degenerative changes in the internal membrane neural epithelium, and detected changes in the microvessels and intrafusal fibers make the possibility of the preservation of many receptors doubtful. The prevalence of destructive degenerative changes in regenerating neural structures and impaired axon-Schwann cell interactions impede the formation of normal neuromuscular and sensory contacts already limited due to the random nature of the reinnervation processes.

The pattern of muscle spindle structural arrangement in a replanted limb suggest a marked limitation of the processes of recovery of adequate functional activity in many muscle receptors, which may be responsible for the observed abnormal sensitivity of the replanted limb and for failure of its satisfactory functional restoration [5].

REFERENCES

1. A. V. Volodina, N. S. Gurko, and O. M. Pozdnyakov, in: *Anatomicophysiological and Pathomorphological Asprcts of Microsurgery and Gunshot Injury* [in Russian], Leningrad (1990), pp. 95-96.
2. A. N. Studinskii, M. M. Umnova, I. L. Novoselov, *et al.*, *Dokl. Acad. Nauk SSSR*, **301**, № 1, 222-225 (1988).
3. H. Tamar, *Principles of Sensory Physiology*, C. C. Thomas (1972).
4. R. W. Banks and D. Barker, *J. Physiol.* (lond), **345**, 97 (1983).
5. S. Z. Pogers, *Develop. Biol.*, **94**, 265-284 (1982).
6. J. J. A. Scott, *Brain Res.*, **563**, № 1/2, 195-202 (1991).

Ultrastructural and Electron-Cytochemical Alterations of Large Intestine Cells in Viral Diseases

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UDC 616.98−06:616.351−018.1−076.4

Translated from *Byulleten' Eksperimental'noi Biologii i Meditsiny*, Vol. 115, № 4, pp. 436−440, April, 1993
Original article submitted November 4, 1992

Key Words: *large intestine; HIV infection; cell ultrastructure; adenylate cyclase ultracytochemistry*

Research on virus particles and viral inclusions is being successfully carried out using cell culture [4-6]. However, the study of virus-induced alterations *in vivo* has not still received sufficient attention, even though it is an essential step in the investigation of the morphological features of viral effects on cell ultrastructure. We have not found any published data dealing with cell ultrastructure, virus particles, and viral inclusions in various populations of large intestine cells in HIV infection.

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It is common knowledge that viruses do not express metabolic activity their own. But the formation of viral inclusions can markedly change the cellular metabolism [2,9]. For example, the activity of the main metabolic enzyme adenylate cyclase (AC) adequately reflects the processes of virus-induced cell alterations. This is true, specifically, for the RNA-containing viruses such as HIV (retroviruses) and the influenza virus (orthomyxoviruses).

The aim of this investigation was to reveal the salient features of the ultrastructural and cytochemical alterations in various large intestine cells in HIV infection. Considering the well-known fact that diseases of the large intestine are often induced by respiratory

0007-4888/93/0004-0470$12.50

virus particles, in particular, by the influenza virus [1], we performed a differential study of the specific alterations of large intestine cells during experimental influenza.

MATERIALS AND METHODS

Bioptates of the rectum and sigmoid flexure were obtained by aspiration biopsy from AIDS patients (men and women 25-40 years old - 5 cases in all). Two of these patients had diarrhea. The controls were specimens of the same parts of the large intestine taken from three patients with gastric ulcers. In 5 cases the large intestine from deceased persons of the same age was studied.

Experimental influenza was induced on BALB/c mice by intranasal inoculation of the influenza virus (A/H3N2/Aichi/68) in a sublethal dose of 3 to 30 ID/0.1. Specimens were removed from the large intestine 3 and 7 days after infection. For transmission electron microscope study the samples were fixed in a glutaraldehyde - paraformaldehyde mixture (0.5% and 4%, respectively) buffered with 0.05 M Na-cacodylate with glucose, then washed, postfixed with 1% OsO_4 solution in the same buffer, dehydrated,

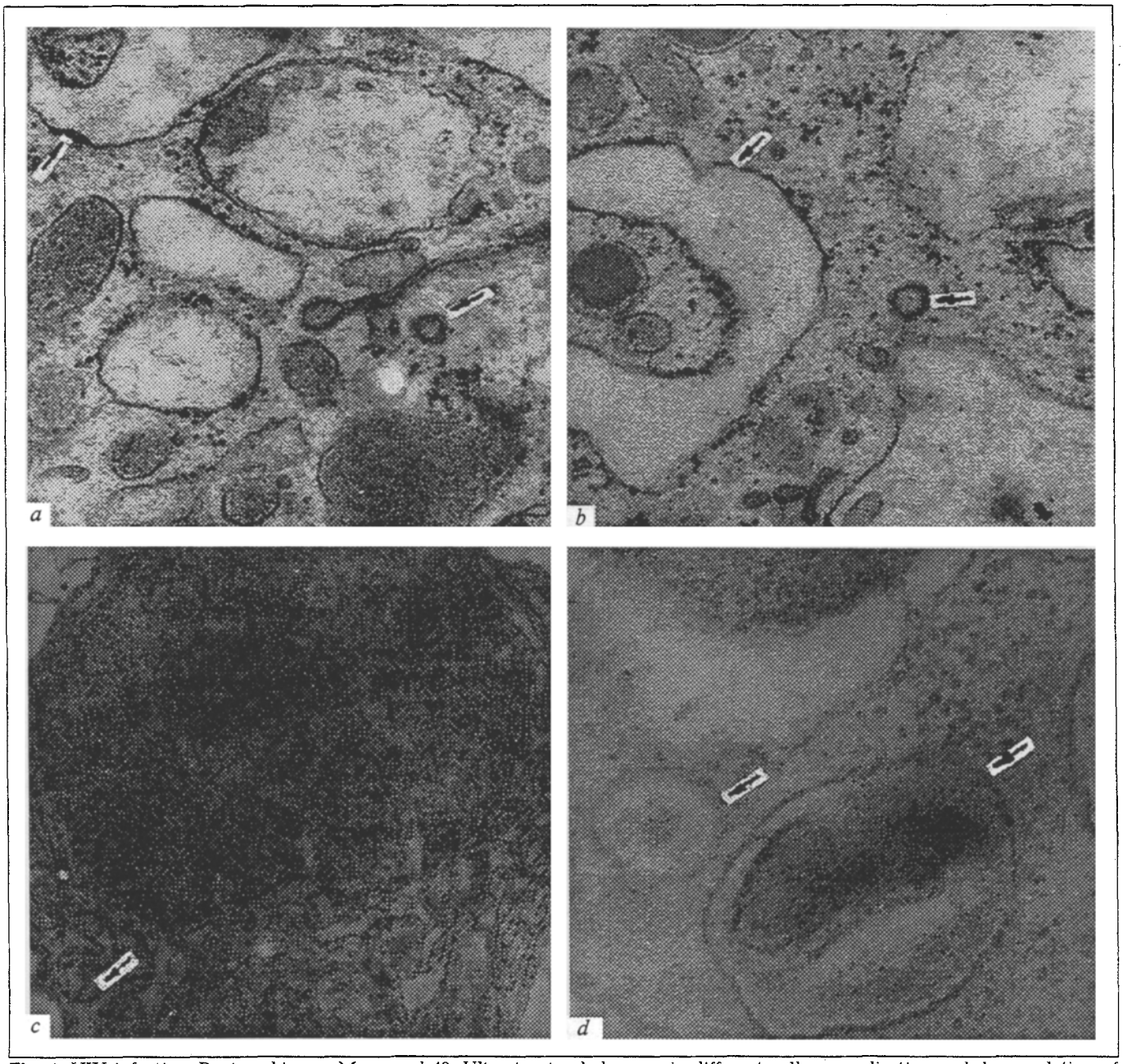

Fig. 1. HIV infection. Rectum biopsy. Man aged 40. Ultrastructural changes in different cells: vacuolization and degranulation of GER with invaginations; different stages of MGS formation (arrows); viral particles and inclusions (*a*, *b*) in bordered epitheliocytes, in plasmocyte (*c*), and in lymphocyte (*d*). ×50,000 (*a*, *b*, *d*), ×21,000 (*c*).

and embedded in Westopal W. A modified method was used [3] for the ultracytochemical demonstration of AC. Ultrathin sections were examined under a JEM-100C electron microscope (Japan).

RESULTS

The specimens of HIV-altered large intestine exhibit moderate lymphoid-plasmocytic infiltration, marked edema of the stroma, spasmodic contraction of the arteries, and dilatation of the veins. The muscle layers are markedly swollen, and ganglionic cells of the intramural nerve plexus between the longitudinal and circular muscle layers exhibit pronounced dystrophic changes. The goblet cells at different stages of the secretory cycle exhibit an increased secretory activity. They have fine dark secretory granules and show some dystrophic and destructive features. These processes spread throughout the cell organelles: the cisternae of the granular endoplasmic reticulum are markedly dilated and degranulated, the perinuclear space and interdigitations of the lateral plasmalemma

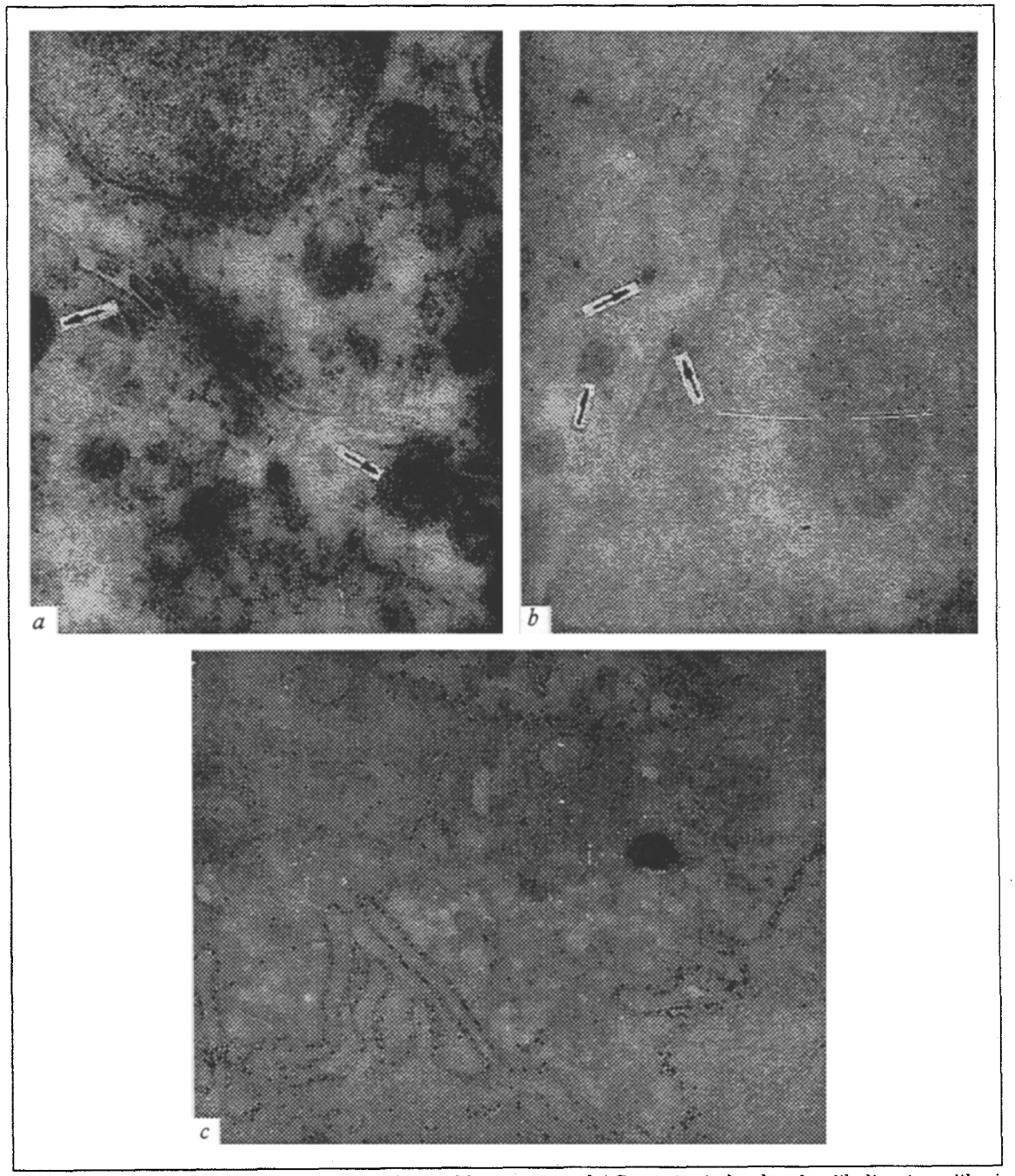

Fig. 2. HIV infection. Rectum biopsy. Woman aged 37. Weakly pronounced AC activity in bordered epitheliocytes with viral particles and inclusions (arrows) (*a*, *b*). High AC activity in control (rectum, woman aged 38). ×32,000 (*a*), ×28,000 (*b*, *c*).

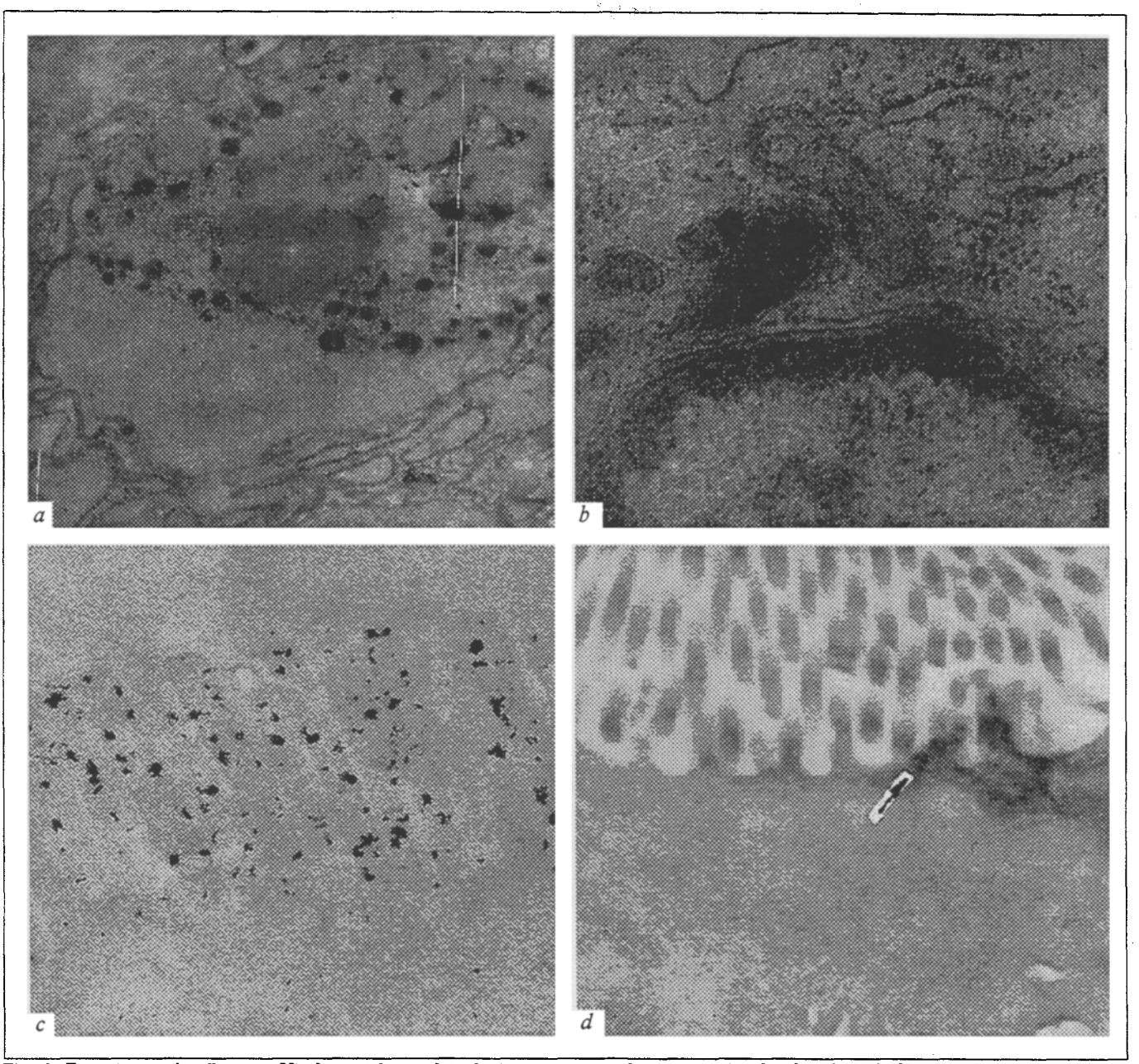

Fig. 3. Experimental influenza. Viral particles and inclusions in mouse large intestine bordered epitheliocytes 3 days after virus inoculation (*a, b*). High AC activity in control mice (*c*); lowered AC activity in experimental influenza in the cells with viral particles (*d*). ×28,000 (*a–d*).

are also dilated, there is local and sometimes general lightening of the hyaloplasm, and destruction of mitochondria and the Golgi apparatus.

Both virus particles and viral inclusions are observed in the bordered epitheliocytes. Virus particles are mainly localized in the apical part of the cells, where the microfilaments are disoriented. There may also be viral inclusions within the microvilli. Virus particles are often incorporated into the viral inclusions, microbodies, and altered mitochondria as osmiophilic, various-shaped structures (Fig. 1, *a, b*); sometimes there is damage of the host organelle membrane in such incorporations (Fig 1, *a*).

An ultrastructural specific feature of HIV infection is the formation of tubuloreticular and tubulocircular structures [10-12]. In our view, these structures should be called membranous granular structures (MGS) as this term more directly reflects their composition. MGS consist of both membranous and granular components of the intracellular organelles and may also include virus particles (Fig. 1). It can be assumed that the formation of all MGS-type structures is induced by alterations of the granular and agranular endoplasmic reticulum (GER, AER). This can be clearly demonstrated on various cells of the intestinal mucosa. In HIV infection we find

MGS of different shape and size, formed from altered components of the reticulum in bonrered epitheliocytes, goblet cells, lymphocytes, plasmocytes, mast cells, eosinophils, and endothelial cells. In addition, destroyed mitochondria, ribosomes, microfilaments, and secretory and lipid granules may be involved in the process. MGS are often found in cells with well-developed GER and AER cisternae. They can be of various shape and size, from tiny, resembling micropinocytic vesicles, to large vacuolar or rod-shaped structures [2]. We found similar structures in the intestinal epithelium, lymphocytes, and plasmocytes while examining both biopsy and autopsy samples.

The study of AC activity revealed a very weak reaction to this key metabolic enzyme, present only as dotlike precipitates in the lateral plasmalemma and nucleolemma of the bordered epithelium cells, whereas in other diseases the enzyme activity remains quite stable (Fig. 2). A weakly pronounced reaction to AC was exhibited in the patients with or without of diarrhea and was assumed to be due to the virus-induced depression of all metabolic processes.

In the experimental influenza study we noted that influenza and HIV virus particles and viral inclusions are of different size and composition. Different cell organelles and granular structures may be included in their composition. Influenza virus particles are smaller in size, rounder, and typically occur in the paranuclear zone as isolated structures or else within viral inclusions associated with lipid granules (Fig. 3, *a, b*). The control animals show sufficiently high AC activity in the apical and lateral plasmalemma of the bordered epithelium, whereas experimental influenza produces the same decrease of AC as does HIV (Fig 3, *c, d*).

The results obtained are probably related to virus-induced changes in the intestinal cells. Virus particle penetration is connected with damage to the cell membrane, which results in an increase of membrane permeability for and accumulation of Ca^{2+} [8]. Similar effects are demonstrated for Na^+ and K^+ [7]. Eventually this leads to some inhibition of membrane AC. Marked cellular edema, an increase of the cell volume, and changes of the lipid metabolism of infected cells lead to cell destruction. Confirming this is the presence of a large number of lipid granules in the cells with virus particles and viral inclusions. In addition to the increase of membrane permeability, a decreased synthesis of phosphatidylcholine, which is a structural membrane component intimately connected with AC, and also of diacylglycerol, one of the secondary messengers for protein kinase C [9], are observed. The changes in the protein-lipid composition of the membrane result in a decrease of AC activity with a concurrent increase of water and electrolyte secretion. We contend that even in the absence of opportunistic infections in the gastrointestinal tract, there are significant alterations in various populations of intestinal cells which impair the absorption and step up the secretion of both the secretory cells and the bordered epitheliocytes.

Thus, we identified four groups of HIV-induced morphological alterations. The first group involves dystrophic and destructive changes, observed in the large intestine epithelium and stroma. Virus particles and viral inclusions revealed in various intestinal cells make up the second group. The third group presents MGS peculiar to HIV infection. And, finally, the fourth group of changes consists in the marked decrease of AC activity in the large intestine epitheliocytes.

REFERENCES

1. A. E. Ali-Riza, T. G. Barkhina, and Yu. G. Parkhomenko, *Byull. Exp. Biol.*, **114**, № 10, 337-342 (1992).
2. T. G. Barkhina, T. G. Shipakina, and V. E. Kondrat'ev, *Ibid*, **105**, № 3, 354-357 (1988).
3. T. G. Barkhina and Yu. G. Parkhomenko, in: *Current Topics in Modern Histopathology* [in Russian], Moscow (1990), pp. 85-88 .
4. A. F. Bykovskii and N. V. Klitsunova, *Dokl. Akad.Nauk SSSR*, **224**, № 1, 226-227 (1975).
5. V. M. Zhdanov and S. Ya. Gaidamovich, *General Virology* [in Russian], Moscow (1982).
6. V. D. Solov'ev, Ya. E. Khesin, and A. F. Bykovskii, *Essays in Viral Cytopathology* [in Russian], Moscow (1979).
7. R. F. Garry, A. A. Gottlieb, and K. P. Zuckerman, in: *4th International Conference of AIDS, Abstract*, New York (1988), p. 22.
8. S. Gupta and B. Vayuvegula, *J. Clin. Immunol.*, **7**, 486-489 (1987).
9. W. S. Lynn, A. Tweedale, and M. W. Cloyd, *Virology*, **163**, 43-51 (1988).
10. C. Pedersen, T. Horn, J. Junge, *et al., APMIS*, **97**, 249-252 (1989).
11. G. S. Sidhu, R. E.Stahl, W. El-Sadr, *et al., Hum. Path.*, **16**, № 4, 377-386 (1985).
12. R. Ullrich, M. Zeitz, W. Heise, *et al., Ann. Intern. Med.*, **111**, № 1, 15-21 (1989).