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STUDIES ON THE ULTRASTRUCTURE OF KERATOACANTHOMA IN MAN

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Abstract. Studies on the ultrastructure of lesions from two cases of KA during development and mature stages permit following conclusions: (*a*) An evident increase in the number of KH and tonofibrils may suggest an accelerated and more intense keratinization. (*b*) The increased number of the endoplasmic reticulum and ribosomes suggests an intensification of the metabolic processes in the cells participating in the process of keratinization. (*c*) No virus-like elements were found either in the nuclei or in the cytoplasm.

During the last twenty years the clinical as well as histological criteria of the diagnosis of keratoacanthoma (KA) have been the subject of studies of many authors (5). Special attention was given to the benign course of KA and its considerable histological similarity to highly-differentiated squamous-cell carcinomas (22).

Many authors (10, 11, 18, 19, 20, 24) could produce by application of chemical carcinogens tumours markedly resembling KA in experimental animals. There is, however, no agreement of views as to whether the tumours produced in experimental animals should be regarded as KA or as squamous-cell carcinoma (4).

The most frequently expressed view on the aetiology of KA is the opinion that the growth of the tumour is induced by a factor of viral origin. This view has been advanced on the basis of positive results of tissue cultures inoculated with filtrates of the tissue of KA (6, 9, 12, 13), and demonstration by electron microscope of the so-called spiral bodies, regarded as virus particles (7, 8, 9, 25, 26). Further investigations showed (1, 2, 17, 23) that these bodies are non-specific elements present in many tissues, i.a. in the normal skin. They are now called "spheroids"–"sphaeridien" (3). Thus, no reliable proof of viral aetiology of KA is yet available, although further investigations on this problem are in progress.

In the course of the development of KA, keratinization is accelerated, resulting in the development of the characteristic keratinous plug penetrating deeply into the corium with variously intense proliferation of the epidermis about it. The process of keratinization is normal (16).

The ultrastructure of KA induced by carcinogens in experimental animals was the subject of a very careful analysis by Prutkin (18). This author demonstrated the presence of increased numbers of keratohyalin granules and tonofibrils in the period of growth and maturation of KA, and a marked increase of the endoplasmic reticulum and keratinosomes in the period of tumour regression. In this investigation Prutkin failed to observe virus-like particles.

In the available literature we could find no reports on similar investigations carried out in cases of KA in man, apart from some fragmentary observations made during search for virus-like particles (1, 21, 23).

The aim of the present investigation was to study the ultrastructure of KA in man and to compare these observations with the data obtained by Prutkin in his investigations on experimental animals.

MATERIAL AND METHOD

Investigations were carried out in two cases of KA. *Case 1.* Patient S. S., aged 67, had senile diabetes treated by diet alone. Several months before her admission to the hospital, the patient "squeezed out a small purulent pustule" on her right cheek, followed by a small dome-shaped nodule, about 1.5 cm in diameter. The surface of the nodule was papillary, and a large keratinous plug occupied its central part. Its histological picture was characteristic of KA. In the keratinous plug of the tumour, dense accumulations of a PAS-positive substance were present. Only a part of the tumour was taken for histological examinations; the remainder regressed spon-

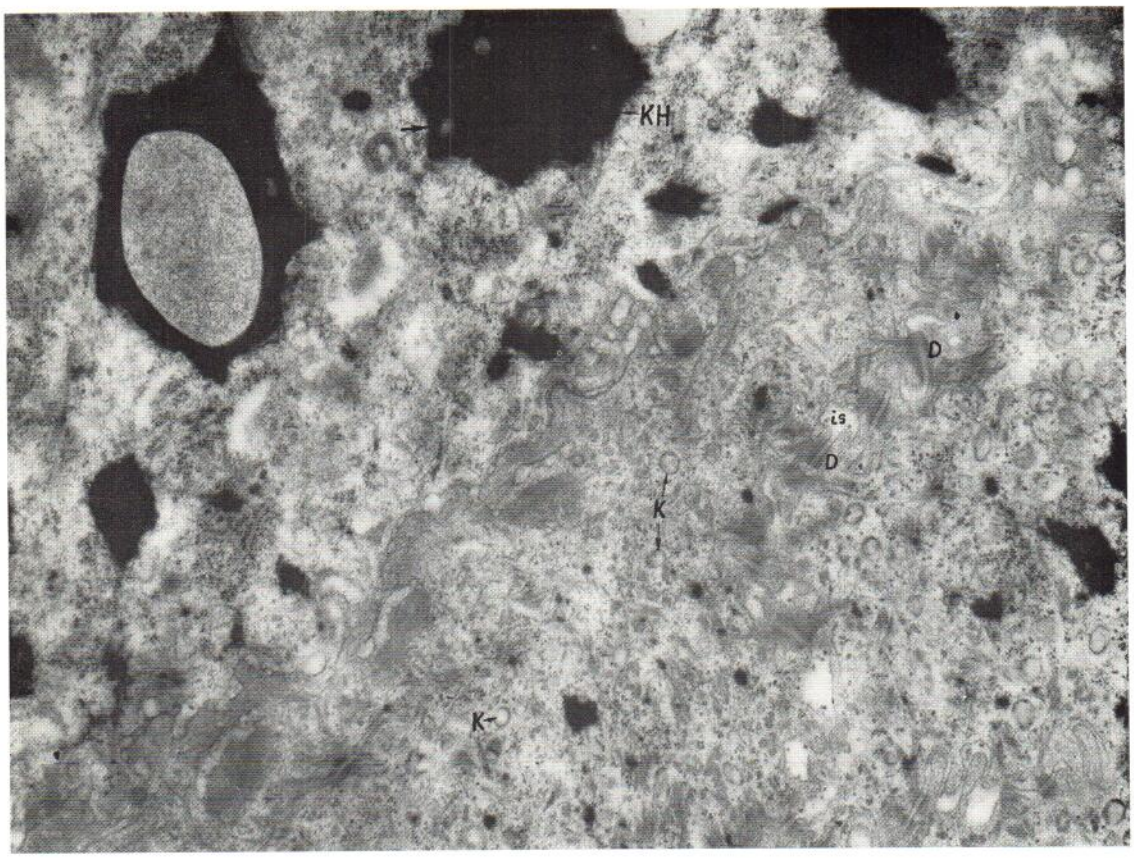

Fig. 1. KA. The prickle-cell layer at the border of the granular layer. Numerous large keratohyalin granules (*KH*) visible in several neighbouring cells, some with vacuoles (→). Their shape is star-like or irregular. The intercellular spaces (*is*) are narrow. Desmosomes (*D*) are normal. The number of keratinosomes (*K*) is moderately increased. × 18,000.

taneously, without treatment, during 1–2 months. Only a smooth scar was found after 1 year during a control examination.

Case 2. Patient J. O., aged 74. Several months before her admission to the hospital she noticed a "small wart" on her right cheek. For some 7–8 weeks prior to her admission the lesion increased progressively in size. During examination, a nodule with a papillary surface with a central keratinous plug, about 1 cm in diameter, was seen. KA was diagnosed clinically, and a portion of the lesion was taken for microscopic examinations.

Histological examination revealed the structure of a KA or a highly differentiated squamous-cell carcinoma. The nodule was excised totally, and a serial histological examination disclosed the features of KA.

The material for electron microscopic investigation was taken simultaneously with that for light microscopy. The first tumour was in the stage of active growth; the second, in the mature stage.

A part of the specimen for electron microscopic examination was cut and fixed in 1% osmium tetraoxide solution in 0.2 M phosphate buffer with addition of 5% saccharose at pH 7.3.

The fixed tissue was dehydrated in alcohols of progressively increasing concentrations and embedded in Epon resin according to the method of Luft (14). The material prepared in this way was cut on a Reichert or Porter-Blum microtome and examined in a JEM 7 electron microscope.

RESULTS

During the examination particular attention was directed to the epidermal cells including, especially, the prickle-cell, granular, and horny layers.

Under normal conditions keratohyalin granules (KH) are present in only one cell layer, immediately below the horny layer.

In the studied material from both cases a considerable increase in number of these granules

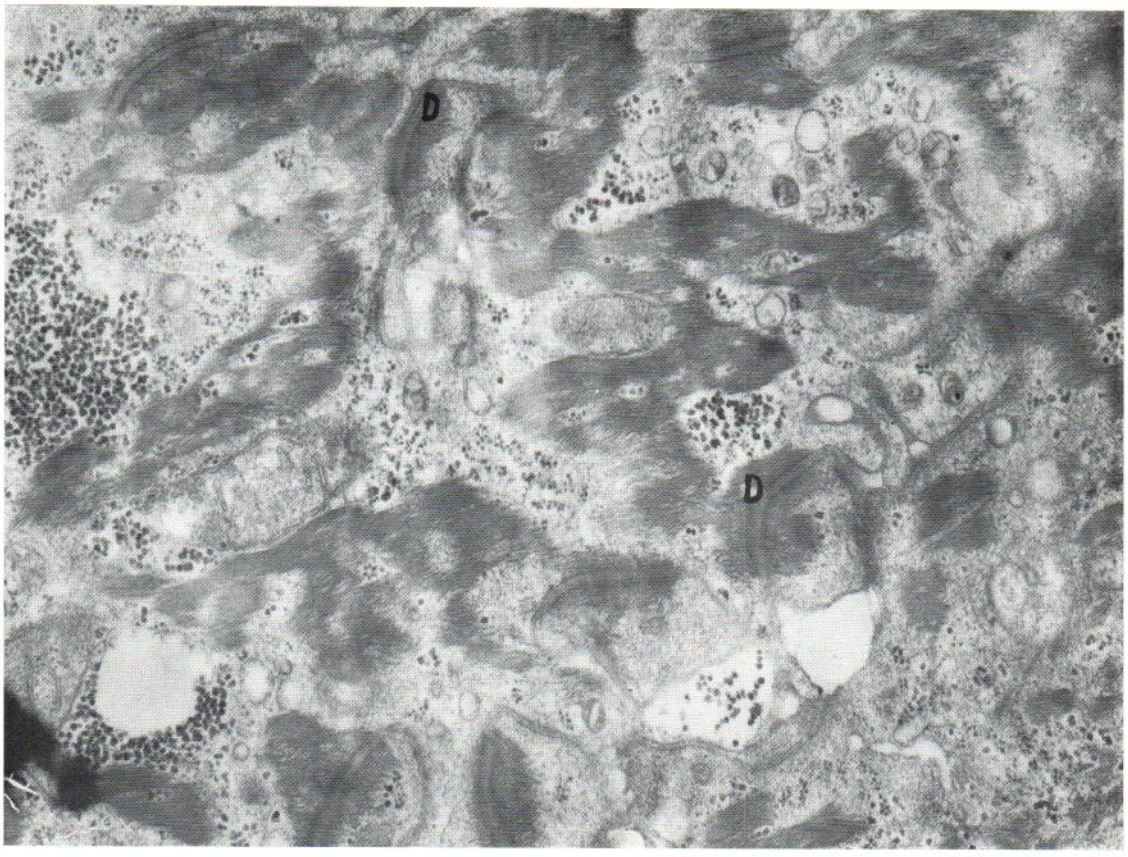

Fig. 2. KA. Prickle-cell layer. A striking increase in the number of tonofibrils forming thick bundles, running in various directions. Their connections with desmosomes (*D*) are clearly visible. Accumulation of granular substance corresponding to glycogen. × 20,000.

was observed, lying in several layers below the keratinized cells. The size of KH surpassed markedly the size of these granules in normal skin. Giant granules were also found, sometimes containing vacuoles (Fig. 1).

The shape of KH was star-like, less frequently round or multi-angular. Many lay in close contact with tonofibrils. The distribution of KH was variable; rather frequently they were arranged in rings around cell nuclei.

The number of tonofibrils was also markedly increased, particularly in case 2 (more mature tumour). The increased production of tonofibrils in the prickle-cell layer led to appearance of large dense accumulations exceeding considerably in size the analogous structures observed in normal epidermis. Their arrangement was variable; some were oriented transversely and some longitudinally in relation to the surface of the horny layer (Fig. 2).

The number of keratinosomes was moderately increased in relation to the normal skin. They were found mainly in the granular layer near the cellular membranes (Fig. 1).

The horny layer was considerably thickened. The keratinized cells were not always arranged parallel as in the normal epidermis. Irregular arrangement was also observed. The degree of development of the horny matrix also lacked uniformity.

The cellular cytoplasm was usually rather rich in organelles. The considerably increased number of rough surfaced endoplasmic reticulum should be stressed (Fig. 3). Similarly, the number of free RNA bodies (ribosomes) was significantly increased (Fig. 3).

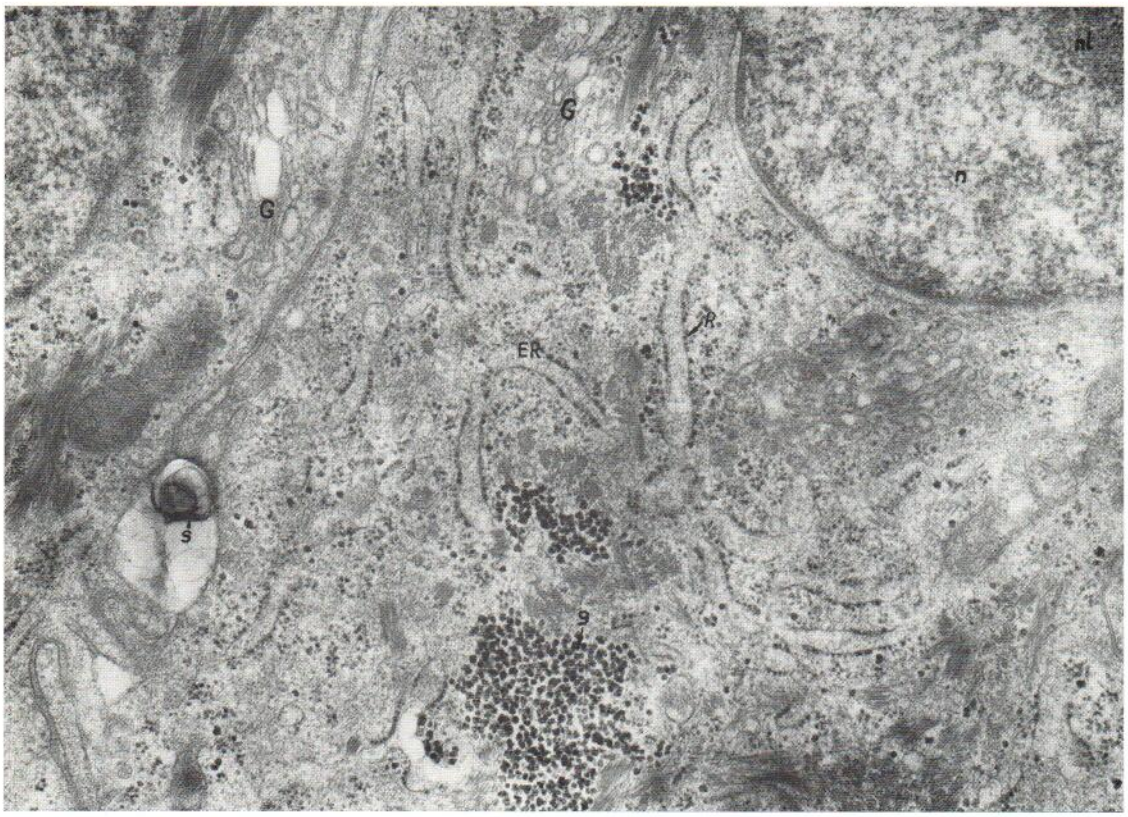

Fig. 3. KA. Fragments of cells in the lower part of the prickle-cell layer. Large numbers of rough endoplasmic reticulum (*ER*) and free ribosomes (*R*). *G*, Golgi zone; *g*, glycogen; *s*, spiral body; *n*, nucleus; *nl*, nucleolus. × 23,000.

The Golgi apparatus was well developed (Fig. 3). The number and the structure of mitochondria did not differ from normal (Fig. 3).

In the cytoplasm of many cells, accumulation of a granular material (probably glycogen) was striking and sometimes very large (Fig. 2 and 3).

The intercellular spaces in the prickle-cell layer were usually narrow and the cellular membranes were folded. The desmosomes were well preserved and unchanged (Fig. 1). Only exceptionally were they slightly widened.

The nuclei of these cells were large, contained a large nucleolus (Fig. 4), and sometimes two nucleoli.

In some cells the nucleus was very large and occupied most of the cell body, leaving only a narrow rim of cytoplasm poor in organelles.

In the examined specimens from both patients, no structures were observed which could resemble viruses. The so-called spheroids were not seen in the nuclei.

DISCUSSION

The increase in the number of KH, tonofibrils and keratinosomes suggests an acceleration of the process of keratinization, corresponding to the stage of growth of the lesion (15).

A similar increase in the amount of endoplasmic reticulum and in the number of ribosomes suggests an increased metabolic activity of the cells, in which the process of intense keratinization is about to begin.

Prutkin (18) in his investigations of KA induced in experimental animals found analogous phenomena in the first two stages of the disease. It may be said then that the ultrastructure of KA in man and in experimental animals is very similar.

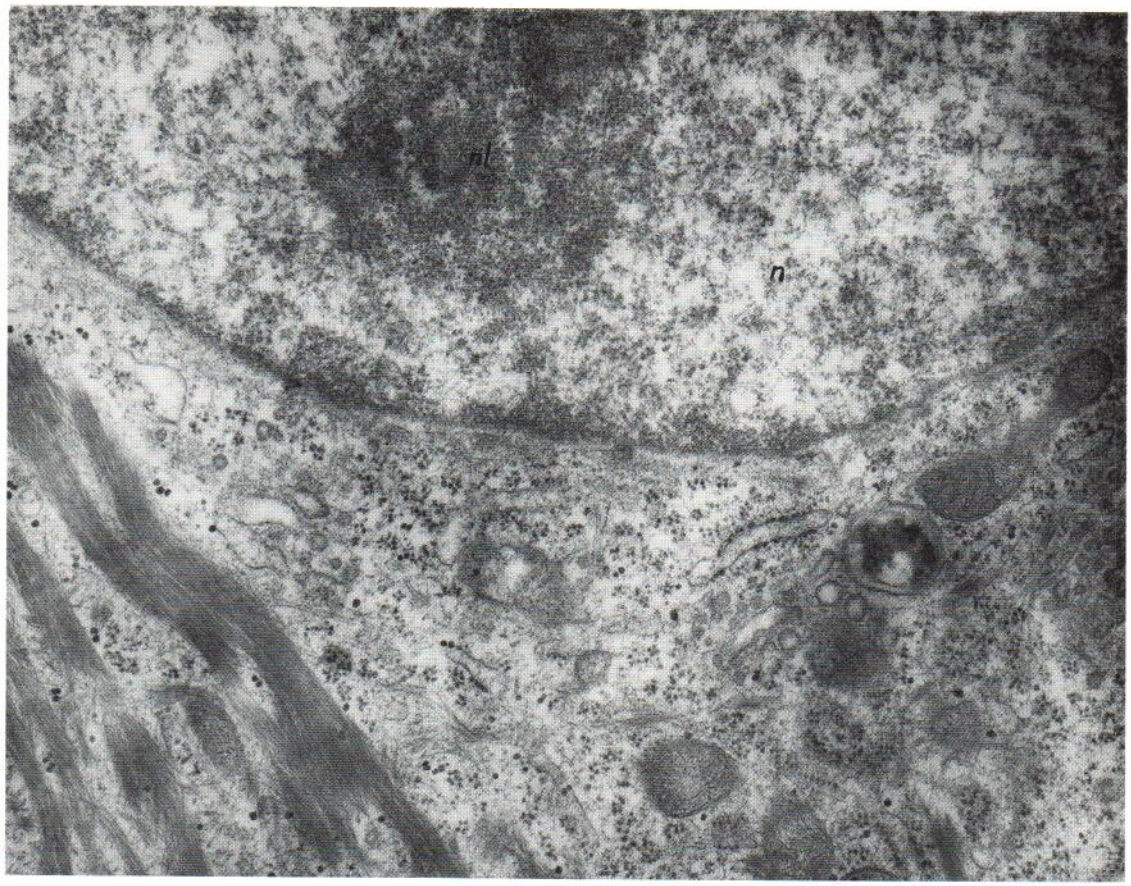

Fig. 4. KA. Fragment of cell in the lower part of the prickle-cell layer. Very large nucleus (*n*) with nucleolus (*nl*). The nuclear chromatin is irregularly arranged. × 25,000.

Similarly, Sagbiel (21) observed in humans KA an increased production of KH. This author stressed the presence of large cells as a feature of a developing lesion. He also described a marked dilatation of the intercellular spaces and folding of the cell membranes. A similar finding was reported by Prutkin in the third stage of KA, i.e. the stage of regression. In our material the intercellular spaces were not very large; it should, however, be stressed that the tumours were not examined during the stage of regression. It is known also that even during the process of normal keratinization the intercellular spaces undergo dilatation with progressing keratinization and with migration of the cells towards the horny layer (15).

The amount of glycogen in the cells exceeding that observed under normal conditions confirms similar observations in light microscopy.

Like Bierwolf & Thormann (1) we observed enlarged nuclei with large nucleoli. Large nuclei, often with double nucleoli, are a characteristic feature of cells actively producing proteins.

Bierwolf & Thorman (1) also regarded the increase in the number of tonofibrils in the prickle-cell layer as an inconstant phenomenon. On the other hand they found a decreased number of desmosomes, which could be regarded as evidence of a less compact arrangement of the cells. This observation has not been confirmed by other authors. In our investigations the number of desmosomes was not decreased and showed no abnormalities.

All these phenomena seem not to be specific for the process of keratinization in KA. Similar phenomena of varying intensity may be found in dermatoses associated with hyperkeratosis or dyskeratosis. Therefore comparative studies on

the ultrastructure of squamous-cell carcinoma of high grade differentiation and also of senile keratosis may be of great importance. Such investigations are now in progress.

Bierwolf & Thormann express a similar opinion, that the ultrastructure of KA shows no characteristic features.

In agreement with other investigations, the presence of virus-like particles in KA could not be established.

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