Ultrastructural and Biosynthetic Characteristics of Epidermal Cells in Patients with Psoriasis Treated with Antiproliferative Drugs

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Biopsy specimens from patients with psoriasis treated with antiproliferative drugs (methotrexate, etretinate) were analyzed. Acanthosis and papillomatosis of the epidermis and inflammatory reaction of the derma decreased. A tendency to normalization of epidermocyte ultrastructure with regeneration of tonofilaments and normal keratinization was noted. An important role in the morphogenesis of this process belongs to interrelationships of two competitive types of biosynthesis, DNA and RNA synthesis, providing epidermocyte proliferation and intracellular regeneration, respectively. Structural and functional composition optimal for the epidermis is formed under conditions of complex treatment with antiproliferative drugs combined with photochemotherapy.

Key Words: *psoriasis; epidermocytes; DNA and RNA synthesis; electron microscopy; autoradiography*

Many fragments in the chain of pathogenetic events of psoriasis are now known [4,7,8-11,14], but the general concept of psoriasis morphogenesis has not yet been formulated. Various models of psoriasis etiopathogenesis are discussed; one of the last model presents psoriasis as a typical pathological process: inflammation under conditions of activated antioxidant defense and increased expression of apoptosis receptors [12]. We consider that oxidative stress of keratinocytes which have not passed through all stages of differentiation serves as the triggering mechanism for the formation of defective horny layer, the key component of psoriatic process.

The epidermis-derma interactions are important components in the morphogenesis of psoriasis. They represent a variant of parenchymatous-stromal interactions reflecting synchronism of epidermal and dermal reactions [5] and realized, for instance, via apoptosis [13]. However both pro- and antiapoptosis genes are expressed in psoriasis [15]. These two morphogenetic aspects (cellular and tissue) form the basis of patho- and morphogenesis of psoriatic disease [6].

Comparative pathomorphological study of skin biopsy specimens with evaluation of biosynthetic reactions of epidermocytes during therapy by different methods acquires special importance, as it helps to evaluate the treatment efficiency and detect the key components in the interaction between two biosynthesis types, DNA and RNA production, determining the proliferation and differentiation responses.

We carried out ultrastructural and autoradiographic analysis of epidermocytes in biopsy specimens from patients with psoriasis treated with antiproliferative drugs.

MATERIALS AND METHODS

A complex pathomorphological analysis of skin biopsy specimens was carried out in 232 patients with pso-

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riasis. Skin biopsy specimens were collected at the same time of the day (from 11.00 to 13.00) before and 1 month after the beginning of therapy.

Patients were treated with methotrexate (cytostatic) twice a week in a dose of 5 mg for 4-5 weeks (n=60), methotrexate+photochemotherapy (n=122) [2], or etretinate (synthetic aromatic retinoid, Tigason) in a daily dose of 25 mg (n=50); in 10 patients of this group etretinate treatment was combined with photochemotherapy.

For light microscopy, skin specimens were fixed in 10% neutral formalin; paraffin sections were stained with hematoxylin and eosin and Pearls reaction, by Van Gieson method with poststaining of elastic fibers with Weigert resorcin-fuchsine, and periiodic acid Schiff-reaction was carried out. For electron microscopy the samples were fixed in 4% paraformaldehyde and postfixed in 1% OsO_4. After standard treatment, the specimens were embedded in epon-araldite mixture. Semithin sections were stained with Azur II. Ultrathin sections were contrasted with uranyl acetate and lead citrate and examined under a JEM 101 electron microscope.

Metabolic and proliferative activity of epidermal and dermal cells was evaluated by autoradiography [8] with tritium-labeled DNA (^{3}H-thymidine) and RNA precursors (^{3}H-uridine). Quantitative evaluation of autoradiographs included estimation of labeled cell index and evaluation of the label density.

RESULTS

Light optic structure of the skin before therapy was typical of psoriasis in all analyzed biopsy specimens. Pronounced hyper- and parakeratosis, acanthosis, papillomatosis, and degenerative changes in epidermocytes were evaluated for each case (Fig. 1, *a*). The derma was characterized by hyperemia of the papillary layer vessels, edema, and inflammatory cell reaction of different degree, presented mainly by lymphocytes and macrophages, sometimes neutrophilic granulocytes, plasmocytes, and eosinophils.

Inflammatory cell infiltration was located mainly perivascularly, sometimes was directed towards the epidermis. Inflammatory infiltrate involved the interface between the epidermis and derma, which was paralleled by edema and active penetration of neurophilic granulocytes and lymphocytes into the epidermis. Migrating lymphocytes formed Munro microabscesses in the upper layers of the epidermis. A characteristic change in the derma was a drop in the number of elastic fibers and their destruction primarily in the papillary layer.

One month after the beginning of therapy light microscopy showed stereotypical positive shifts in biopsy specimens: acanthosis, papillomatosis, and inflammatory cell infiltration of the derma decreased (Fig. 1, *b*). No keratosis was seen in the majority of cases; perivascular infiltration was mainly mononuclear and did not involve the adjacent derma.

Electron microscopic data before treatment showed a complex of stereotypical ultrastructural changes in epidermocytes. Epidermal basal cells had signs of degeneration: devastation and partial condensation of cytoplasmatic matrix, destruction of mitochondrial cristae, decreased number of ribosomes and tonofilaments. Osmiophilic residual structures were detected in dilated intercellular spaces.

Changes in the ultrastructural organization of the epidermal stratum spinosum were uniform and consisted in the formation of a wide empty perinuclear cytoplasm zone (Fig. 2, *a*), presented mainly by small-granular nonstructural matrix and by more electron-dense peripheral zone with cell organelles and scanty fragmented tonofilaments. Some epidermocytes of the stratum spinosum underwent necrobiosis and necrosis (cytolysis); there were degenerating electron-dense nonstructured cells.

There was no clear-cut interface between the prickle-cell and granular layers of the epidermis due to similarity in their ultrastructure. The structure of granular epidermocytes indicated their insufficient differentiation (intact nucleus, mitochondria, cytoplasmatic reticulum profiles). Many cells of the granular layer contained tonofilaments which only at some places showed a trend to concentration and aggregation with subsequent formation of polymorphic granules differing from normal keratohyalin granules. Rudiments of cell nuclei, fragments of other cell organelles, and tonofilament bundles were seen in the horny layer.

One month after the start of treatment electron microscopy (Fig. 2, *b*) showed clear-cut signs of morphological stratification of the epidermis due to normalization of keratinization and decreased degeneration, the degree of these shifts depended on the treatment protocol.

After methotrexate therapy there were many degeneratively changed epidermocytes, which was due to cytopathic effect of the drug. Keratohyalin granules were seen in granular epidermocytes. Combined treatment with methotrexate and photochemotherapy attenuated epidermocyte degeneration (in comparison with methotrexate monotherapy); keratinocyte structure tended to normal due to regeneration of cytoplasmatic organelles.

Basal epidermocytes looked like cells with high biosynthetic activity; the nuclei occupied the greater part of the cytoplasm, many of them contained large nucleoli; profiles of the granular cytoplasmatic reticulum, ribosomes, polysomes were clearly seen among

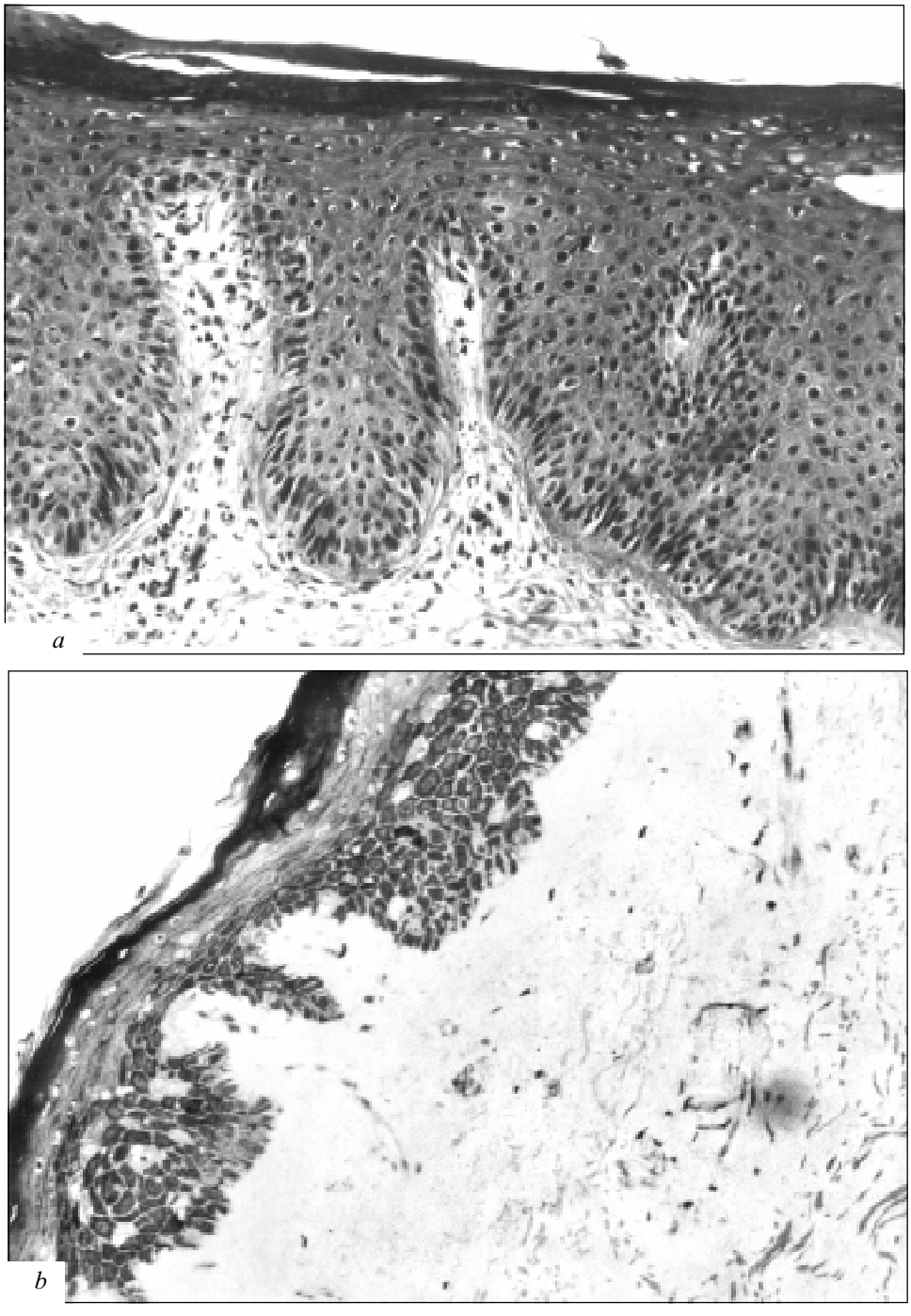

Fig. 1. Optic microscopic changes in the forearm skin biopsy specimens from psoriatic patients. *a*) before treatment: marked acanthosis, dystrophic changes in epidermocytes, inflammatory cell infiltration of the derma. Hematoxylin and eosin staining, ×600; *b*) after treatment: decreased acanthosis, no inflammatory cell infiltration. Semithin section, Azur II staining, ×320.

cytoplasmatic organelles; there were abundant tonofilament bundles. Cells with clarified perinuclear cytoplasm were seen among spinous epidermocytes, but there were no essential changes in the structure of these cells; tonofilaments were located mainly at the cytoplasm periphery, their number in the cells greatly varied. Signs of normalization of keratin formation were clearly seen in the granular layer. Ultrastructure of horny layer cells corresponded to that in normal epidermis.

A characteristic feature of the epidermis in patients treated with etretinate was normalization of epidermocyte ultrastructure and keratinization process. Basal layer cells had large euchromic nuclei, there

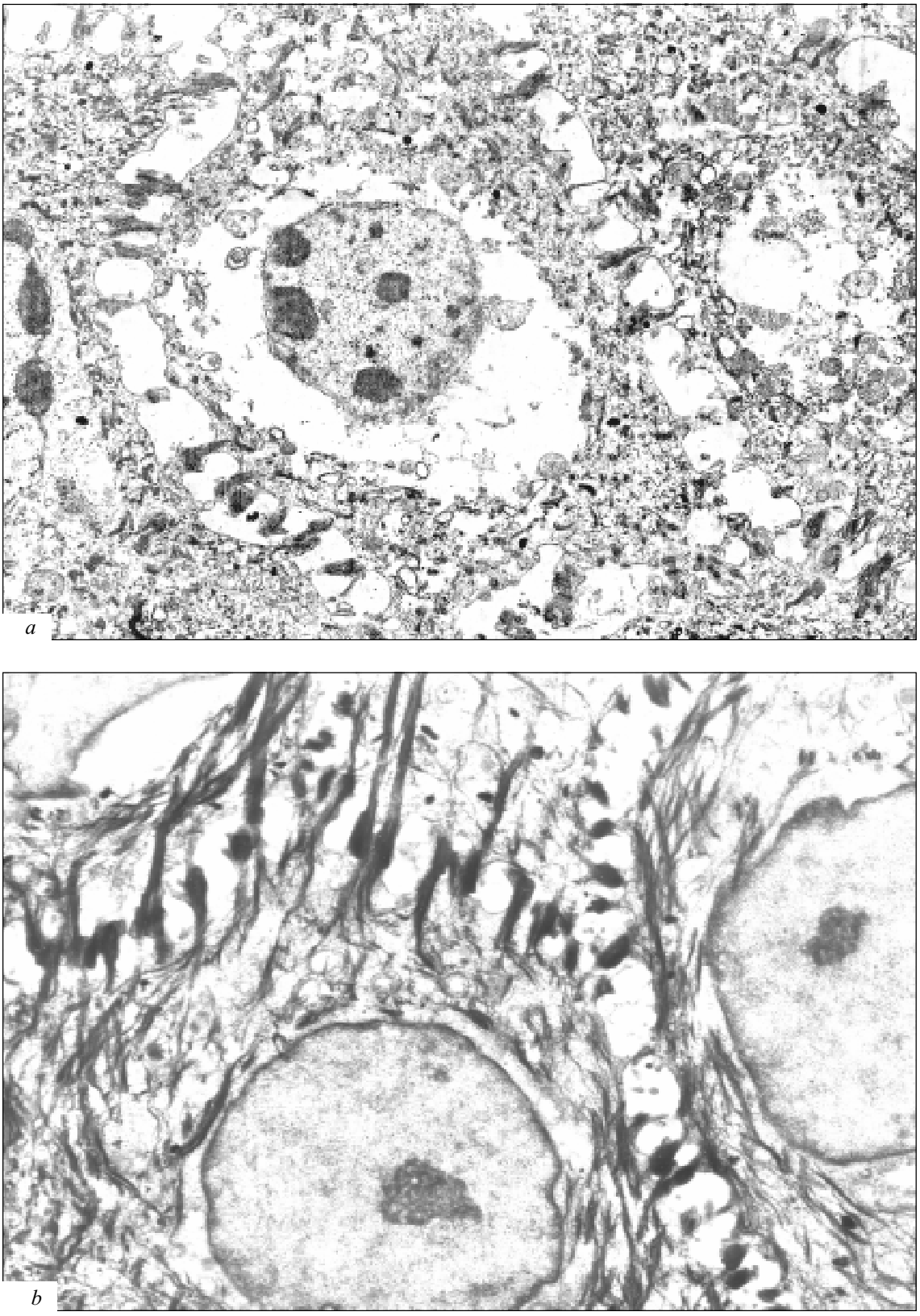

Fig. 2. Ultrastructural changes in spinous epidermocytes in patients with psoriasis. *a*) before treatment: chromatin aggregation, cytoplasm devastation, degradation of cytoplasmatic organelles, reduction of filamentous structures, ×3330; *b*) after therapy: clearly seen nucleoli, many tonofilament bundles, restoration of cell-cell contacts, ×3300.

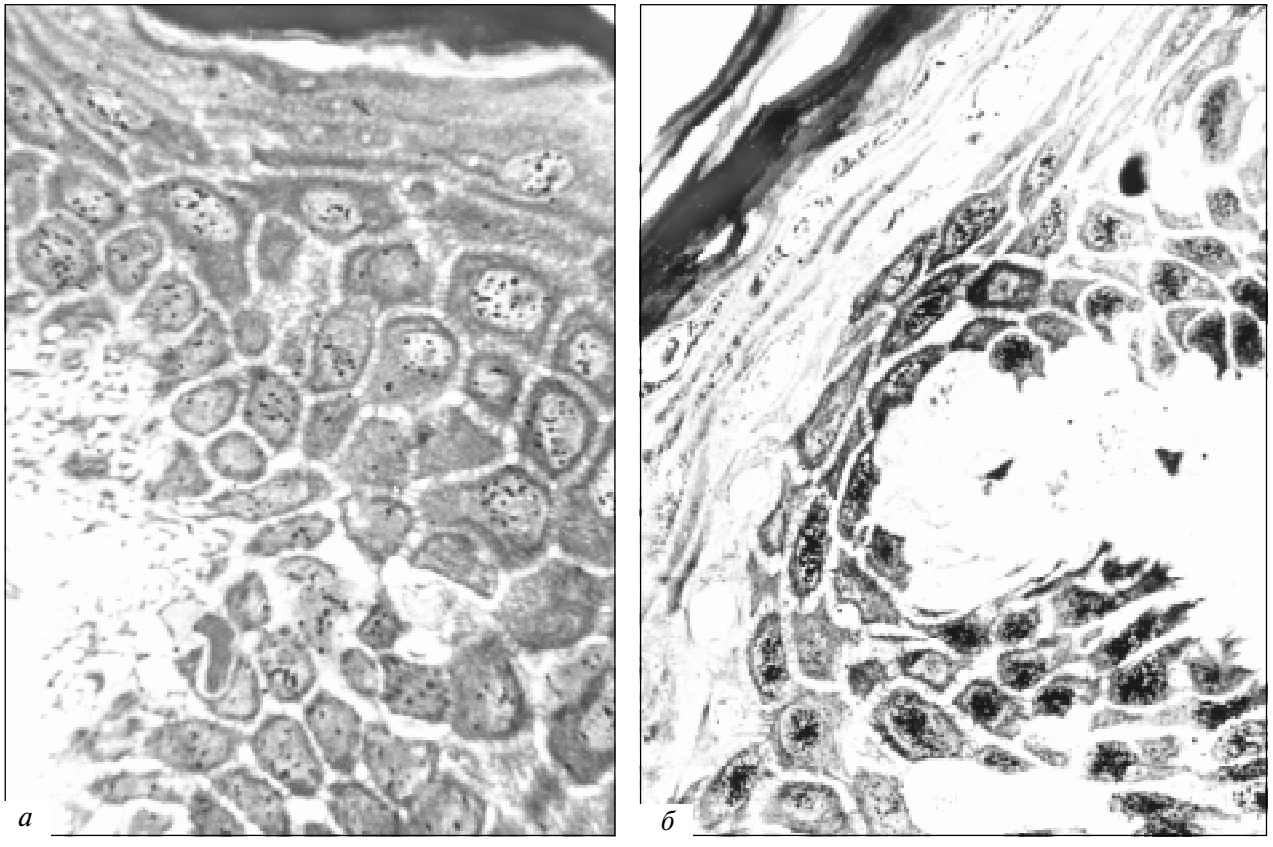

Fig. 3. Radioautographic changes in the forearm skin biopsy specimens from patients with psoriasis. Semithin sections, Azur II staining. Incubation with ^{3}H-uridine. *а*) before treatment: low label density in epidermal cells, ×1000; *b*) after treatment: high biosynthetic activity of epidermocytes, ×800.

were polymorphic mitochondria, granular cytoplasmatic reticulum profiles, polysomes, and numerous tonofilament bundles in the cytoplasm. The cytoplasm of spinous epidermocytes contained numerous mitochondria, cisterns of the granular cytoplasmatic reticulum, and numerous polysomes. All cells contained abundant tonofilaments, concentrated mainly along the periphery and oriented concentrically. There were signs of differentiation of the granular layer epidermocytes, in some cells tonofilaments fused into polymorphic conglomerations of low electron density. One month after etretinate treatment combined with photochemotherapy the ultrastructure of cells populations in all epidermal layers returned to normal.

Autoradiographic analysis of RNA synthesis before therapy (Fig. 3, *а*) showed pronounced structural and metabolic heterogeneity of epidermocytes. Metabolically active epidermocytes were located mainly in the basal layer (small groups or solitary cells), the index of ^{3}H-uridine-labeled cells being 12.3-51.9%; label density was 14-32 silver grains per cell. ^{3}H-uridine label was found in the basal layer epidermocytes, labeled cell index being 1.1-6.8%, label density 5-16 silver grains/cell and in some cells extremely high.

Metabolic reactions in epidermal cell populations decreased more markedly than proliferative in patients treated with methotrexate (4-fold and more in 50% cases). A notable decrease in RNA synthesis led to suppression of plastic processes in epidermocytes and, hence, to their degeneration and impaired differentiation. Complex therapy with methotrexate and photochemotherapy led to a more pronounced decrease of the epidermocyte proliferative reactions (4-fold in 27% cases) in comparison with metabolic reactions. Decreased proliferation in the presence of a certain level of metabolic reactions promoted normalization of the epidermocyte structure and differentiation.

Proliferative reactions of the epidermis essentially decreased in patients treated with etretinate (^{3}H-thimidine label was detected only in few cells) and plastic processes sharply increased (95-100% epidermocytes incorporated ^{3}H-uridine) (Fig. 3, *b*). Etretinate provided optimal structural and functional composition in the DNA/RNA synthesis ratio in the epidermal cell populations.

Hence, imbalance between two major types of biosynthesis, DNA and RNA synthesis ensuring cell proliferation and intracellular regeneration, plays an

important role in the pathogenesis of psoriasis. Decreased level of proliferative reactions in the presence of high level of metabolic (plastic) processes is an indicator of optimal effects of drug therapy on the epidermis.

Distorted relationships between such fundamental reactions of cell populations as proliferation, differentiation, regeneration is reflected in the morphogenesis of psoriasis, which is eventually seen in tissue (epithelial) changes: degeneration, atrophy, and acanthosis. The corresponding molecular and cellular effects are realized in the course of biosynthetic reactions, including nucleic acid production and competitive composition (competitive depression or expression of protein synthesis) under conditions of disease development [5].

Study of keratinization and apoptosis in squamous-cell lung cancer showed different variants of epithelial cell death: apoptosis, keratinization, necrosis, *etc*., keratinization being an individual, other than apoptosis type of programmed cell death and a variant of cell death through differentiation [3].

Our results of psoriasis therapy with antiproliferative drugs suggest that normalization of the epidermis structure is paralleled by restoration of the balance between cell proliferation and death via epidermocyte differentiation, for which restoration of normal ratio of nucleic acid biosynthesis is essential. The most demonstrative results in this respect were obtained with etretinate (particularly in combination with photochemotherapy), which, in addition to the antiproliferative effect, normalized the hornification process and stabilized cell membrane structures [1].

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