattern", in which chain 1 was deficient (Fig. 11(b)).

Tryptic Digestion of Whole Epidermis

The fibrous protein of psoriatic scale has a deficient or absent chain 1. It is possible that this structural abnormality is the result of post-synthetic modification of the protein by proteolytic digestion. This possibility was tested by performing a tryptic digestion of human epidermis. Whole tissue rather than purified prekeratin was used as it was believed that this would more closely parallel the conditions *in vivo*.

Fig. 12 shows the effect of tryptic digestion of human epidermis. Prolonged digestion resulted in the formation of increased amounts of low molecular weight polypeptides, and by 2 hr, most of the fibrous protein

chains were no longer visible. Between 5 min. and 10 min., both chain 1 and chain 3 appeared to be digested, while chain 2 remained intact.

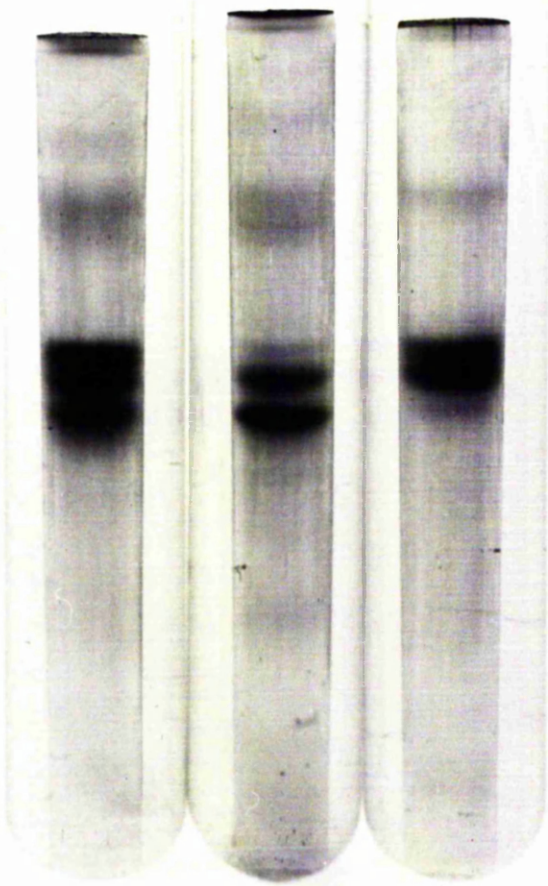
Tryptic digestion of whole epidermis failed to produce a psoriatic fibrous protein structure, and would tend not to favour proteolytic digestion of chain 1 during psoriatic keratinisation.

Figure 11: The Structure of the Epidermal Fibrous Protein in Involved and Uninvolved Horny Layer of Psoriatics

(a) Uninvolved Callus

(b) Involved Callus

(c) Uninvolved Stratum Corneum



(a) (b) (c)

Figure 12: Tryptic Digestion of Human Epidermis

(a) Control at 37°C

(b) Control at 4°C

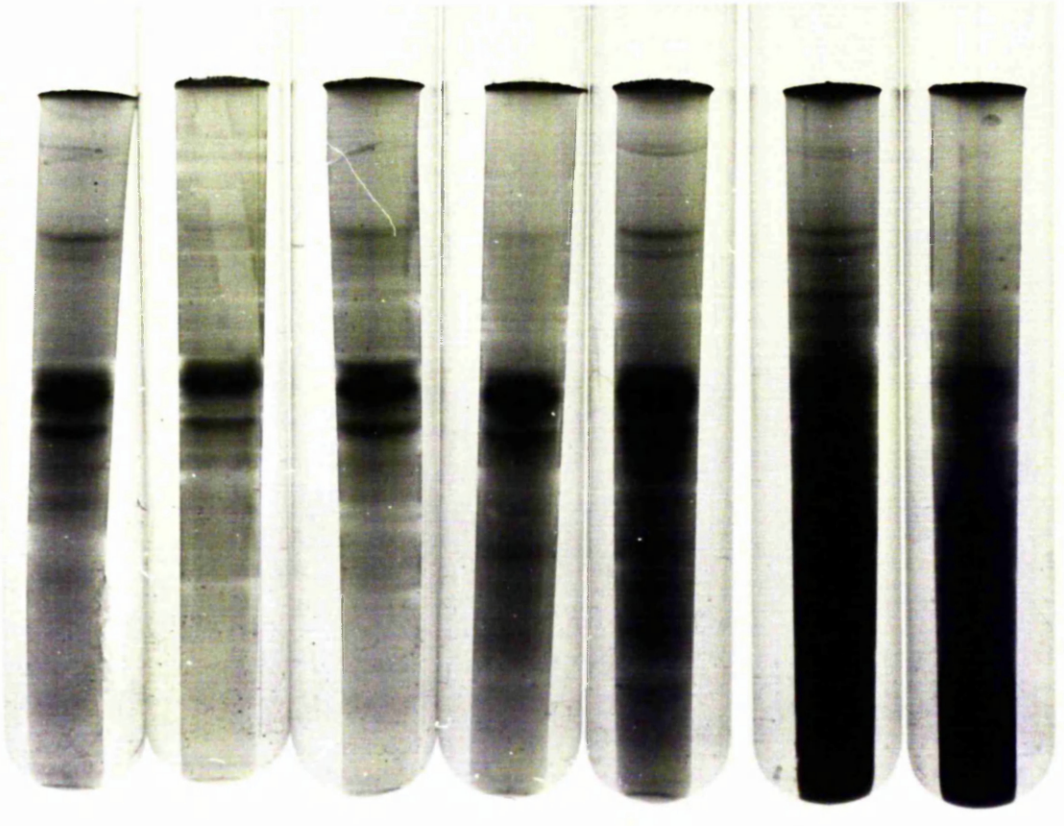
(c) Normal extraction

(d) 2 min. digestion

(e) 5 min. "

(f) 10 min. "

(g) 2 hr. "



(a) (b) (c) (d) (e) (f) (g)

2.4 DISCUSSION

The fibrous protein of psoriatic scale had an abnormal structure in which the 70,000 molecular weight chain was deficient or absent, and this was in agreement with the preliminary study of Skerrow (1977b). This deficiency was observed in both affected stratum corneum and plantar callus, despite the structural dissimilarity of the fibrous protein from these horny layers in normal epidermis. Description of the structural abnormalities of the psoriatic fibrous protein depends upon the normal control chosen. This is in contrast to a previous chemical analysis (Flesch & Esoda, 1957), in which the differences present in psoriatic scales were constant regardless of the control tissue used.

In a previous structural study, Bauer (1972) was unable to detect any abnormality of the psoriatic fibrous protein and an α-fibrous protein from psoriatic scale was compositionally similar to the protein from normal epidermis (Baden & Bonar, 1968). Peptide mapping studies (Rothberg, 1960) detected a number of differences between normal and psoriatic keratin fractions, suggested to reflect defective fibrous protein synthesis in the pathological epidermis.

Although the effect of treatment on clinical remission of psoriasis has been widely studied (Young, 1970; Weinstein & Velasco, 1972; Wolff et al, 1977), its affect on the structure of the epidermal fibrous protein has not

been previously reported. Topical therapy was found here, to cause a gradual change in the molecular structure of the fibrous protein. The recovery process started in patients A and B involved an increase in the intensity of the 70,000 molecular weight chain and was completed in patient C by the disappearance of the 55,000 molecular weight chain. The final fibrous protein structure closely resembled that of normal stratum corneum.

The loss of the 55,000 molecular weight chain during treatment was analogous to the structural change which accompanied cornification in normal epidermis, believed to be due to modification of the fibrous protein by digestion or cross-linking. Indeed, during the recovery of patient C there was an increase in the level of low molecular weight material detected in the psoriatic scale extracts, which appears to be correlated with the disappearance of the 55,000 molecular weight chain. This chain, which was shown to be trypsin sensitive, may then be subject to proteolytic digestion.

The structure of the fibrous protein from the living layers of psoriatic epidermis is unknown, and thus it is not clear whether the abnormal structure detected in psoriatic scales persists at all levels of the involved tissue or is the result of defective cornification. The 70,000 molecular weight chain was also found to be susceptible to tryptic digestion and a modification of this type may occur during psoriatic keratinisation, increased

proteolytic activity being detected in the upper layers of the pathological epidermis (Herrmann,1976). The 70,000 molecular weight chain was not subject to modification during normal cornification, but Fräki & Hopsu-Havu (1976) found that the proteases of psoriatic epidermis differed from those of the normal tissue.

Impaired protease and hydrolase activities have been detected in psoriatic epidermis, resulting in low free amino acid levels (Flesch et al, 1962), high peptide levels (Wheatley & Farber, 1961) and the presence of undigested subcellular organelles in the parakeratotic stratum corneum. This may be due to defective enzyme release from the lysosomes (Reid & Jarrett, 1967) or the increased rate of epidermal turnover (Van Scott & Ekel, 1963) and does not favour enzymic modification of the 70,000 molecular weight chain of the fibrous protein in psoriasis.

The 70,000 molecular weight chain was apparently structurally unaltered in psoriasis, and simply decreased in amount. A similar situation has been observed in the thalassemias, pathological conditions of Man resulting from decreased synthesis of either the α or β chains of globin (Comings, 1970). This synthetic imbalance is the result of a genetic disturbance possibly causing supression of mRNA translation (Heywood et al, 1965) or defective mRNA production (Clegg et al, 1968). A similar repression of the synthesis of the 70,000 molecular weight chain in psoriasis would yield the observed abnormal fibrous

protein structure. Voorhees et al (1968) proposed that the absence of keratohyalin granules in psoriatic epidermis was due to repression of the keratohyalin cistrons. Tsuji & Cox (1977) found significant enlargement of the nuclei and nucleoli in psoriasis and a decrease in the amount of repressed DNA sequences, which is in contrast to the proposed increase in repressed structural protein cistrons.

In a review of the available genetic data on psoriasis, Kimberling & Dobson (1973) concluded that an autosomal dominant mode of inheritance was most likely and although the genetic predisposition is a major factor in determining the occurrence of eruptions, appropriate environmental factors are also necessary in the majority of cases. Dominant disorders are normally associated with defects of non-enzymic proteins (McKusick, 1971) and this is in agreement with the proposed primary role for the defective fibrous protein detected in psoriatic tissue.

Increased DNA synthesis and thymidine incorporation (Newbold, 1972) and enhanced activity of the pentose monophosphate shunt (Gordon & Johnson, 1967) have been detected in the uninvolved skin of psoriatics. There was also an increase in the level of the acid hydrolases (Jarrett, 1973) and in the lipid content (Madden, 1941). All these studies were in contrast to the analysis of the fibrous protein of uninvolved horny layer of psoriatics, which was indistinguishable from that of the normal tissue.

It is suggested that psoriasis most probably results from a defect of keratinisation in which there is production of an abnormal fibrous protein, and that this leads to the defective cellular architecture and increased cell proliferation characteristic of the disease. This defect may directly involve the structural gene coding for the 70,000 molecular weight chain of the fibrous protein or is involved in the modification of a repressor protein.

The alternative possibility that the fibrous protein is subject to post-synthetic modification by either digestion or cross-linking seems less likely, but cannot be ruled out. Trypsinisation of epidermis failed here, to produce a psoriatic protein pattern and in the psoriatic epidermis with its greatly increased rate of turnover it seems unlikely that a specific cross-linking mechanism for the fibrous protein would be present or elaborated.

CHAPTER 3: THE COMPOSITION OF CORNIFIED CELL PROTEINS

3.1 INTRODUCTION

The fibrous protein of the epidermal tonofilaments has been isolated and purified from the living layers of both cows' nose (Matoltsy, 1965) and human epidermis (Skerrow, 1977a). Bovine prekeratin is a multichain protein of two, three chain subunits, with an α-helix content of about 40%. Two discrete three chain α-helical regions about 20 nm in length are present in each subunit, and these are rich in the helix-favouring amino acids, glutamic acid, alanine and leucine whilst the helix-inhibiting residues, proline, glycine and serine are preferentially located in the non-α-helical regions (Skerrow et al, 1973).

The cystine content of the epidermal fibrous protein is very low, only 8.8 residues/1000 residues for bovine prekeratin (Skerrow, 1972), and is distributed between α-helical and non-α-helical regions (Skerrow et al, 1973). It has been shown that there is no interchain disulphide bond stabilisation of the living cell fibrous protein (Skerrow, 1974, 1977a) although intrachain disulphide bonds have been detected in bovine prekeratin (Steinert, 1975).

The cornified cells are resistant to the action of CASC buffer, and extraction of the intracellular contents requires the use of denaturing solvents. The stability of the horny layer has been generally considered to reflect disulphide bond stabilisation of the fibrous protein.

Baden et al (1976) found that the fibrous protein of bovine stratum corneum, isolated in the absence of a reducing agent, was a high molecular weight aggregated

material stabilised by interchain disulphide bonds. This stabilisation of the fibrous protein apparently occurs during cornification since no disulphide bonding of the living cell protein has been detected (Baden et al, 1973a, 1976).

In contrast, Steinert (1975) found that the same amount of fibrous protein polypeptides could be extracted from the horny cells of bovine epidermis in the absence as in the presence of a reducing agent, although mechanical disruption of the horny layer was required in the former case. The stability of the cornified cells was attributed to disulphide bonding of the cell envelope protein, which agreed with the chemical study of Matoltsy & Matoltsy (1966).

The keratohyalin granules of the epidermis are believed to form the interfilamentous matrix of the horny cells (Lavker & Matoltsy, 1970). Intact granules isolated from newborn rat epidermis (Matoltsy & Matoltsy, 1970) had a high cystine content, which was subsequently also detected in the keratohyalin granules by electron microscope autoradiography (Matoltsy, 1975).

Keratohyalin isolated by an alternative extraction procedure was shown to be histidine rich, cystine poor (Hoober & Bernstein, 1966; Gumucio, 1967) while Ugel (1969, 1971) extracted a ribonucleoprotein, thought to be keratohyalin, in which the protein component was rich in serine, arginine, glycine and histidine (Ugel & Idler, 1972).

It has been calculated that the matrix of the horny cells contains about ten times more sulphur than the fibrous protein (Matoltsy, 1976) and this is not consistent with a histidine rich, cystine poor protein being the major component of keratohyalin granules. The suggestion that the histidine rich protein may be derived from the interstitial space of the keratohyalin granules (Matoltsy, 1975) awaits further investigation.

Thus,determination of the amino acid composition of epidermal proteins has been of value in determining the nature of the bonding forces present in these proteins, and has provided an insight into their possible function during the keratinisation process.

The preparation of proteins for amino acid analysis requires an initial acid hydrolysis and this may cause degradation of some residues. Tyyptophan is totally destroyed during acid hydrolysis. Serine, threonine and tyrosine are particularly susceptible to attack, and lysine, arginine and histidine may be partially destroyed. Temperature control is of particular importance during hydrolysis, and slight variations may cause an alteration in both the rate of decomposition and the release of amino acids.

The cystine content of a protein is generally measured after performic acid oxication. This converts cystine to cysteic acid, in which sulphur is in a stable oxidation state and allows accurate measurement of

this amino acid. Performic acid may also react with serine and threonine and forms several derivatives of tryptophan in which the indole ring is opened (Hirs, 1967).

The aim of this study was to compare the amino acid contents of callus, stratum corneum and psoriatic scale with that of purified prekeratin, and to attempt to correlate differences in amino acid composition with the differences in polypeptide chain composition described in Chapters 1 and 2.

3.2 MATERIALS AND METHODS

3.2.1 MATERIALS

Plantar callus, stratum corneum and psoriatic scale were obtained as described in Chapters 1 and 2.

3.2.2 METHODS

Amino Acid Analysis

Tissue samples were subjected in duplicate to amino acid analysis as described in Chapter 1. Cystine and cysteine were determined after performic acid oxidation.

3.3 RESULTS

The Composition of Human Prekeratin

The amino acid composition of human prekeratin is similar to that of the bovine fibrous protein (Skerrow, 1977a). Glycine, serine and glutamic acid were present in large amounts, while proline, methionine and cystine were present only in small amounts.

The Composition of the Horny Cell Proteins

There is an overall compositional similarity between normal and psoriatic horny layer (Table 4). As is the case for prekeratin, glycine, serine and glutamic acid are the most abundant and proline, methionine and cystine the least abundant amino acids in the cornified cells. The proline contents of psoriatic scale and normal stratum corneum are higher than those of prekeratin and plantar callus.

However, there were large differences between the glycine and serine contents of the tissues. Not only were large quantitative differences present, but in addition, the levels of these amino acids varied in an exact relationship to the intensity of chain 1 of the fibrous protein, and were the only residues to do so. Thus, callus with its increased proportion of chain 1 had high contents of glycine and serine while much lower levels were present in psoriatic scale, which had little or no chain 1. Prekeratin and normal stratum corneum had values between the two and were also intermediate in their contents of chain 1.

Table 4. Amino Acid Analysis.

Composition (residues/1000 residues) of:-

(a) Callus

(b) Stratum corneum

(c) Prekeratin (Skerrow, 1977a)

(d) Psoriatic scale

Amino Acid	(a)	(b)	(c)	(d)
Lysine	61.0	52.5	54.6	70.6
Histidine	20.2	26.3	14.4	20.3
Arginine	47.7	36.1	53.9	46.4
Aspartic Acid	78.6	68.6	87.2	87.8
Threonine	38.6	43.9	43.9	54.3
Serine	145.8	130.5	108.9	81.5
Glutamic Acid	121.7	114.2	119.2	118.8
Proline	22.3	48.6	22.7	56.7
Glycine	208.7	176.0	173.8	90.9
Alanine	39.2	53.5	53.5	65.3
Valine	34.9	43.5	49.8	60.7
Methionine	10.2	17.9	8.8	16.0
Isoleucine	36.4	36.5	45.9	47.1
Leucine	61.0	59.8	86.8	85.2
Tyrosine	33.9	33.7	26.7	35.8
Phenylalanine	28.0	37.0	36.3	35.6
$\frac{1}{2}$ cystine	13.6	21.4	13.3	27.0

3.4 DISCUSSION

The amino acid composition of human prekeratin is similar to that of the bovine protein (Skerrow, 1977a). Both have low cystine contents, and lack interchain disulphide bonds (Skerrow, 1974, 1977a).

There have been few previous analyses of human horny layer, and neither that of callus quoted by Flesch (1958) nor of psoriatic scale by Zahnd & Citron (1960) is in good agreement with the present results. In the latter case, this may be due to a hydrolysis time of only 15-20 hours and the subsequent elution of amino acids from a home made system of ion exchange columns, with manual addition of ninhydrin. An analysis of psoriatic scale by Liss & Lever (1963) was however, in agreement with the results obtained here, except for the increased glutamic acid and decreased cystine levels of their study. Baden et al (1973b) failed to distinguish between normal stratum corneum and callus, but the amino acid analysis of their α-fibrous protein, isolated from human horny layer closely resembles the present whole tissue analysis of plantar callus.

Despite the remarkable overall compositional similarity between prekeratin and both normal and psoriatic horny layer, the glycine and serine levels are particularly variable. The changes in the contents of these amino acids correspond with the changing

intensity of the 70,000 molecular weight chain of the fibrous protein, previously detected in these tissues.

In an analysis of previous data, a similar change in the levels of glycine and serine can be seen in a comparison of the compositions of α-fibrous proteins isolated from normal epidermis and psoriatic scale (Baden & Bonar, 1968) and from normal horny layer (Baden et al, 1973b). In a similar analysis of the individual polypeptide chains of bovine prekeratin (Baden et al, 1973a) glycine and serine were unevenly distributed, with higher amounts in the heavier molecular weight band. However, the fibrous protein chain composition found by Baden et al (1973a) was not in agreement with that of bovine prekeratin characterised by Skerrow (1972, 1974) and found to be structurally and compositionally similar to human prekeratin (Skerrow, 1977a). It is difficult, therefore, to compare their results with those of the present study.

Whole tissue amino acid analyses are derived from all the horny cell proteins and thus, the changing glycine and serine levels may not be associated with the fibrous protein. However, neither keratohyalin (Table 2) nor the cornified envelope protein (Matoltsy & Matoltsy, 1966) are rich in these amino acids, and a basic protein isolated from the horny cells of newborn rat (Dale, 1977) had a glycine and serine content similar to that of human prekeratin. It is possible then, that these amino

acids are preferentially located in the 70,000 molecular weight chain of the fibrous protein.

Neither glycine nor serine has any helix-favouring properties, and they are predominantly localised in the non-α-helical regions of bovine prekeratin (Skerrow et al, 1973). The possible function or benefit of such an uneven residue distribution is not clear. Little is known about aggregation of the fibrous protein into filaments, and it is suggested that the glycine and serine residues located in the non-α-helical regions of the fibrous protein may be involved in filament assembly.

Recently, it has been observed that the DNA levels of the nuclei decrease during keratinisation while the nuclear volume remains the same (Rowden, 1975). Suzuki et al (1977) have postulated that the space thus created in the nucleus becomes filled with newly synthesised protein. It is suggested that the DNA is completely replaced by protein and that this protein mass survives cornification. Thus, changes in these 'nuclear proteins' in normal and psoriatic epidermis may also account for the observed differences in composition.

CHAPTER 4: THE EFFECT OF EXTRACTION CONDITIONS ON CORNIFIED CELL PROTEINS

4.1 INTRODUCTION

Fibrous proteins apparently derived from the tonofilaments have been extracted from mammalian epidermis under a number of conditions. Using CASC buffer it has been possible to isolate the fibrous protein of the living cell layers in an undenatured form (Matoltsy, 1965). The horny cell layer is however resistant to the action of CASC buffer, and denaturing solvents are used in the extraction of horny cell fibrous proteins.

The fibrous protein of the living cell layers lacks inter-chain disulphide bonds (Skerrow, 1974, 1977a) but the nature of the bonding in the horny cell fibrous protein is in dispute. Fibrous protein fractions have been isolated both in the presence (Baden & Bonar, 1968; Huang et al, 1975); Baden et al, 1976) and in the absence of a reducing agent (Roe, 1956; Flesch & Esoda, 1957; Steinert, 1975). In many cases the isolated protein represents only a small amount of the total cell protein, while in other cases no quantitative analysis was performed, which makes interpretation of such data extremely difficult. Thus, although Roe (1956) and Flesch & Esoda (1957) were able to extract the fibrous protein of horny cells in the absence of a reducing agent, the amount of material isolated was so small that the remaining unextracted protein may well

have been disulphide bond stabilised.

The extraction of considerably more material from horny cells in the presence of reducing agent than in its absence (Baden & Bonar, 1968) need not necessarily indicate that the fibrous protein is disulphide bonded since both the protein of the cornified envelope (Matoltsy & Matoltsy, 1966) and the interfilamentous matrix protein (Matoltsy & Matoltsy, 1970) are believed to be stabilised by disulphide bonds. Indeed, in a study of bovine stratum corneum, Steinert (1975) found that the same amount of fibrous protein polypeptides could be extracted in the absence as in the presence of a reducing agent, provided the horny cells were disrupted mechanically in the former case. This indicated that the envelope protein and not the fibrous protein was disulphide bonded.

In contrast, Baden et al (1976) found that fibrous protein isolated from either bovine or human horny layer was a high molecular weight material which could be separated into individual polypeptide chains, when treated with a reducing agent.

There has been little work done on the extraction of fibrous proteins from psoriatic scales. Roe (1959) isolated a psoriatic fibrous protein which was indistinguishable from the normal protein and an α-fibrous protein extracted from psoriatic scale was

compositionally similar to the fibrous protein of whole epidermis. Flesch & Esoda (1957) found that the fibrous protein of psoriatic scale could be extracted in the absence of a reducing agent and that the amount of material isolated was three times as much as could be extracted from callus.

In addition to the effect of reducing agents, Baden & Bonar (1968) found that both the pH and the temperature of the extraction were important in determining the amount of material isolated from horny cells. The proteins isolated under different conditions were apparently very similar.

In the present study both the effect of pH and the presence of a reducing agent on the extraction of horny cells, has been studied. The efficiency of a particular extraction procedure is monitored by determining the amount of material isolated (dry weight analysis) and the composition of the isolated material determined by polyacrylamide gel electrophoresis.

4.2 MATERIALS AND METHODS

4.2.1 MATERIALS

Tissue Samples

Callus, stratum corneum and psoriatic scale were obtained as described in Chapters 1 and 2.

4.2.2 METHODS

Dry Weight Analysis, pH7.2

Tissue samples (20 mg dry weight callus or psoriatic scale) were immersed in 2 ml 50mM Tris-HCl, pH7.2 containing 6M urea. Samples to be reduced contained in addition, 2% ($^{v}/_{v}$) 2-mercaptoethanol. After extraction at 4°C for 24 hr., the samples were homogenised for 2 min. with an Ultra Turrax homogeniser, sonicated for 30 sec. and left to stand at 4°C for 2 hr. The extracts were then centrifuged at 50,000 x g for 10 min., and the supernatant retained. The pellet was then re-extracted under the same conditions, homogenised for 2 min., sonicated for 30 sec. and centrifuged at 50,000 x g for 10 min. to produce a second supernatant and final pellet. The supernatants were dialysed extensively against distilled water over a period of 48 hr. and the pellets washed with several changes of distilled water for the same time. The final samples were oven dried to constant weight, overnight at 110°C.

Dry Weight Analysis, pH10.6

Tissue samples (20 mg dry weight callus or psoriatic scale) were immersed in 2 ml 10mM Glycine-NaOH, pH10.6 containing 6M urea. 2% ($^{v}/_{v}$) 2-mercaptoethanol was added to samples to be reduced. Addition of 2-mercapto-ethanol to the buffer caused the pH to drop to about 9, and this was corrected by the addition of NaOH, before making up the 6M urea solution. Samples were incubated at 37°C for 2 hr. and extracted in an identical manner to those at pH7.2.

Extraction of Tissue with Urea Solutions, pH7.2

Tissue samples (20 mg callus, psoriatic scale or stratum corneum) were immersed in 2 ml 50mM Tris-HCl, pH7.2, containing 6M urea, 2% ($^{v}/_{v}$) 2-mercaptoethanol and extracted as described in Chapter 1. 2-mercaptoethanol was omitted from some samples, and in others,extracted in the absence of reducing agent, 1% 2-mercaptoethanol was added prior to electrophoresis.

Extraction of Tissue with Urea Solutions, pH10.6

Tissue samples (20 mg callus, psoriatic scale or stratum corneum) were immersed in 2 ml 10mM Glycine-NaOH, pH10.6 containing 6M urea, 2% ($^{v}/_{v}$) 2-mercaptoethanol. Addition of 2-mercaptoethanol cause the buffer pH to fall to about 9 and this was corrected by addition of

NaOH, before making up the 6M urea solution. After incubation at 37°C for 2 hr., the samples were extracted as described in Chapter 1 for pH7.2 samples. After addition of solid SDS, the final supernatants were acidified to pH7, before boiling and dialysis.

Electrophoresis was performed as described in Chapter 1.

4.3 RESULTS

Extraction of Plantar Callus

The results of dry weight analysis of callus fractions are shown in Table 5.

(a) Effect of pH

In the presence of 2-mercaptoethanol more material could be extracted from plantar callus at pH10.6 than at pH7.2, but in the absence of a reducing agent the pH of the extraction buffer had little effect on the extraction of callus.

(b) Effect of Reducing Agent

Both at pH10.6 and at pH7.2, considerably more material could be isolated from plantar callus in the presence of 2-mercaptoethanol than in its absence. Re-extraction of the initial pellets in the presence of a reducing agent, isolated an additional, smaller amount of material. In the absence of reducing agent re-extracted pellets normally yielded about the same or an increased amount of material.

Polypeptide Composition of Callus Extracts

Fig. 13 shows the polypeptide composition of human callus extracted under a number of conditions.

Considerably more material was present in the extracts obtained in the presence of a reducing agent. The major

components of all the extracts were the three polypeptide chains of the fibrous protein. The same polypeptides were present in all the extracts and varying the pH of the extraction buffer or adding 2-mercaptoethanol simply varied the amount of these polypeptides extracted. Addition of reducing agent to material extracted in its absence did not alter either the amount or the nature of the isolated polypeptides.

Extraction of Psoriatic Scale

Dry weight analysis of psoriatic scale is shown in Table 6.

(a) Effect of pH

Both in the presence and in the absence of 2-mercaptoethanol, more material was extracted from psoriatic scale at pH10.6 than at pH7.2. More material was isolated by re-extraction of the initial pellets at pH7.2 than at pH10.6.

(b) Effect of Reducing Agent

At pH10.6 and at pH7.2 addition of 2-mercaptoethanol to the extraction buffer increased the amount of material extracted.

Polypeptide Composition of Psoriatic Scale Extracts

The same polypeptides were extracted from psoriatic scale under all conditions (Fig. 14), and the major

bands were those of the fibrous protein. At identical loadings the sample extracted at pH7.2 was only just visible, while the extract obtained at pH10.6 in the presence of 2-mercaptoethanol was clearly overloaded.

Addition of reducing agent to the material extracted in its absence did not alter the polypeptide composition or the amount of the extracted protein.

Extraction of Stratum Corneum

Insufficient material prevented quantitative analysis of stratum corneum, but qualitatively the order of extraction as judged on polyacrylamide gels was similar to that for psoriatic scale. The two polypeptide chains of the fibrous protein were the major constituents of all extracts.

Table 5. Dry Weight Analysis of Plantar Callus.

(a) The amount of material extracted was expressed as a % of the starting weight.

Sample	Extraction Conditions	(a) 1st Supernatant %	(a) 2nd Supernatant %	(a) Pellet %
1	6M urea,	38.0	14.0	20.0
2	pH10.6, 2% 2-	47.0	9.0	16.0
3	mercapto-	45.0	4.0	28.0
4	ethanol	51.0	10.0	16.0
1	6M urea,	5.0	6.0	88.0
2	pH10.6	12.0	13.0	42.0
3		1.0	2.0	65.0
4		3.0	14.0	44.0
1	6M urea,	23.0	13.0	38.0
2	pH7, 2% 2-	35.0	6.0	22.0
3	mercapto-	23.0	9.0	41.0
4	ethanol	20.0	6.0	34.0
1	6M urea,	2.0	1.0	67.0
2	pH7	1.0	1.0	67.0
3		1.0	3.0	54.0
4		4.0	1.0	38.0

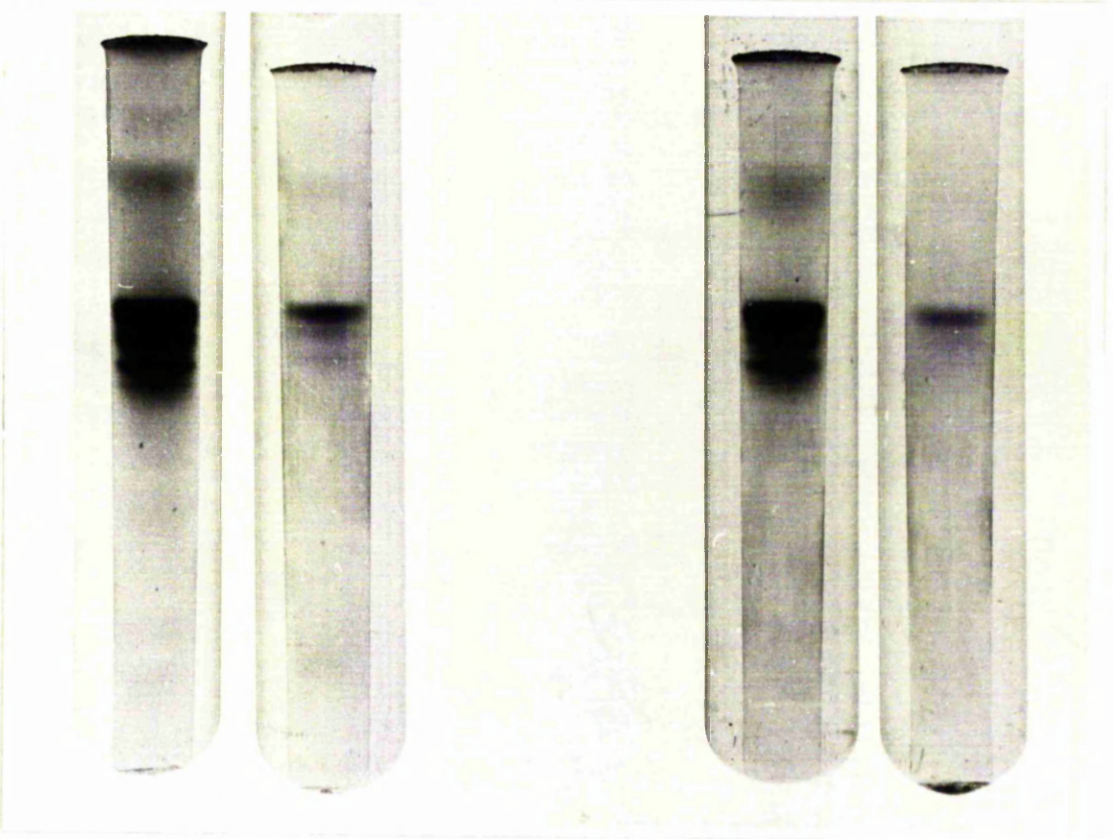
Figure 13. Urea Extraction of Plantar Callus

(a) Tissue extracted at pH10.6 in the presence of 2-mercaptoethanol.

(b) Tissue extracted at pH10.6.

(c) Tissue extracted at pH7.2 in the presence of 2-mercaptoethanol.

(d) Tissue extracted at pH7.2.



(a) (b) (c) (d)

Table 6. Dry Weight Analysis of Psoriatic Scale

(a) The amount of material extracted was expressed as a % of the starting weight.

Sample	Extraction Conditions	(a) 1st Supernatant %	(a) 2nd Supernatant %	(a) Pellet %
1	6M urea,	45.6	1.2	5.5
2	pH10.6, 2%	45.9	1.2	5.1
3	2-mercapto-	42.4	4.9	4.5
4	ethanol	57.0	8.0	2.5
5		42.0	12.0	1.5
1	6M urea,	35.9	1.8	15.5
2	pH10.6	30.2	0.7	8.0
3		29.3	8.9	8.0
4		48.5	7.0	2.0
5		28.0	5.0	4.0
1	6M urea, pH7,	24.6	5.1	8.0
2	2% 2-mercapto-	31.8	6.3	6.0
3	ethanol	18.3	10.0	9.0
4		40.0	8.0	8.0
5		14.5	11.5	29.0
1	6M urea, pH7	19.7	5.4	5.5
2		22.4	3.4	16.0
3		11.7	5.0	27.0
4		14.0	10.0	8.0
5		3.5	7.0	25.0

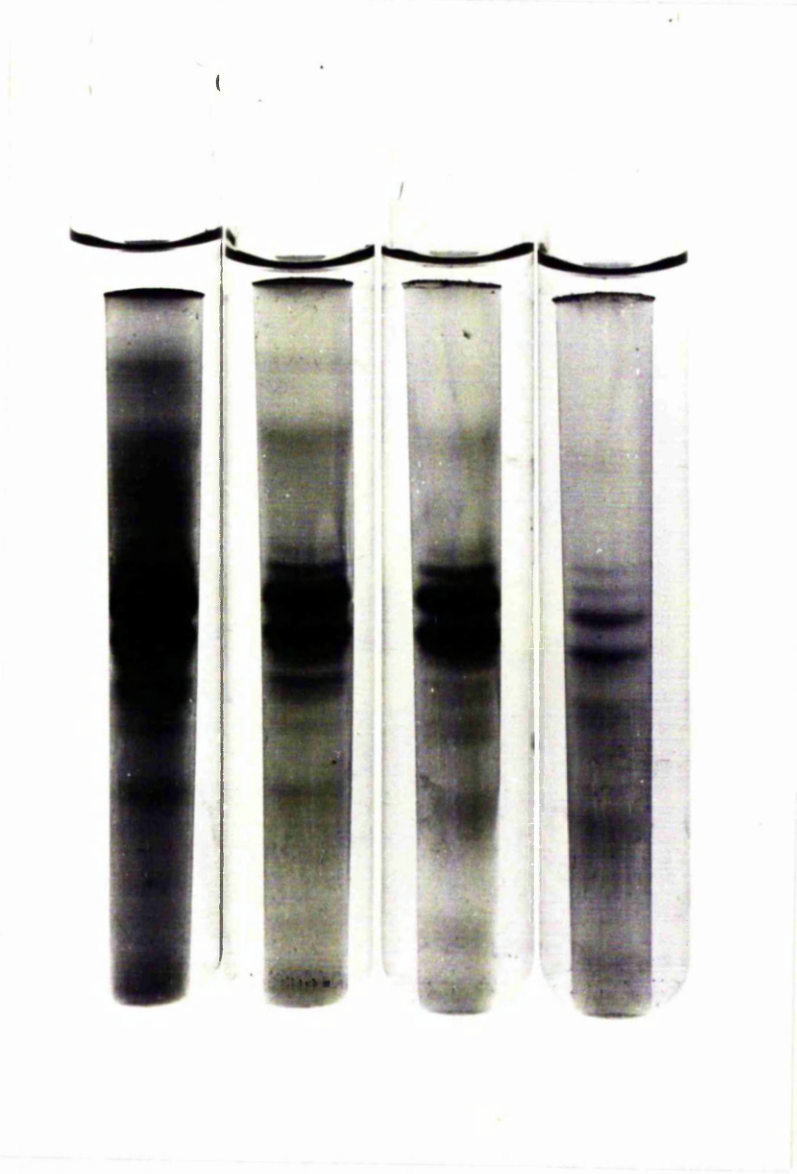
Figure 14. Urea Extraction of Psoriatic Scale

(a) Tissue extracted at pH10.6 in the presence of 2-mercaptoethanol

(b) Tissue extracted at pH10.6

(c) Tissue extracted at pH7.2 in the presence of 2-mercaptoethanol

(d) Tissue extracted at pH7.2



(a) (b) (c) (d)

4.4 DISCUSSION

Plantar callus and psoriatic scale differ with respect to their extractability with 6M urea solutions. The amount of material extracted from these horny layers is dependent upon both the pH of the extraction buffer and the presence of a reducing agent.

Alteration of the conditions of extraction of callus changed the amount but not the polypeptide composition of the isolated material. The major constituents of all the extracts were the three polypeptide chains of the fibrous protein. Efficient extraction of plantar callus required the presence of a reducing agent, and in its absence only small amounts of material could be extracted. Subsequent reduction of material extracted in the absence of a reducing agent did not cause either qualitative or quantitative alteration of the isolated ploypeptides, and no high molecular weight, disulphide bonded fibrous protein such as was found by Baden et al (1976) was detected in the present study.

Steinert (1975) found that in the absence of a reducing agent, the epidermal tonofilaments of bovine stratum corneum could be dissociated, but not released from the horny cells. After vigorous mechanical disruption of the horny layer, the fibrous protein polypeptides lacking interchain disulphide bonds could

be isolated in the same quantity as obtained in the presence of reducing agent. The release of the fibrous protein was apparently prevented by disulphide bond stabilisation of the cornified envelope protein, shown to be rich in cystine (Matoltsy & Matoltsy, 1966).

Similar disruption of the cells of plantar callus failed to release the fibrous protein polypeptides and this suggests that in the human horny layer some cell protein, probably either that of the interfilamentous matrix or of the cell envelope is disulphide bond stabilised.

Matoltsy & Matoltsy (1970) found that the keratohyalin granules which form part of the inter-filamentous matrix of the horny cells (Lavker & Matoltsy, 1970) were sulphur rich, and the stability of the horny cell layer has been attributed to disulphide bonding of the matrix protein (Matoltsy, 1975). Such a possibility would agree well with the results of the present work. The fibrous protein isolated from human callus in the absence of a reducing agent lacked interchain disulphide bonds, but it is possible that the remaining unextracted material is stabilised in this way.

Extraction of psoriatic scale yields the same polypeptides under all conditions, and the chains of the fibrous protein are easily identified. The psoriatic

fibrous protein apparently lacks interchain disulphide bonds, since large amounts of material may be extracted in the absence of a reducing agent. There is apparently, however, some disulphide bonded protein present in psoriatic scale since slightly more material may be isolated from the pathological tissue in the presence than in the absence of 2-mercaptoethanol.

Almost the same amount of material can be isolated from psoriatic scale and from callus in the presence of a reducing agent, but considerably more material is extracted from the pathological tissue when reducing agent is absent. This is in agreement with a previous quantitative analysis by Flesch & Esoda (1957). The ease of extraction of psoriatic scales would agree well with the proposed disulphide bond stabilisation of the interfilamentous matrix protein since the keratohyalin granules which form this matrix are absent from psoriatic epidermis (Brody, 1962).

Preliminary qualitative studies of normal stratum corneum showed that a large quantity of material could be extracted from this tissue in the absence of a reducing agent. The main components of stratum corneum extracts were the two polypeptide chains of the fibrous protein. The difference in the extractability of callus and stratum corneum presumably reflects differences in the disulphide bonding of the membrane or matrix proteins or possibly of the fibrous protein itself. The

intercellular cement of the horny layer is apparently stronger than the horny cells (Huber & Christophers, 1977) and changes in the composition of the intercellular material would also affect the extraction of the horny cell proteins. Plantar callus is apparently more highly stabilised by disulphide bonds than normal stratum corneum.

The stratum corneum is, however, generally regarded as being more stabilised than callus. Kligman (1964) found that the cells of callus were more readily dissociated by mechanical trauma or chemicals and that the cell envelopes of callus were more readily dissolved in alkali. Callus is many times more permeable to water and chemicals than the stratum corneum, and the protection given by callus is mainly attributed to its thickness. In the present study the cells of callus were found to be highly resistant to denaturing solvents while the cells of both psoriatic scale and normal stratum corneum were much more readily extracted.

The fibrous proteins of both normal and psoriatic horny layers are apparently lacking in interchain disulphide bonds, although disulphide bonding of some other horny cell protein, possibly that of the interfilamentous matrix or of the cell envelope may prevent release of the fibrous protein. The possibility that

the increased amount of material extracted from horny cells in the presence of reducing agent, was due to reduction of interchain disulphide bonds of the fibrous protein cannot be excluded.

GENERAL DISCUSSION

The fibrous protein prekeratin, from the living cell layers of human epidermis consists of three different polypeptide chains of molecular weights 70,000, 63,000 and 55,000 (Skerrow, 1977a). The three chains of the fibrous protein are retained in plantar callus, but their intensities are considerably altered and in particular, the 70,000 molecular weight chain is present in a much higher proportion. The stratum corneum contains only two fibrous protein polypeptides, the 55,000 molecular weight chain having been apparently modified during differentiation. The structural differences between the fibrous proteins of callus, stratum corneum and the living cell layers have not been previously detected, and indicate that during normal keratinisation, the fibrous protein is subject to structural alteration.

The most likely explanation of these structural changes appears to be post-synthetic modification of the fibrous protein by either cross-linking or proteolytic digestion. In normal epidermis only the 55,000 molecular weight chain is subject to modification, and in the stratum corneum this chain is completely absent. Chain modification in plantar epidermis is more extensive involving both 55,000 and 63,000 molecular weight chains, but the process is less complete since a small number of these chains are

present in callus.

The possibility that there is increased synthesis of the 70,000 molecular weight chain in plantar epidermis cannot be excluded, and in future work, it is hoped to test this possibility by analysing the fibrous protein from the living layers of plantar epidermis.

The fibrous protein polypeptides are the major constituents of both living and horny cell extracts. However, in extracts of foreskin epidermis an additional polypeptide is prominent. It has an unusual pale-blue staining property and a molecular weight of 45,000. Its amino acid composition does not correspond with any previously analysed epidermal protein and its function is not clear.

Only the 63,000 and 55,000 molecular weight chains of the fibrous protein are prominent in extracts of psoriatic scale, while the 70,000 molecular weight chain is deficient or absent. This protein structure is found both in affected callus and stratum corneum.

The absence of the 70,000 molecular weight chain may again be due to post-synthetic modification. However, the impaired protease activity of psoriatic epidermis which leads to incomplete digestion of the subcellular organelles, does not favour proteolytic digestion of the fibrous protein in the pathological tissue. Specific cross-linking of the 70,000 molecular weight

chain seems unlikely in psoriatic tissue, where both the cell cycle time (Weinstein & Frost, 1968) and the transit time for keratinocytes through the epidermis (Van Scott & Ekel, 1963) are significantly decreased.

The available structural information tends to favour decreased synthesis of the 70,000 molecular weight chain and this may be due to a genetic defect in psoriatics, affecting either the fibrous protein itself or repressor proteins involved in the control of fibrous protein synthesis. In this respect, psoriasis closely resembles the thalassemias, in which there is defective synthesis of either the α or β chains of globin (Comings, 1970). If the abnormal fibrous protein of psoriasis is the result of defective synthesis, then this structure should persist at all levels of the affected epidermis and future work will be concerned with a structural analysis of the living cell fibrous protein.

Effective treatment of psoriatics causes changes in the structure of the fibrous protein. There is an increase in the intensity of the deficient 70,000 molecular weight chain accompanied by a decrease in the intensity of the 55,000 molecular weight chain, finally resulting in a fibrous protein structure almost identical to that of normal stratum corneum. The

fibrous protein of the uninvolved stratum corneum of psoriatics is indistinguishable from that of the normal tissue. The recovery process has not been studied in involved plantar epidermis but the presence of a deficient 70,000 molecular weight chain in involved callus and the presence of a normal fibrous protein structure in uninvolved callus suggests that treatment would cause the production of a normal fibrous protein in this tissue also.

It is suggested that treatment of psoriasis overcomes the block in the synthesis of the 70,000 molecular weight chain which then gradually increases in intensity. As the psoriatic epidermis returns to normal, the fibrous protein is then subject to either digestion or cross-linking such as is believed to occur in normal tissue, resulting in the formation of stratum corneum and callus fibrous protein structures. The production of an abnormal fibrous protein in psoriatic epidermis could be the primary cause of the disease.

Callus, stratum corneum and psoriatic scale show an overall similarity in amino acid content and are closely related to purified prekeratin. The glycine and serine contents of these horny layers are however strikingly different, and they vary in an exact relationship to the intensity of the 70,000 molecular weight chain of the fibrous protein. In callus there

is an increase in the proportion of the 70,000 molecular weight chain and this tissue has high glycine and serine contents while psoriatic scale has only a low content of these residues and a deficient 70,000 molecular weight chain. As expected prekeratin and stratum corneum with their intermediate 70,000 molecular weight chain level, have glycine and serine contents between those of callus and psoriatic scale. The finding that no other epidermal protein so far isolated has a glycine and serine content as high as that of the fibrous protein favours the present suggestion that these residues are preferentially located in the 70,000 molecular weight chain of the fibrous protein. The function of these residues is not known, but it is possible that they may be involved in filament assembly.

Extraction of the horny cell layers at different pH values and in the presence and absence of a reducing agent alters the amount but not the polypeptide composition of the isolated material. The major constituents of all horny cell extracts are the polypeptide chains of the fibrous protein. In a quantitative analysis, it is found that a large amount of fibrous protein polypeptides can be extracted from psoriatic scale in the absence of a reducing agent, which indicates a lack of interchain disulphide

bonding of the psoriatic protein. However, only a small amount of fibrous protein lacking interchain disulphide bonds is extracted from callus in the absence of a reducing agent. It is suggested that the release of the fibrous protein is prevented by disulphide bond stabilisation of the interfilamentous matrix protein or of the cell envelope, although disulphide bonding of the fibrous protein itself cannot be excluded.

This preliminary study of the fibrous proteins of normal and pathological horny cell layers has revealed a large number of structural differences not previously detected. The work is now the basis for a more detailed analysis of these fibrous proteins and of the processes which bring about the structural changes. In this way a better understanding of the process of keratinisation will be gained. The initial cause of psoriasis which remains completely unknown, despite many years of study of the pathological epidermis may well be closely related to the production of an abnormal fibrous protein in this tissue.

REFERENCES

Abell, C.W. & Monahan, T.M.(1973) J. Cell Biol. 59, 549-558.

Adachi, K. & Yamasawa, S. (1966) J. Invest. Dermatol. 46, 473-479.

Allen, T.D. & Potten, C.S.(1974) J. Cell Sci. 15, 291-319.

Asquith, R.S., Otterburn, M.S., Buchanan, J.H., Cole, M., Fletcher, J.C. & Gardner, K.L. (1970) Biochim. Biophys. Acta. 207, 342-348.

Astbury, W.T. & Street, A.(1931) Phil. Trans. Roy. Soc. 230A, 75-100.

Baden, H.P. & Bonar, L.(1968) J. Invest. Dermatol. 51, 478-483.

Baden, H.P. & Goldsmith, L.A. (1972) Clin. Res. 20,415.

Baden, H.P., Goldsmith, L.A. & Fleming, B. (1973a) Biochim. Biophys. Acta. 317, 303-311.

Baden, H.P., Goldsmith, L.A. & Fleming, B. (1973b) Biochim. Biophys. Acta. 322, 269-278.

Baden, H.P., Lee, L.D. & Kubilus, J. (1976) J. Invest. Dermatol. 67, 573-576.

Balinsky, B.I. (1970) An Introduction to Embryology 3rd Edn., Saunders, Philadelphia and London.

Barrière, H., Litoux, P. & Geraut, C.(1974) Dermatologica 149, 257-265.

Bauer, F.W.(1972) Dermatologica 144, 217-228.

Bauer, F.W. & De Grood, R.M.(1975) Br. J. Dermatol. 93, 225-227.

Beavo, J.A., Hardman, J.G. & Sutherland, E.W. (1971) J. Biol. Chem. 246, 3841-3846.

Bernstein, I.A.(1964) In: The Epidermis (Montagna, W. & Lobitz, J.C., eds.) Academic Press, New York and London. pp.471-482.

Billingham, R.E. & Silvers, W.K. (1967) J. Exp. Med. 125, 429-446.

Braverman, J.H. & Yen, A.(1977) J. Invest. Dermatol. 68, 53-60.

Breathnach, A.S. & Wyllie, L.M.A.(1966) J. Invest. Dermatol. 47, 58-60.

Brody, I. (1960) J. Ultra. Res.4, 264-297.

Brody, I. (1962) J. Ultra. Res.6, 304-323.

Brody, I., Mishima, Y. & Matsunaka, M. (1974) J. of Cutaneous Path. 1, 33-46.

Bullough, W.S. (1962) Biol. Rev. 37, 307-342.

Bullough, W.S. (1975) Biol. Rev. 50, 99-127.

Buxman, M. & Wuepper, K.D.(1975) J. Invest. Dermatol. 65, 107-112.

Cairns, J.M. & Saunders, J.W. (1954) J. exp. Zool. 127, 221-247.

Christophers, E.(1971) J. Invest. Dermatol. 57, 241-246.

Christophers, E. Wolff, H.H. & Laurence, E.B.(1974) J. Invest. Dermatol. 62, 555-559.

Christophers, E. & Braun-Falco, O. (1970) Br. J. Dermatol. 82, 268-275.

Clegg, J.B., Weatherall, D.J., Na-Nakorn, S. & Wasi, P. (1968) Nature 220, 664-668.

Cohen, S. (1972) J. Invest. Dermatol. 59, 13-16.

Comings, D.E. (1970) In: Genetic Disorders of Man (Goodwin, R.M., ed.) Little, Brown & Co., Boston. pp. 143-198.

Constable, H., Cooper, J.R., Cruickshank, C.N.D. & Mann, P.R. (1974). Br. J. Dermatol. 91, 39-48.

Cooper, M.F., McGrath, H. & Shuster, S. (1976). Br. J. Dermatol. 94, 369-378.

Cox, A.J. (1969) J. Invest. Dermatol. 53, 428-435.

Crick, F.H.C. (1953) Acta. Crystallogr. 6, 689-697.

Dale, B.A.(1977) Biochim. Biophys. Acta. 491, 193-204.

Dale, B.A. & Stern, I.B.(1975) J. Invest. Dermatol. 65, 220-222.

Dawber, R.P.R., Marks, R. & Swift, J.A. (1972) Br. J. Dermatol. 86, 272-281.

Delescluse, C., Colburn, K.H., Duell, E.A. & Voorhees, J.J. (1974) Differentiation 2, 343-350.

Elgjo, K. (1972) J. Invest. Dermatol. 59, 81-83.

Epstein, E. & Maibach, H. (1965) Arch. Dermatol. 92, 462-468.

Fisher, L.B. & Wells, G.C. (1968) Br. J. Dermatol. 80, 235-240.

Fisher, L.B. & Maibach, H.I. (1971) Arch. Dermatol. 103, 39-44

Flesch, P. (1958) J. Invest. Dermatol. 31, 65-73.

Flesch, P. & Esoda, E.C.J. (1957) Arch. Dermatol. 76 393-401.

Flesch, P., Hodgson, C. & Esoda, E.C.J. (1962) Arch. Dermatol. 85, 476-484.

Fräki, J.E. & Hopsu-Havu, V.K. (1976) Arch. Derm. Res. 256, 113-126.

Fraser, R.D.B., MacRae, T.P., Millward, G.R., Parry, D.A.D., Suzuki, E. & Culloch, P.A.(1971). Applied Polymer Symposium No. 18, 65-83.

Fraser, R.D.B., MacRae, T.P. & Rogers, G.E. (1972). Keratins, Their Composition, Structure and Biosynthesis. Charles C. Thomas, Springfield, Ill.

Freinkel, R.K.(1960) J. Invest. Dermatol. 34, 37-42.

Froelich, J.E. & Rachmeler, M. (1972) J. Cell Biol. 55, 19-31.

Fry, L. & McMinn, R.M.H.(1968) Br. J. Dermatol. 80, 373-383.

Fukuyama, K., Nakamura, T. & Bernstein, I.A. (1965) Anat. Rec. 152, 525-536.

Fukuyama, K. & Epstein, W.L. (1966) J. Invest. Dermatol. 47, 551-560.

Fukuyama, K. & Epstein, W.L.(1967) J. Invest. Dermatol. 49, 595-604.

Gans, O. (1923) Deut. Med. Wochschr. 49, 16.

Gelfant, S. & Candelas, G.C.(1972) J. Invest. Dermatol. 59, 7-12.

Green, H. (1977) Cell 11, 405-416.

Griffin, A.C. (1959) J. Invest. Dermatol. 32, 367-372.

Griffiths, W.A.D. & Marks, R. (1973) J. Invest. Dermatol. 61, 251-254.

Goldsmith, L.A. & Baden, H.P. (1973) Fed. Proc. Fed. Am. Soc. Exp. Biol. 32, 472.

Goodwin, P., Hamilton, S. & Fry, L. (1974) Br. J. Dermatol. 90, 517-524.

Gordon, M. & Johnson, W.C., (1967) Arch. Dermatol. 95, 402-407.

Gumucio, J., Feldkamp, C.S. & Bernstein, I.A. (1967) J. Invest. Dermatol. 49, 545-551.

Halprin, K.M. & Okawara, A. (1966) J. Invest. Dermatol. 46, 51-69.

Hammar, H. (1970) J. Invest. Dermatol. 54, 121-125.

Heaphy, M.R. & Winkelmann, R.K. (1977) J. Invest. Dermatol. 68, 177-186.

Herrmann, J.P. (1976) Arch. Derm. Res. 255, 231-236.

Heywood, D., Karon, M. & Weissman, S. (1965) J. Lab. Clin. Med. 66, 476-482.

Hirs, C.H.W. (1967) Methods Enzymol. 11, 199-203.

Hondius Boldingh, W. & Laurence, E.B. (1968) Eur. J. Biochem. 5, 191-198.

Hoober, J.K. & Bernstein, I.A. (1966) Proc. Nat. Acad. Sci. U.S.A. 56, 594-601.

Hopsu-Havu, V.K. & Jansen, C.T. (1969) Acta derm-venereol 49, 458-467.

Hsia, S.L. (1971) Essays Biochem. 7, 1-38.

Huang, L-Y., Stern, I.B., Clagett, J.A. & Chi, E.Y. (1975) Biochemistry 14, 3572-3580.

Huber, C. & Christophers, E.(1977) Arch.Derm. Res. 257, 293-297.

Im, M.S.C. & Adachi, K. (1966) J. Invest. Dermatol. 47, 823-828.

Ingram, J.T. (1954) Br. Med. J. 2, 823-828.

Jarrett, A.(1971) Br. J. Dermatol. 84, 545-553.

Jarrett, A. (1973) The Physiology and Pathophysiology of the Skin. Vol. 1. Academic Press, London.

Jarrett, A. & Witham, K.M.(1961) Br. J. Dermatol.73, 110-119.

Judewicz, N.D., De Robertis, E.M. & Torres, H.N.(1973) Biochem. Biophys. Res. Commun. 52, 1257-1262.

Karasek, M.A. & Charlton, M.E.(1971) J. Invest. Dermatol. 56, 205-210.

Kimberling, W. & Dobson, R.L.(1973) J. Invest. Dermatol. 60, 538-540.

Kirschenbaum, D.N.(1972) Analyt. Biochem. 49, 248-266.

Kligman, A.M.(1964) In: The Epidermis (Montagna, W. & Lobitz, W.C., eds.) Academic Press, New York and London pp. 387-430.

Lagerholm, B. (1965) Acta. derm-venerol 45, 99-122.

Lavker, R. (1975) J. Cell Biol. 63, 186a.

Lavker, R. & Matoltsy, A.G.(1970) J. Cell Biol. 44, 501-512.

Leavell, V.W., Mersack, I.P. & Smith, C. (1973) Arch. Dermatol. 107, 467.

Lee, L.D., Fleming, B.C., Waitkus, R.F. & Baden, H.P. (1975) Biochim. Biophys. Acta. 412, 82-90.

Liss, M. & Lever, W.F. (1963) J. Invest. Dermatol. 40, 45-49.

Lomholt, G. (1963) Psoriasis. Prevalence, spontaneous

course and genetics. A census study on the prevalence of skin diseases on the Faroe Islands; G.E.C.Gad, diss. Copenhagen.

MacDonald, E.J., Griffin, A.C. & Hopkins, G.E. (1963) J. Invest. Dermatol. 41, 213-223.

Mackenzie, I.C. & Linder, J.E. (1973) J. Invest. Dermatol. 61, 245-250.

Mackenzie, I.C.(1975) J. Invest. Dermatol. 65, 45-51.

Madden, J.L. (1941) Archs. Derm. Syph. 44, 655-666.

Mahle, C. & Orfanos, C.E.(1977) Br. J. Dermatol. 96, 215-223.

Mann, F.R. & Constable, H. (1977) Br. J. Dermatol, 96, 421-426.

Matoltsy, A.G.(1964) Nature 201, 1130-1131.

Matoltsy, A.G. (1965) In: Biology of Skin and Hair Growth (Lyne, A.G. & Short, B.F. eds.) Angus & Robertson, Sydney, pp.291-303.

Matoltsy, A.G. (1975) J. Invest. Dermatol. 65, 127-137.

Matoltsy, A.G. (1976) J. Invest. Dermatol. 67, 20-25.

Matoltsy, A.G. & Matoltsy, M.N.(1966) J. Invest. Dermatol. 46, 127-129.

Matoltsy, A.G. & Matoltsy, M.N. (1970). J. Cell Biol. 47, 593-603.

Matoltsy, A.G. & Bednarz, J.A. (1975) J. Ultra. Res. 53, 128-132.

McKusick, V.A. Mendelian Inheritance in Man. William Heinemann Medical Books Ltd., London.

Melbye, S.W. & Karasek, M.A. (1973) Exp. Cell Res. 79, 279-286.

Menton, D.N. & Eisen, A.Z. (1971a) J. Ultra. Res. 35, 247-264.

Menton, D.N. & Eisen, A.Z. (1971b) J. Invest. Dermatol. 57, 295-307.

Mercer, E.H. & Maibach, H.I. (1968) J. Invest. Dermatol. 51, 215-221.

Mier, P.D. & Van den Hurk, J.J.M.A. (1976a) Br. J. Dermatol., 94, 219-220.

Mier, P.D. & Van den Hurk, J.J.M.A. (1976b) Br. J. Dermatol 95, 271-274.

Michell, R.H. (1975) Biochim. Biophys. Acta. 415, 81-148.

Newbold, P.C.H. (1972) Br. J. Dermatol. 86, 87-90.

Nicolaides, N. & Rothman, S. (1955). J. Invest. Dermatol. 24, 125-129.

Odland, G.F. & Reed, T.H. (1967) In: Ultrastructure of Normal and Abnormal Skin (Zelickson, A.S.,ed.) Henry Kimpton, London. pp. 54-75.

Pastan, I. (1972) Sci. Am. 227, 97-105.

Pauling, L., Corey, R.B. & Branson, H.R. (1951) Proc. Nat. Acad. Sci. U.S.A. 37, 205-211.

Pauling, L. & Corey, R.B. (1953) Nature, 171, 59-61.

Padace, F.J., Muller, S.A. & Winkelmann, R.K. (1969) Acta. derm -venereol, 49, 390-400.

Petrozzi, J.W., Kaidbey, K.H. & Kligman, A.M. Arch. Dermatol. 113, 292-296.

Potten, C.S. (1974) Cell Tissue Kinet. 7, 77-88.

Prunieras, M., Delescluse, C. & Regnier, M.(1976) J. Invest. Dermatol. 67, 58-65.

Reaven, E.P. & Cox, A.J. (1965) J. Invest. Dermatol. 45, 422-431.

Reig, J. & Jarrett, A.(1967) Arch. Dermatol. 95, 632-641.

Regnier, M., Delescluse, C. & Prunières, M. (1973) Acta. derm-venereol 49, 241-247.

Rheinertson, R.P. & Wheatley, V.R.(1959) J. Invest. Dermatol. 32, 49-59.

Rheinwald, J.G. & Green, H. (1975) Cell 6, 331-344.

Rice, R.H. & Green, H. (1977) Cell11, 417-422.

Roe, D.A. (1956) J. Invest. Dermatol 27, 1-8.

Roe, D.A. (1959) Arch. Dermatol. 80, 210-219.

Roelfzema, H., Van den Hurk, J.J.M.A. & Mier, P.D. (1976) Dermatologica 152, 337-339.

Rothberg, S. (1960) J. Invest. Dermatol. 34, 197-206.

Rothberg, S., Crounse, R.G. & Lee, J.L. (1961) J. Invest. Dermatol. 37, 497-505.

Rowden, G. (1967) J. Invest. Dermatol. 49, 181-197.

Rowden, G. (1968) J. Invest. Dermatol. 51, 51-61.

Rowden, G. (1975) J. Invest. Dermatol. 64, 1-8.

Rudall, K.M. (1952) Adv. Protein Chem. 7, 253-290.

Rudall, K.M. (1968) Compr. Biochem. 26B, 559-591.

Sheppard, J.R. Proc. Nat. Acad. Sci. U.S.A. 68, 1316-1320.

Skerrow, D. (1972) Biochim. Biophys. Acta. 257, 398-403.

Skerrow, D. (1974) Biochem. Biophys. Res. Commun. 59, 1311-1316.

Skerrow, D. (1977a) Biochim. Biophys. Acta. 494, 447-451.

Skerrow, D. (1977b) In: The Ichthyoses (Marks, R. & Dyer, P.J., eds.) H.T.P. Press Ltd., Lancaster pp. 41-48.

Skerrow, D., Matoltsy, A.G. & Matoltsy, M.N. (1973) J. Biol. Chem. 248, 4820-4826.

Spearman, R.I.C. & Hardy, J.A. (1976) Acta. Anat. 94, 476-480.

Steinert, P.M. (1975) Biochem. J. 149, 39-48.

Steinert, P., Lichti, U. & Hennings, H. (1977) J. Invest. Dermatol. 68, 237.

Sun, T-T. & Green, H. (1976) Cell 9, 511-521.

Suzuki, H. & Fukuyama, K. & Epstein, W.L. (1977) Cell Tiss. Res. 184, 155-167.

Swanbeck, G. & Thyresson, N. (1965) Acta. derm-venereol 45, 344-348.

Telner, P. & Fekete, Z. (1961) J. Invest. Dermatol. 36, 225-230.

Tezuka, T. & Freedberg, I.M. (1972) Biochim. Biophys. Acta. 263, 382-396.

Tsamboas, D., Kalofoutis, A., [illegible], J., Miras, C. & Capetanakis, J. (1977) Br. J. Dermatol 97, 135-138.

Tsuji, T. & Cox, A.J. (1977) J. Invest. Dermatol. 69, 205-210.

Ugel, A.R. (1969) Science 166, 250-251.

Ugel, A.R. (1971) J. Cell Biol. 49, 405-422.

Ugel, A.R. & Idler, W. (1972) J. Cell Biol. 52, 453-464.

Van Scott, E.J. & Ekel, T.M. (1963) Arch. Dermatol. 88, 373-381.

Voorhees, J.J., Chakrabarti, S.G. & Bernstein, I.A., (1968) J. Invest. Dermatol. 51, 344-354.

Voorhees, J.J., Duell, E.A. & Kelsey, W.H. (1972) Arch. Dermatol. 105, 384-386.

Voorhees, J.J. & Mier, P.D. (1974) Br. J. Dermatol. 90, 223-227.

Voorhees, J.J., Duell, E.A., Creehan, P., Stawiski, M. & Harrell, E.R. (1976) In: Current Problems in Dermatology Vol. 6 (Mali, J. W.H., ed.) S. Karger, London pp. 107-153.

Vukas, A., Velfl, D. & Gligora, M. (1977) Dermatologica 154, 277-283.

Weber, G. In: The Epidermis (Montagna, J. & Lobitz, W.C., eds.) Academic Press, New York and London. pp. 453-463.

Weber, K. & Osborn, M. (1969) J. Biol. Chem. 244, 4406-4412.

Weber, K., Pringle, J.R. & Osborn, M. (1972) Methods Enzymol. 26, 3-27.

Weinstein, G.D. & Frost, P. (1968) J. Invest. Dermatol. 50, 254-259.

Weinstein, G.D. & Velasco, J. (1972) J. Invest. Dermatol. 59, 121-127.

Wheatley, V.R. & Farber, E.M. (1961) J. Invest. Dermatol. 36, 199-211.

Wolff, K., Fitzpatrick, T.B., Parrish, J.A., Gschnait, F., Gilchrest, B., Honingsmann, H., Pathak, M.A. & Tannenbaum, L. (1977) Arch. Dermatol. 112, 943-948.

Young, E. (1970) Br. J. Dermatol. 82, 510-515.

Zahnd, H. & Citron, M. (1960) Arch. Derm. 81, 936-939.

Zetterberg, G. & Swanbeck, G. (1971) Acta. derm-venereol 51, 45-49.