# Allergic Dermatoses Associated with Chronic Opisthorchiasis during Antihelminthic Therapy

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We studied skin biopsy specimens from patients with allergic dermatoses associated with opisthorchiasis. Structural changes in the epidermis and derma were presented by degenerative and destructive changes in epitheliocytes with a pronounced membranolytic component, hemodynamic disturbances in the derma, disorganization of the connective tissue, and progressive fibrosis. Combination therapy including antihelmintic drug produced a positive effect, which was associated with induction of regenerative processes in basal epidermal cells, stabilization of the basal membrane, and normalization of the dermoepidermal junction. Our findings indicate that opisthorchiasis plays an important role in the pathogenesis of allergic dermatoses.

**Key Words:** *allergic dermatoses; opisthorchiasis; skin biopsy; electron microscopy*

Opisthorchiasis is a common regional disease endemic to West Siberia [1,6]. This territory is a pesthole of opisthorchiasis, which is associated with peculiar natural and social conditions. In most people the disease results from repeated infections and superinvasion determined by life and nutrition habits. Most individuals have opisthorchiasis for several years or decades before diagnosis [7].

The prevalence of opisthorchiasis in the Lower Irtysh-Middle Ob reaches is 70-90%. In patients with chronic opisthorchiasis (CO) somatic and infectious diseases are extremely severe or torpid and often follow a chronic course [3,4]. However, the clinical course of dermatoses associated with CO remains unclear.

Despite a great number of morphological assays on dermatopathology [5,8,9], pathomorphological characteristics of dermatoses associated with opisthorchiasis remain unknown. In this respect it interesting to compare structural changes in the epidermis and derma in patients with combined diseases receiving various therapeutic treatments. This assay will allow us not only to evaluate the efficiency of therapy, but also to estimate factors that play a key role in the pathomorphogenesis of allergic dermatoses associated with CO.

Here we studied the morphogenesis of allergic dermatoses associated with CO in patients receiving various corrective therapy.

## MATERIALS AND METHODS

We examined 124 patients with chronic allergic dermatoses (atopic, seborrheic, and microbial eczema and allergic dermatitis) associated with CO. After treatment of dermatosis and 1-2-week preparative therapy, the patients received antihelmintic drugs chloxyl ($n$=32), biltricide ($n$=38), or poputril (70% extract of aspen bark, $n$=28). Antihelminthic drugs were not given to 26 patients.

Skin biopsy specimens were obtained under local anesthesia with chloroethyl or Novocain 1 month after the start of combination therapy. For light microscopy the specimens were fixed in 10% neutral formalin. The staining procedure for paraffin sections included

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hematoxylin-eosin and Perls stain. Staining was performed by the method of van Gieson. Elastic fibers were stained with Weigert resorcin fuchsin. The periodic acid-Schiff (PAS) reaction in combination with azure-eosin staining was performed. Preparations stained by the Giemsa method were used for bacterioscopy. Specimens for electron microscopy (2 $mm^3$) were fixed in 4% paraformaldehyde, postfixed in 1% $OsO_4$, and embedded in Epon-araldite mixture. Semithin sections were stained with Schiff reagent and azure II. Ultrathin sections were contrasted with uranyl acetate and lead citrate and examined under a JEM 1010 electron microscope.

## RESULTS

Allergic dermatoses often accompanied *Opisthorchis* invasion. These diseases ran a severe course with frequent exacerbations, unstable remissions, and regular recurrences. Lesions were revealed in a large area of the skin. Severe and complicated allergic dermatoses were found in 40.4% patients. Most patients were resistant to standard therapy. The patient's complaints were associated with pain, dyspepsia, and general toxic syndrome. In 63% patients CO was characterized by a latent course and mild symptoms. Invasion was moderate in 64% patients and insignificant in 36% patients (3362.8±1131.9 and 677.1±206.3 eggs/g feces, respectively). The severity of skin lesions corresponded to the degree of helminth invasion.

One-month combination therapy of dermatosis and CP was followed by recovery from the disease, improvement of the state, and attenuation of symptoms in 42, 47, and 11% patients, respectively. For patients not receiving antihelminthic drugs these parameters were 28, 22, and 50%, respectively. After 6-12 months exacerbation of dermatosis was observed in 18% patients receiving combination therapy and 94% patients not treated with antihelminthic drugs. After 1 month of combination therapy we observed a decrease in the area of skin lesions in patients with allergic dermatoses receiving chloxyl (from 15.1±0.5 to 2.2± 0.2%), biltricide (from 14.6±0.7 to 1.9±0.4%), and poputril (from 18.4±1.4 to 1.2±0.6%). In patients not receiving antihelminthic drugs the area of damaged skin decreased from 15.8±0.4 to 1.8±0.6%.

It should be emphasized that antihelminthic drugs chloxyl and biltricide are highly toxic and have contraindications. These disadvantages are less pronounced in various plant preparations, including poputril. Poputril produced a greater therapeutic effect compared to other antihelminthic preparations. Poputril is well tolerated, causes only insignificant side effects, and ensures better dehelminthization compared to chloxyl and biltricide (95.6, 85.7, and 91.7%, respectively).

We revealed similar pathological changes in skin specimens from patients with allergic dermatoses. The structure of the epidermis was preserved. Focuses of acanthosis and papillomatosis (Fig. 1, *a*) interspersed with regions of smoothed dermal papillae. The granular layer of the epidermis was sometimes absent. In 50% specimens we revealed focal spongiosis, which was especially pronounced in the wall of hair follicles.

Basal cells were heterogeneous. Most cells had pyknotic nuclei and amorphous dense cytoplasm. Some epitheliocytes had signs of increased functional activity and contained numerous free ribosomes, polysomes, and small mitochondria with the dense matrix. The spinous layer included a considerable number of degenerating cells with pyknotic nuclei and optically loosened perinuclear zone of the cytoplasm, which resulted from alteration and reduction of cytoplasmic organelles constituting the protein-synthesizing (granular cytoplasmic reticulum, free ribosomes, and polysomes) and energy-producing compartment (mitochondria). The inhibition of intracellular plastic processes was accompanied by a sharp decrease in the number of filaments. Individual and chaotically oriented small bundles of tonofibrils were localized in peripheral zones of the cytoplasm. We observed reduction of desmosomes and irregular, but pronounced enlargement of the intercellular space. Partial lysis of the cytoplasm, destruction of specific cytoplasmic organelles, and vacuolization of preserved membrane structures were typical of epitheliocytes in the spinous layer (Fig. 2, *a*, *b*).

Granular epidermal cells had polymorphic nuclei and granules of keratohyalin. The cytoplasmic matrix containing considerable amounts of membrane and amorphous components had different electron density. Our findings indicate that the degree of keratinization varied. In the corneal layer of the epidermis characterized by abnormal keratinization regions of parakeratosis and dyskeratosis alternated with loosely packed keratin lamellae. The cytoplasm of parakeratotic cells was eosinophilic and PAS-positive and contained the pyknotic nucleus. In patients with microbial eczema focuses of parakeratosis included bladders filled (Fig. 1, b) with the exudate, neutrophils, lymphocytes, and cellular detritus.

In the papillary layer of the derma we revealed diffuse of focal hyperemia, edema, and perivascular mononuclear infiltrates with different density and distribution (Fig. 1, *c*). In patients with allergic dermatitis focuses of dermatoid changes in the connective tissue were formed in the apical zone of papilla. The perifocal zone was characterized by hyperemia, edema, and mononuclear infiltration. Sclerotic changes were most pronounced in deep layers of the derma. Bundles of collagen fibers differed in the thickness, localiza-

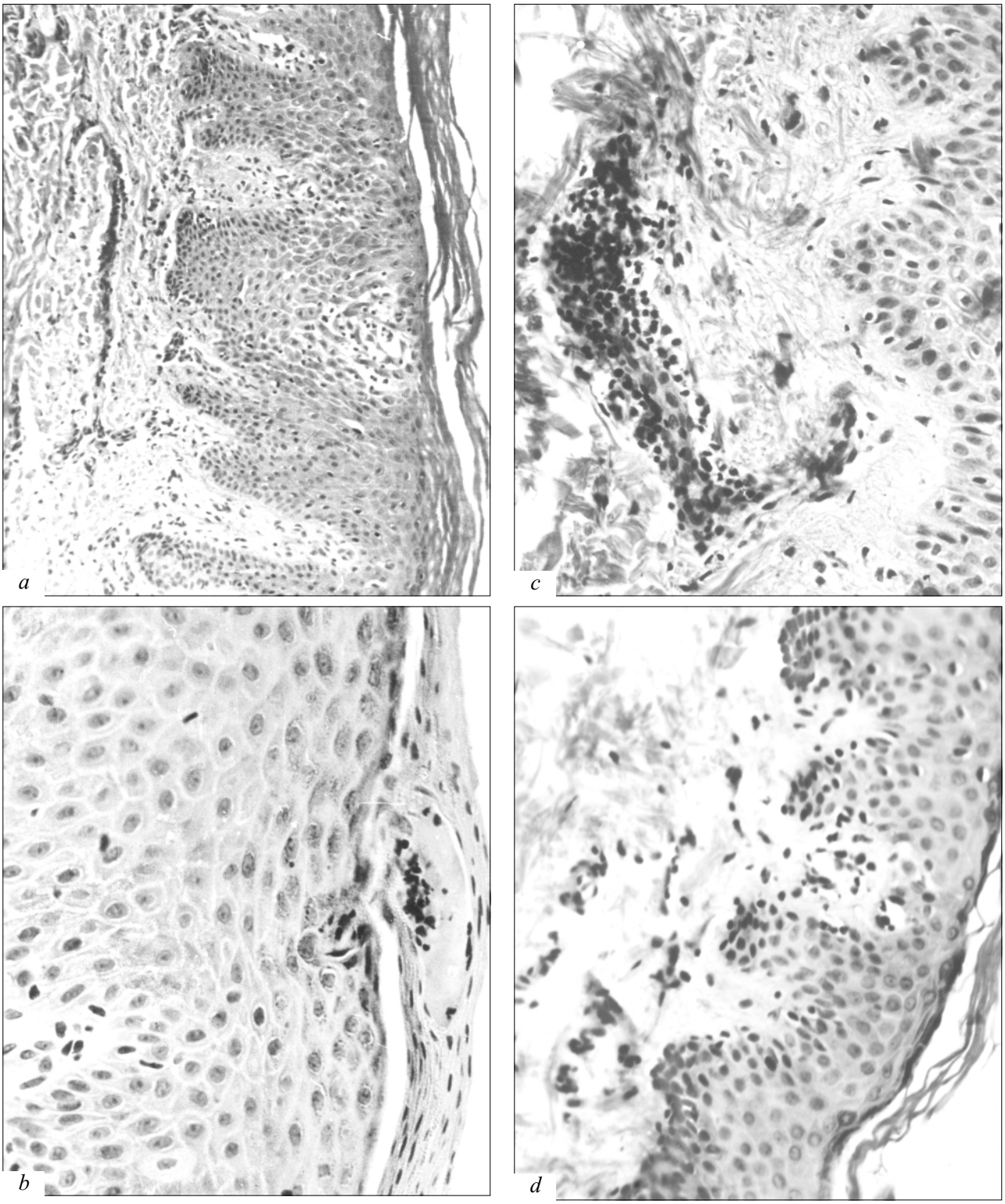


**Fig. 1.** Light microscopy of skin biopsy specimens from patients with allergic dermatoses associated with chronic opisthorchiasis receiving combination therapy. Hematoxylin-eosin staining; ×100 (*a*), ×250 (*b-d*). Microbial eczema before therapy (*a*, *b*): acanthosis, papillomatosis, focal spongiosis in the epidermis, diffuse cellular infiltration of the derma (*a*), intraepidermal bladder filled with lymphocytes and cellular detritus (*b*). Allergic dermatitis before therapy (*c*): pronounced perivascular mononuclear infiltration. Microbial eczema after therapy (*d*): decrease in the severity of acanthosis, papillomatosis, and degenerative changes in epidermal cells, moderate cellular infiltration of the derma.

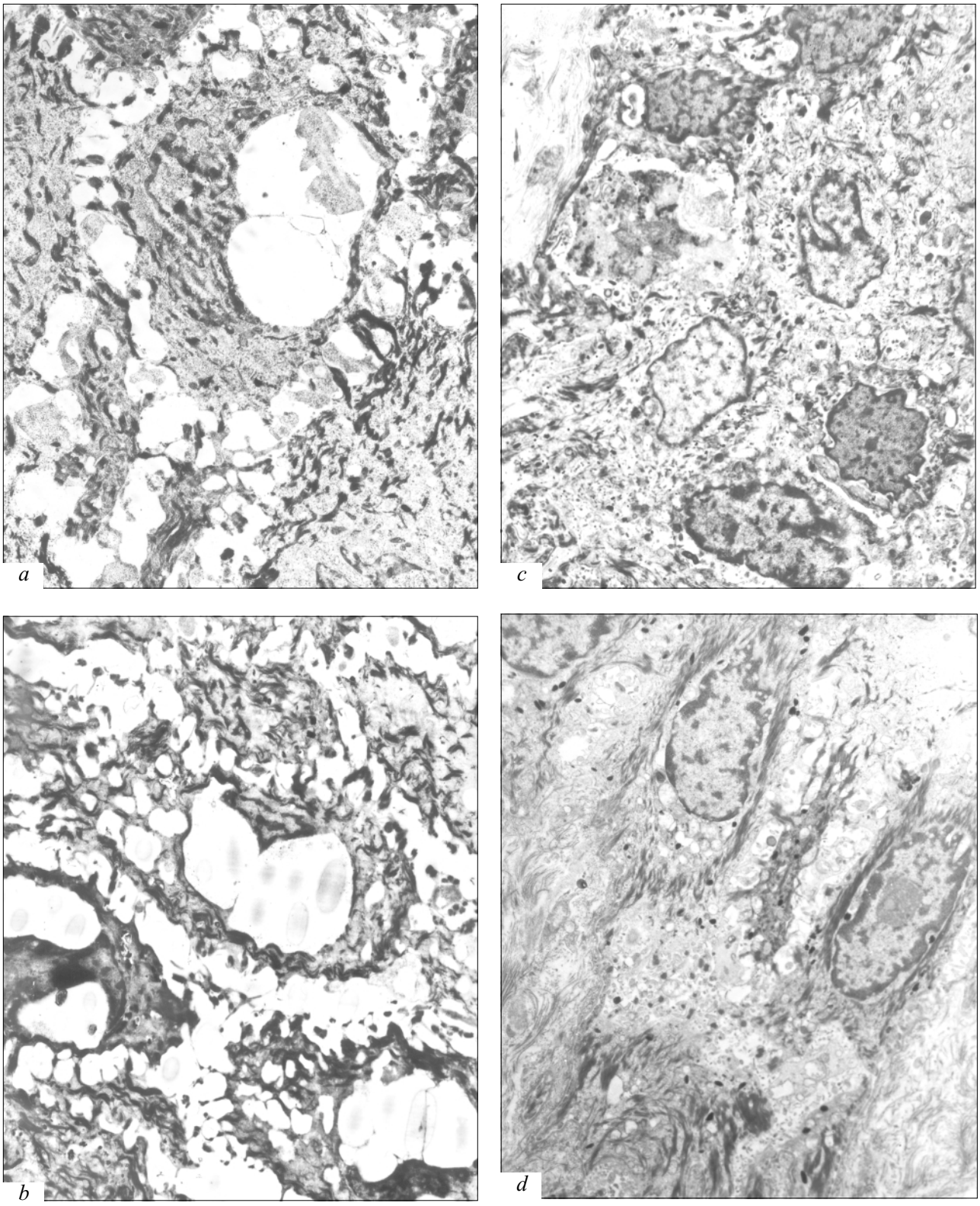


**Fig. 2.** Ultramicroscopic characteristics of the epidermis in patients with allergic dermatoses associated with chronic opisthorchiasis before (*a*, *b*) and after (*c*, *d*) combination therapy. Electronograms. Fragments of the spinous layer in the epidermis (*a*, *b*). Pronounced degenerative and alterative changes in epitheliocytes: perinuclear empties, vacuolization of the cytoplasm, disorganization of intercellular contacts (×6000). Heterogeneity of basal epidermal cells: polymorphism of nuclei and intracellular organization (×3000, *c*). Basal and spinous epidermal cells with numerous bundles of tonofibrils (×4000, *d*).

tion, and tinctorial properties. Elastic fibers were fragmented. Vascular endotheliocytes in the derma were polymorphous. In microvessels these cells were hypertrophic and contained numerous processes of the luminal surface and considerable number of microvesicles. These signs reflect activation of pinocytosis. The perivascular zone contained macrophages, lymphocytes, and degranulating mast cells. In various zones of the derma we found numerous fibroblasts with hyperplasia of the granular cytoplasmic reticulum (fibrillogenesis).

After combination therapy the severity of acanthosis, papillomatosis, and keratinocyte degeneration decreased (Fig. 1, *d*). Signs of epitheliocyte regeneration and recovery of morphological stratification in the epidermis were revealed. Basal epitheliocytes were characterized by pronounced polymorphism. Hyperplasia of organelles responsible for biosynthetic processes was found in most cells (Fig. 2, *c*). Epidermal cells in the spinous layer contained a considerable number of longitudinal tonofibrils, which contributed to the formation of specialized intercellular contacts and decrease in the intercellular distance (Fig. 2, *d*). Keratinocytes in the granular layer accumulated large granules of keratohyalin. Their nuclei underwent degeneration, which reflected normalization of keratinization and terminal differentiation.

The signs of acute hemodynamic disturbances in the derma were not observed. We revealed individual perivascular agglomerates of mononuclear cells. Focuses of fibrinoid changes in the derma were replaced with the granulation tissue. Then these regions underwent fibrosis. We found reduction of elastic fibers in the papillary layer of the derma (hypoelastosis) and fibrosis of the reticular layer due to newly formed collagen fibers.

The basal membrane (BM) underwent changes during combination therapy. Before therapy BM in skin specimens was thickened due to edema and presence of a considerable number of destructed fibers. Otherwise, BM was dense and thinned. In some specimens the dermoepidermal junction was not formed, and BM was visually non-differentiable from the subepidermal layer of the derma. After therapy and recovery of papillary structures in the derma, BM of the epidermis was characterized by moderate electron density due to the presence of irregular flake-like substances and longitudinal thin-fibrous structures.

BM performs various functions, affects the "behavior" of keratinocytes, and modulates polarity, proliferation, migration, and differentiation of cells. BM is involved in the morphogenesis and remodeling of the skin [15]. Although functions of various components in BM are unclear, its structural modification can be associated with hereditary and other pathological changes [11,12].

Disturbances in BM that acts as a pacemaker of the dermoepidermal junction formed by ectodermal (keratinocytes) and mesodermal cells (fibroblasts) play a key role in the morphogenesis of skin diseases. Various protein components produced by basal keratinocytes and fibroblasts of dermal papillae are necessary for the development and functional activity of BM [13,14]. BM is involved in structural integration. Recent studies showed that one components act as signal molecules, while others serve as the target during pathological processes (*e.g.*, antigenic or toxic action of helminths). Other elements of the dermoepidermal junction also play an important role. It should be emphasized that signal transduction triggers various cytoplasmic processes. For example, binding of integrins secreted by basal epitheliocytes can be followed by suppression of apoptosis in epitheliocytes [10].

Parasitic toxemia and antigenemia play a role in the pathogenesis of allergic dermatoses associated with opisthorchiasis. Probably, toxins produce the cytopathic effect on various organs and cause degenerative and destructive changes [2]. These findings explain the therapeutic effect of combination therapy with vermicides during severe and torpid allergic dermatoses. Our results indicate that combination therapy prevents progressive and recurrent dermatoses, causes stable remission or clinical improvement, and produces positive structural changes in the epidermis and derma.

## REFERENCES

1. E. I. Beloborodova, M. I. Kalyuzhyna, Yu. A. Timchenko, *et al*., *Chronic Opisthorchiasis and Digestive System* [in Russian], Tomsk (1996).
2. L. M. Isachkova, N. F. Timchenko, E. P. Nedashkovskaya, and S. D. Raznik, *Byull. Eksp. Biol. Med*., **130**, No. 11, 593-597 (2000).
3. A. V. Lepekhin, V. V. Mefod'ev, and D. M. Dalmatov, *Clinics, Pathogenesis, Epidemiology, and Prevention of Intestinal Infections in Newly Constructed Regions Endemic by Opisthorchiasis* [in Russian], Tomsk (1991).
4. A. V. Lepekhin, G. F. Rogodenko, T. I. Gulina, *et al*., *Clinical Course of Infectious Diseases in Pestholes of Opisthorchiasis* [in Russian], Tomsk (1984).
5. G. I. Nepomnyashchikh, *Boundary Tissues (Mucosal Layers and Skin) in the Morphogenesis of General Pathological Processes* [in Russian], Novosibirsk (1996).
6. A. I. Pal'tsev, *Diseases of Digestive Organs during Chronic Opisthorchiasis* [in Russian], Novosibirsk (1996).
7. S. G. Senchukova, *Pathomorphological Assay of Allergic Dermatoses and Psoriasis in Patients with Chronic Opisthorchiasis*, Abstract of Doct. Med. Sci. Dissertation, Novosibirsk (1999).
8. G. M. Tsvetkova and V. N. Mordovtsev, *Pathomorphological Diagnostics of Skin Diseases* [in Russian], Moscow (1986).
9. V. N. Shilov and V. I. Sergienko, *Byull. Eksp. Biol. Med*., **129**, No. 4, 364-369 (2000).

10. L. Borradori and A. Sonnenberg, *Curr. Opin. Cell Biol*., **8**, 647-656 (1996).
11. R. E. Burgeson and A. M. Christiano, *Ibid*., **9**, 651-658 (1997).
12. U.-F. Haustein, *Dermatol. Online J*., **8**, No. 1, 3 (2002).
13. A. Jelaska, D. Strehlow, and J. H. Korn, *Springer Semin. Immunopathol*., **21**, 385-395 (2000).
14. H. M. Kagan, *Acta Trop*., **77**, 147-152 (2000).
15. R. Timpl, *Curr. Opin. Cell Biol*., **8**, 618-624 (1996).