ERYTHROKERATODERMIA VARIABILIS: AN ULTRASTRUCTURAL STUDY

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Summary

There have been conflicting views as to the precise nature of erythrokeratodermia variabilis. It has been suggested that it is a "retention" hyperkeratosis. Light and electron microscopic studies were undertaken to study the morphological changes that take place in the epidermal cells and their bearing on the pathogenesis in two sisters, suffering from this rare disorder.

Histopathologic changes as seen under the light microscope were non-specific. Ultrastructural studies showed a thickened basement membrane, and presence of large amount of tonofibrils in the basal cells, some aggregating to form clumps. In the suprabasal and lower spinous cells keratohyaline granules were present. The cells of the upper spinous layer were full of keratohyaline material. Normally keratohyaline material is only seen in the granular layer (transforming phase). Membrane-coated granules were decreased in the cells of upper spinous layer.

Formation of keratohyaline granules in the suprabasal and spinous cells (synthetic phase) is possibly a major factor in the pathogenesis of erythrokeratodermia variabilis leading to an early or accelerated process of keratinisation and thus, it is unlikely to be a retention hyperkeratosis.

KEY WORDS: Erythrokeratodermia variabilis, Ichthyosis, Keratinisation, Electron microscopy

Introduction

Erythrokeratodermia variabilis also known as keratosis rubra figurata is a rare ichthyosiform genodermatosis inherited as a variably expressed autosomal dominant trait[1]. There are approximately 130 cases reported in the world literature[2]. Possibly the first case was reported by Rille as keratosis rubra figurata[3]. Mendes Da Costa[4] in 1925 was the first to describe it in a mother and child, and called it erythro-et keratodermia variabilis.

In the majority of cases the disorder manifests in the first year of life and both the sexes are affected. Two distinct morphological components have been described, one erythrodermic and the other hyperkeratotic. There are lot of individual variations as one or the other component may predominate or be altogether absent. The

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lesions are usually seen on face, buttocks and extensor surfaces of extremities. The erythrodermic component may appear for a few hours or may persist indefinitely. It is macular and bright red in colour with sharply demarcated capricious borders. Hyperkeratotic lesions have a similar distributions and appear mostly on the normal and sometimes on erythrodermic areas. These are yellowish-brown in colour with variable thickness and large greasy scales. Palms and soles may show thick keratoderma but hair, teeth and nails are normal.

Not only physical factors like wind, cold and heat but also emotional stimuli and stress play a role in the development and course of this disease. Cases are on record where the lesions have cleared completely at puberty and menopause, suggesting hormonal influence[5].

There have been conflicting views as to the precise nature of the disease and its pathogenesis. Cram[3] because of its varied presentation suggested that it was not a single disease but a group of diseases. Gans and Kochs[6] and Gertler[7] suggested that there was a vascular dysplasia leading to dilatation of dermal papillary vessels resulting in abnormal keratinisation. Schellander and Fritsch[8], however, did not find any difference in the temperature between the affected and normal skin. Furthermore, Brown and Kierland[5] could not evoke any abnormal vascular responses to intradermal injections of histamine, methacholine and nicotinic acid in the normal and abnormal skin. Thus, the theory of vascular hyperemia was no longer tenable. Weber suggested the disorder to be of the nature of a proliferative hyperkeratosis[2]. On the other hand, Schellander and Fritsch[8] using autoradiographic techniques found normal epidermal proliferation rate and suggested that it was a "retention" hyperkeratosis. Vandersteen and Muller[2] reported in an ultrastructural study that the number of membrane coated granules was reduced in the upper layers of stratum malpighii resulting in decreased shedding of the stratum corneum. They concluded that erythrokeratodermia variabilis is a separate entity and not a variant or "forme fruste" of congenital ichthyosiform erythrodermia as suggested by Noordhoek[1].

The whole group of inherited disorders of keratinisation characterised by varying degrees of scaling are ill-understood at the present time. In view of this an ultrastructural study was undertaken to study the morphological changes that take place in the epidermal cells and find out their bearing on the pathogenesis of this rare and interesting disorder.

Material and Methods

Clinical features : Two sisters suffering from erythrokeratodermia variabilis were studied. The onset of the disease in both the cases was from infancy. In the three year old younger sister the lesions were more extensive than in the older sibling, and involved the face, chest, upper back and pubic region in a girdle like fashion. In addition, lesions were present on the hands in a glove like pattern. Scattered well circumscribed plaques were present on feet and knees as well (Fig. 1). The lesions were erythematous and hyperkeratotic with greasy brown scales. An interesting feature was that during periods of stress (viral influenza) her skin lesions got cleared (Fig. 2). Her six year old elder sister also suffered from similar condition but in a much milder form. In her case, lesions were confined to the pubic region and buttocks. The plaques were not as thick as in her younger sister. Scaling and erythema was also minimal (Fig. 3).

Light and Electron Microscopy : In both the cases biopsy was taken from

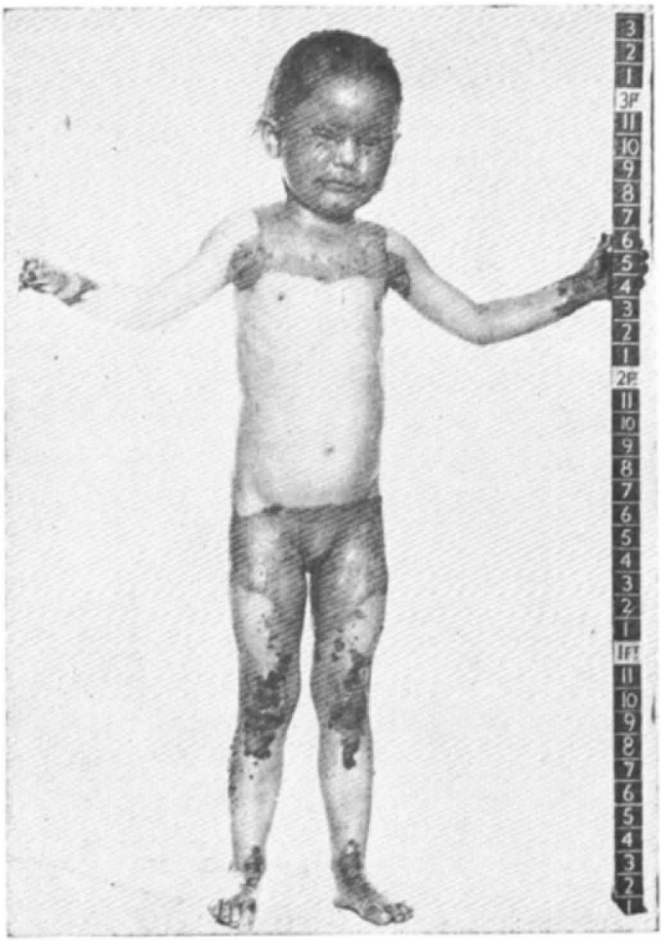

Fig. 1
Lesions of erythrokeratodermia variabilis in younger sister.

the erythemato-hyperkeratotic patches for light and electron microscopic studies. The material for light microscopy was fixed in phosphate buffered neutral formalin (10%). Sections were cut and stained with H & E, periodic acid-Schiff and Masson's trichrome stains.

The specimen for electron microscopy was immediatcly cut into small pieces and fixed in 3% glutaraldehyde in 0.067 M sodium cacodylate buffer (pH 7.4) at 4°C for 3 hours[9]. After fixation, the specimens were washed in several changes of cold 0.067 M cacodylate buffer (pH 7.4) containing 60 mgm/ml sucrose and were allowed to remain in it for 18 hours at 4°C. The specimens were post-fixed in 1% osmium tetroxide in phosphate buffer[10], and then dehydrated through a graded series of ethanols. After dehydration the specimens were put into propylene oxide for half an hour and transferred to 1 : 1 propylene oxide/araldite mixture for further half an hour. After this, the mixture was replaced by araldite and left overnight. Finally, the specimens were placed in fresh araldite and polymerised for 2 days at 60°C.

Sections were cut with glass knives on LKB ultramicrotome III and stained in fresh saturated uranyl acetate in 50% ethanol and then in 0.04% lead citrate. These were viewed in RCA

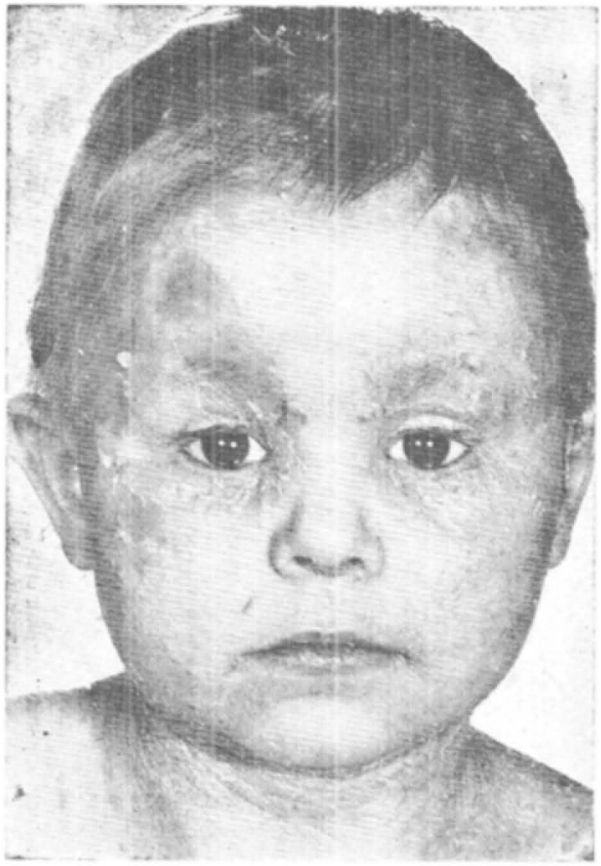

Fig. 2
Lesions clearing after viral influenza.

EMU IV electron microscope with 30 mμ aperture at 50 Kv.

Results

Light microscopic findings : The stratum corneum showed hyperkeratosis and patchy parakeratosis. The granular layer was of normal thickness. Stratum spinosum showed acanthosis and papillomatosis. Papillary vessels and dermal appendages were normal. No changes were observed in the dermis.

Electron microscopic findings : The basement membrane showed thickening. The adjacent keratinocytes were loosely packed with filamentous processes. Villa-like epidermal pegs were seen going downwards into the dermis (Fig. 4).

The basal keratinocytes were markedly electron dense containing an active nucleolus and nucleoli situated at the periphery. The cell cytoplasm contained large number of free ribosomes and scanty endoplasmic reticulum (Fig. 4). A normal complement of other cell organelles were present. Adjacent keratinocytes were joined by desmosomes (Figs. 5 and 6).

The tonofilaments in the basal and suprabasal layer were thickened and had lost their typical fibrillar characteristics (Fig. 5). Some had already started showing presence of ribosomes in between them and aggregation into bundles. Suprabasal layer and cells of stratum spinosum showed accumulation of electron dense homogenous substance called keratohyaline granules in large amounts, so much so that

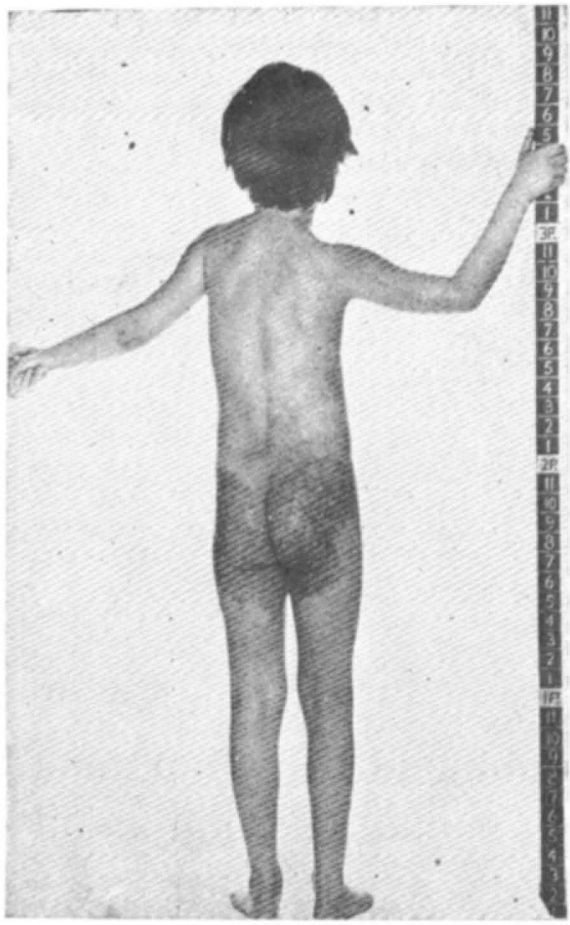

Fig. 3
Lesions of erythrokeratodermia variabilis in elder sister.

they formed a substantial portion of the cell cytoplasm in mid epidermis (Figs. 6 and 7). The free ribosome content of these cells was high. The nucleus and nucleoli were preserved right upto the granular layer.

Membrane coated granules were seen in the lower spinous layer cells (Fig. 5), but they decreased rapidly so that hardly any could be seen at the granular layer levels. The mitochondria, Golgi apparatus and other intracellular organelles showed no variation or abnormalities. The nucleus was lost at the stratum corneum level. Figure nine diagrammatically shows these changes.

The dermis showed normal number of non-myelinated nerves (Fig. 4). The collagen and histiocytes were unremarkable. The endothelial cells of dermal and papillary vessels showed no abnormality.

Discussion

The histopathologic changes as seen under the light microscope are nonspecific. There was hyperkeratosis with patchy parakeratosis acanthosis and papillomatosis, indicating an ichthyotic disorder.

Electron microscopy has provided considerable new information on the intracellular contents and sequence of events leading to keratinisation which were otherwise not visible by the light microscope. The epidermis is mostly composed of keratinocytes which differentiate as they traverse upwards to

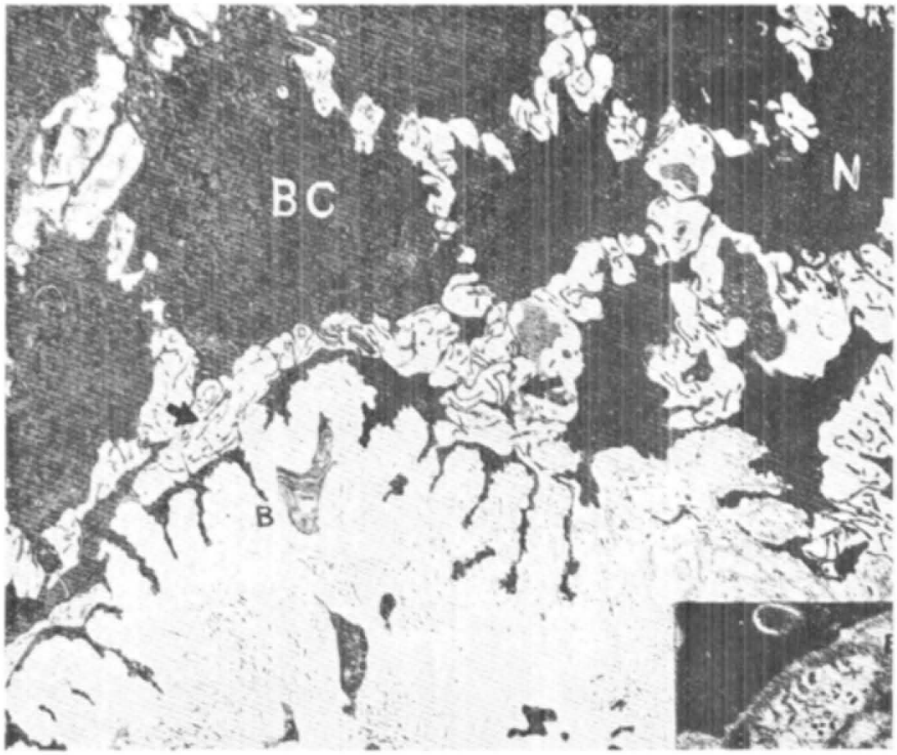

Fig. 4 Electron micrograph showing thickened basement membrane (B), filamentous processes (arrow), electron dense basal cells (BC) and nucleus (N). (reduced from x 4600). Inset shows thickened basement membrane (B) (reduced from 42400).

form stratum corneum. This process involves a number of complex and ill-understood biochemical and structural events. The basal cells which are columnar change their shape and become flattened, lose their nucleus and intracellular organelles, synthesize a sulfur rich protein with a fibrous structure stabilised by disulfide bonds. Brody[11] and Matoltsy[12] suggested that the basal layer and malpighian layer should be called "synthetic phase" and cells of the granular layer "transforming phase".

The epidermis is separated from the dermis by a 300 to 400 Å unit thick basement membrane the fibrils of which run parallel and follow the underside of the epidermis. It is formed by the secretions of not only the dermal fibroblasts as was previously thought but also by epidermal cell secretions[13]. In the present study both the cases showed thickening of the basement membrane (Fig 4). The cells of the basal and spinous layers (synthetic phase) showed marked increase of free ribosomes, the protein synthesizing unit of the cell[14]. The thickening of the basement membrane can be attributed to the increased ribosomal content and increased protein synthesis in epidermal cells. Suprabasal cells showed filamentous projections (Fig. 4), the significance of which is not clear. The desmosomes as described by Odland[15] showed no abnormality (Figs. 6 and 7).

Basal cells contain numerous regularly oriented perpendicular to surface filaments of 50 to 100 Å thickness

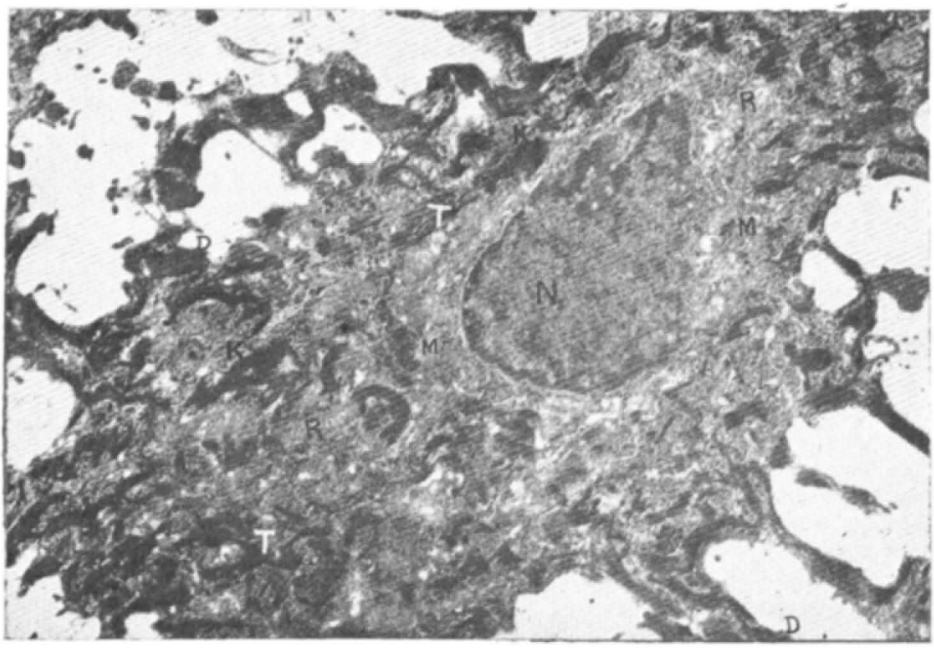

Fig. 5 Electron micrograph of supra-basal keratinocyte showing tonofibrils (T), aggregating to form keratohyaline granules (K), membrane-coated granules (M), desmosomes (D), nucleus (N) and ribosomes (R) (reduced from x 21200).

called tonofibrils or tonofilaments. These are attached to desmosomes and never cross from one cell to another [16,17]. Specific changes take place in these both at the structural as well as at molecular levels culminating in the formation of highly resistant and protective stratum corneum. The tonofilaments increase gradually and sequentially in the spinous cells as they differentiate and mature. These fibrils coalesce to form amorphous matrix which becomes visible at the granular layer level ("transforming cells")[18,19]. In the granular layer it assumes a star shaped electron dense appearance and is called keratin pattern or keratohyaline granules. The formation of these granules was studied by Bell and Kellum[20] who suggested their formation as a result of aggregation of RNA particles around tonofibrils. Keratohyaline granules increase in number and thickness in the granular layer and practically fill the cytoplasm at the stratum corneum level forming the interfilamentous protein matrix of the mature keratin. Thus, these play a very vital role in the process of keratinisation. In both the cases studied the tonofilaments in the basal layer were coarse and scattered all over the cell cytoplasm and had lost their perpendicular orientation, and had started aggregating into bundles to form keratohyaline granules in the suprabasal and lower spinous layers (Fig. 5). The cells of the mid-epidermis were practically filled with keratohyaline granules, unlike in the normal state of keratinisation where these are present only in the granular layer (Figs. 6 and 7). Their formation in the cells of "synthetic phase" suggests an early or an accelerated process of keratinisation. This is possibly the major factor in the pathogenesis of erythrokeratodermia variabilis. As far as the authors are aware there is only one other study on the ultrastructure of

erythrokeratodermia variabilis[2] and no comments on this very interesting finding is made in that study.

Selby[21] described persence of granules in the transitional layer. Odland[22] showed that the granules were membrane coated and first appeared in the lower spinous layers. These are 100 to 300 nm. in size and disappear with the formation of keratohyaline material. These were called keratinosomes or Odland bodies and were studied in great details by Frei and Sheldon[23], Farbman[24] and Matoltsy and Parakkal[25,26]. Matoltsy and Parakkal[25] called them membrane coated granules, a term by which these are now commonly known. For many years their significance and function were not known. Odland[22] suggested that these play an important role in keratinisation. Wolff and Schreiner[27] suggested that these are epidermal lysosomes. Hashimoto[28] showed that these contained acid phosphatases. Matoltsy and Parakkal[25] and Matoltsy[29] showed that at the granular layer level the membrane coated granules move out to the periphery of the "transforming cells" and discharge their contents into the cellular spaces. The discharged contents spread over the surface of the horny cells and provides a barrier and protection to the cells of the stratum corneum from keratolytic agents. During this phase cellular lysosomes also discharge their contents which dissociate the cell nucleus and intracellular organelles leaving tonofila-

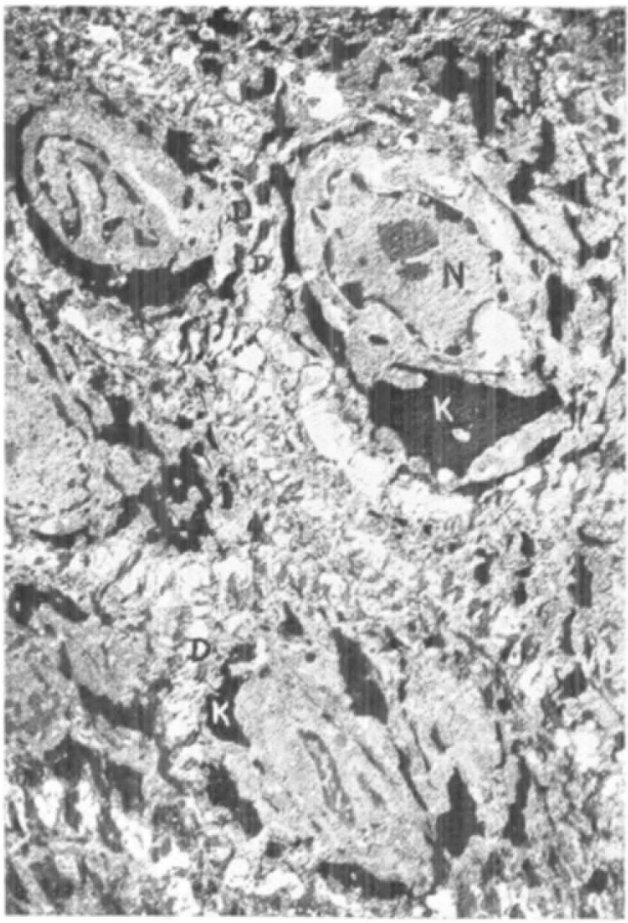

Fig. 6
Electron micrograph showing keratinocytes of upper stratum spinosum having massive accumulation of keratohyaline material (K), nucleus (N) and desmosomes (D) (reduced from x 9200).

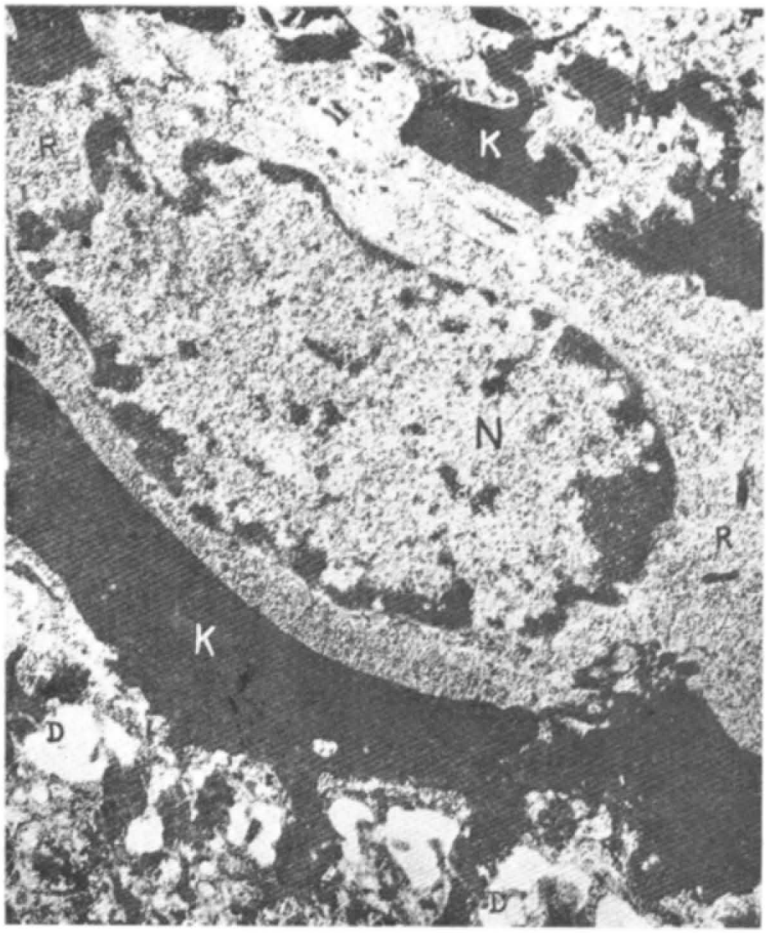

Fig. 7
Electron micrograph of keratinocyte in upper spinous layer showing nucleus (N). Cytoplasm is full of keratohyaline material (K), Ribosomes(R) and desmosomes (D) can be seen (reduced from x 32000).

ments and keratohyaline material intact.

Vandersteen and Muller[2] reported that the number of membrane coated granules was decreased in the spinous layer in erythrokeratodermia variabilis leading to decreased shedding of stratum corneum. This seems rather unlikely as the contents of membrane coated granules form rather a protective covering over horny cells and in their absence these cells, if anything, would be more vulnerable. The findings of the present study regarding membrane coated granules corroborated their findings[2]. The reduced number of membrane coated granules in spinous layer (Figs. 6 and 7) can be explained by the findings of Odland[22,30] who has shown that these granules disappeared when the keratohyaline material was fully formed in the transitional cells. In both these cases the formation of keratohyaline material took place in the lower spinous cells and that explains their absence in the granular layer.

Vandersteen and Muller[2] found increased number of unmyelinated nerves in the papillary dermis, which they related to epidermal abnormality. However, in both of our cases excess of unmyelinated nerves was not found. The dermal vessels also showed no abnormality.

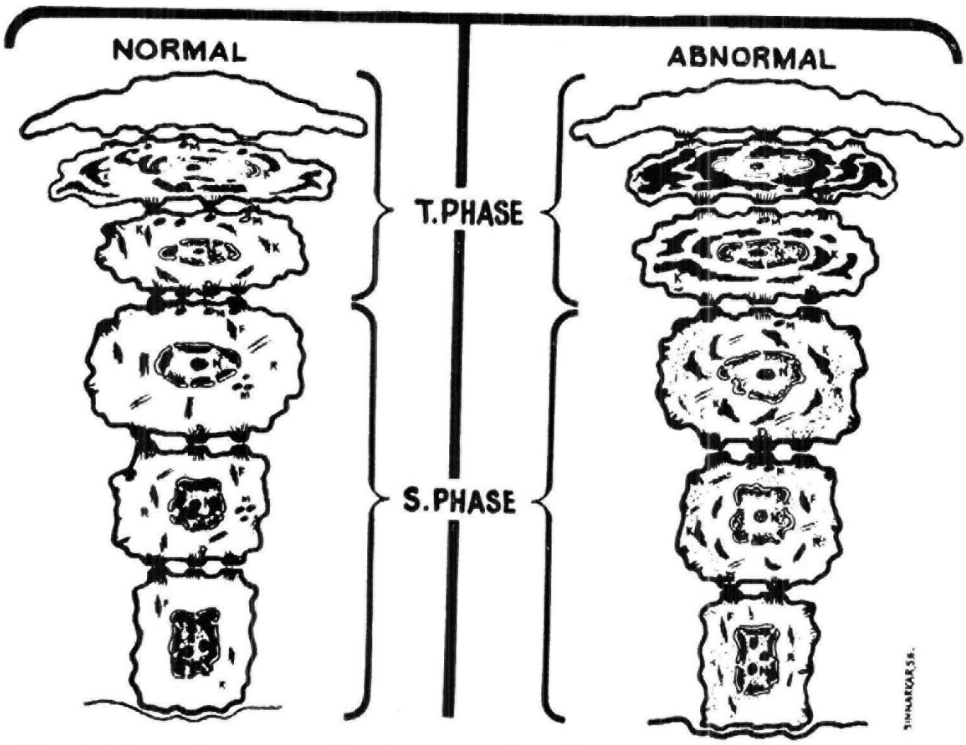

Fig. 8 Schematic representation of events taking place in normal keratinocyte and in erythrokeratodermia variabilis. T=Transitional phase. S=Synthetic phase F=Tonofilaments. K=Keratohyaline granules. R=Ribosomes. M=Membrane-coated granules. D=Desmosomes. N=Nucleus.

Thus, the results of this study suggest that erythrokeratodermia is a separate entity and not a "forme fruste" of congenital ichthyosiform erythrodermia. The ultrastructural study shows that there is an accelerated and early formation of keratohyaline granules in the cells of "synthetic phase", and thus it is unlikely to be a retention hyperkeratosis.

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