## REFERENCES

1. F. Arguello, R. B. Baggs, A. E. Eskenazi, *et al., Int. J. Cancer,* **48**, № 4, 583-590 (1991).
2. T. Baron and J. F. Dore, *In Vivo,* **6**, № 1, 77-79 (1992).
3. E. A. Bayer and M. Wilchek, *Methods Biochem. Anal.,* **26**, 2-15 (1990).
4. M. Belew and J. S. Whitehead, *Biochem. J.,* **167**, 621-628 (1977).
5. I. J. Fidler, *Proc. Amer. Ass. Cancer Res.,* **32**, 486-487 (1991).
6. A. Locshin, B. C. Giovanella, P. D. Deipolyi, *et al., Cancer Res.,* **45**, 345-350 1985).
7. B. M. Mueller and R. A. Reisfeld, *Cancer Metastasis Rev.,* **10**, № 3, 193-200 (1991).
8. X. Xie, N. Brunner, G. Jensen, *et al., Clin. Exp. Metastasis,* **10**, № 3, 201-210 (1992).


# MORPHOLOGY AND PATHOMORPHOLOGY

# Ultrastructural Analysis and Autoradiography of Gastric Mucosa Bioptates in Chronic Active Hepatitis

**D. L. Nepomnyashchikh**

UDC 616.36-002.2-07:616.33-018.73]-076.4

Translated from *Byulleten' Eksperimental'noi Biologii i Meditsiny,* Vol. 118, № 8, pp. 190-193, August, 1994
Original article submitted May 30, 1994

The results of gastrobiopsy are studied for a general pathological process in the liver. It is shown that the atrophic-sclerotic reactions predominating in the gastric mucosa are characterized by disturbances in the mucocyte ultrastructure attended by a reduction of cytoplasmic organelles, a marked decrease of biosynthesis in parenchymatous cells, and stroma collagenization, which is a systemic manifestation of regenerative-plastic insufficiency.

**Key Words:** *stomach; electron microscopy; regenerative-plastic insufficiency*

The seminal studies of Aruin *et al.* [1], Ugolev *et al.* [7], Uspenskii [8], and Yasinovskii [9] have shed much light on the structural and functional reactions of the digestive system in physiology and pathology. However, studies performed on clinical models, assessing the systemic reactions which reflect the interaction between organs of the digestive system in health and pathology, are few and far between [2,3]. The importance of such an approach is to be stressed, as it allows for assessment of cell and tissue heterogeneity, taking into account the integrity of structural and metabolic responses. The aim of the present study was to perform a complex morphological analysis of gastric mucosa specimens in chronic active hepatitis.

Laboratory of General Pathology, Research Institute of Regional Pathology and Pathomorphology, Siberian Division of the Russian Academy of Medical Sciences, Novosibirsk. (Presented by D. S. Sarkisov, Member of the Russian Academy of Medical Sciences)

## MATERIALS AND METHODS

Seventy-two gastric mucosa (GM) specimens obtained during fibrogastroscopy were morphologically investigated. A larger part of each specimen was used for the preparation of paraffin sections (fixing in 10% neutral Formalin and hematoxylin-eosin staining in combination with Perls reaction, Van Gieson staining, and Schiff reagent). A

0007-4888/94/0008-0885$12.50 

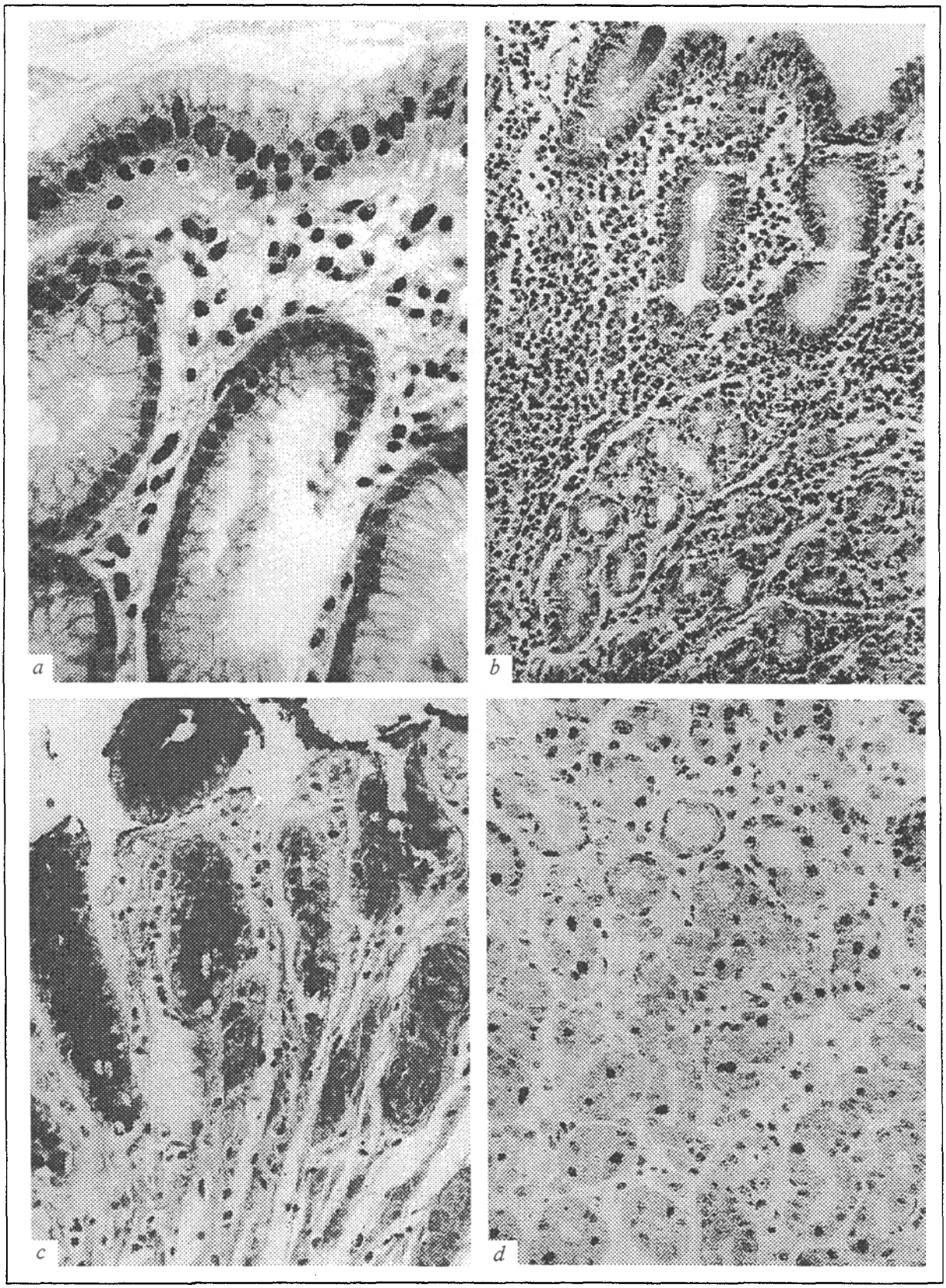


**Fig. 1.** Light-optic changes in stomach specimens in chronic active hepatitis. *a*) edema and moderate polymorphonuclear cellular infiltration of GM; hematoxylin-eosin staining, ×400; *b*) thick diffuse inflammatory reaction of GM; hematoxylin-eosin staining, ×200; *c*) diffuse atrophy of glands and stromal sclerosis; Van-Gieson staining, ×350; *d*) RNA synthesis in cells of fundal glands; semithin section; incubation with $^3$H-uridine; azure II staining, ×300.

smaller part of each specimen was fixed in 4% paraformaldehyde and postfixed in 1% osmium tetroxide; after dehydration specimens were embedded in Epon-Araldite. Semithin sections were stained with azure II and Schiff reagent. Ultrathin sections obtained on an LKB III ultratome were double-contrasted and examined in a JEM 100B electron microscope (accelerating voltage 60 kV).

Paraffin sections stained after Giemsa without differentiation and after Gram-Weigert, as well as semithin sections stained with azure II, were used in bacterioscopic analysis of stomach specimens. Autoradiography of GM specimens was performed by *in vitro* incubation with $^3$H-thymidine and $^3$H-uridine [6].

Gastrobiopsy was performed in patients who had completed the obligatory course of clinical and laboratory investigations, including, in the majority of cases, liver biopsy, the results of which yielded a diagnosis of chronic active hepatitis. The state of GM was judged from a complex assessment of clinical, endoscopic, and morphological data.

## RESULTS

Light-optic microscopy showed pathological changes of the GM in all specimens examined. These changes occurred in the epithelium, where mucocyte dystrophy with disturbed secretory function, dysplasia, sometimes metaplasia, and atrophy were found. In the lamina propria of the GM inflammatory-cellular infiltration of varying severity was observed (Fig. 1, *a* and *b*) against the background of focal or diffuse sclerosis. In some cases atrophy of the luminal epithelium and glands, as well as stromal sclerosis, occurred in the absence of cell infiltration (Fig. 1, *c*). In this case autoradiography showed a marked reduction of metabolic and proliferative activity of epithelial structures (Fig. 1, *d*).

Electron-microscopic examination of surface epitheliocytes showed three degrees of ultrastructural changes, and accordingly three groups of specimens were analyzed. In the first group of specimens the regular distribution of the microvilli was disturbed; the outer cytoplasmic membrane was found to invaginate into the luminal epitheliocytes; an enlargement of the intercellular spaces, notably in the basal portion of luminal epitheliocytes, was frequently observed along with intraepithelial migration of lymphocytes, macrophages, and plasmacytes. In some cases the bacteria *H. pylori* were found at the luminal surface of mucous cells (Fig. 2, *a*). A moderate enlargement of the cytoplasmic reticulum, focal destruction of mitochondrial cristae, and clearing of their matrix were noted in the mucocyte ultrastructure. On the whole, the maximum changes were observed in cells at the apex of the gastric ridges, i.e., in the upper part of the gastric pits.

In gastric biopsy specimens of the second group irregular contours of the surface were clearly observed, which was due to deformation of the gastric ridges, diverse sizes of surface epitheliocytes, and disturbed distribution of microvilli. In some cells the central part of the surface seemed to be elevated; cytoplasmic outgrowths sometimes formed (Fig. 2, *b*); bacteria were frequently encountered in the intercellular spaces and at the bottom of the gastric pits. Many of them had direct contact with the epitheliocyte membrane, but they were never observed in the cytoplasm [10-12]. The basal portions of cells were separated from each other with enlarged intercellular spaces, where lymphocytes and, more seldom, granulocytes were localized (Fig. 2, *c*). In the lamina propria lymphocytes, plasmacytes, and macrophages were also found. Specialized glandular cells were characterized by a smaller size and a reduced number of cytoplasmic organelles.

The number of surface cells with disturbances in the ultrastructure was higher in this group of specimens than in the first group. We observed dark cells with a poorly structured electron-dense cytoplasm containing solitary secretory granules or devoid of these. Along with this, epitheliocytes with partially altered cytoplasmic organelles and vacuolization of the cytoplasm were found.

In gastric specimens of the third group a large number of epitheliocytes almost devoid of microvilli with electron-dense cytoplasm, in which cytoplasmic organelles were poorly distinguishable, was the most notable feature (Fig. 2, *d*). Sometimes we were able to distinguish a few mucous cytoplasmic granules with a fibrillar structure, which formed saucer-shaped aggregates. Other epitheliocytes exhibited ultrastructural characteristics of goblet cells with fine-grained granules; we encountered cells with fibrillar and fine-grained granules.

Changes in the surface epitheliocytes in different forms of chronic gastritis reflect different degrees of ultrastructural changes. Invaginations of the cell surface, frequently with attached bacteria but without signs of phagocytosis, and intercellular edema with transepithelial cell migration are typical of the first degree of changes. More severe damage to mucocytes is characterized by the presence of electron-dense epitheliocytes with saucer-shaped aggregates formed by mucous granules. Evidently, these cells are part of the ultrastructural manifestation of so-called "intestinal metaplasia." Their relation to goblet enterocytes is difficult to

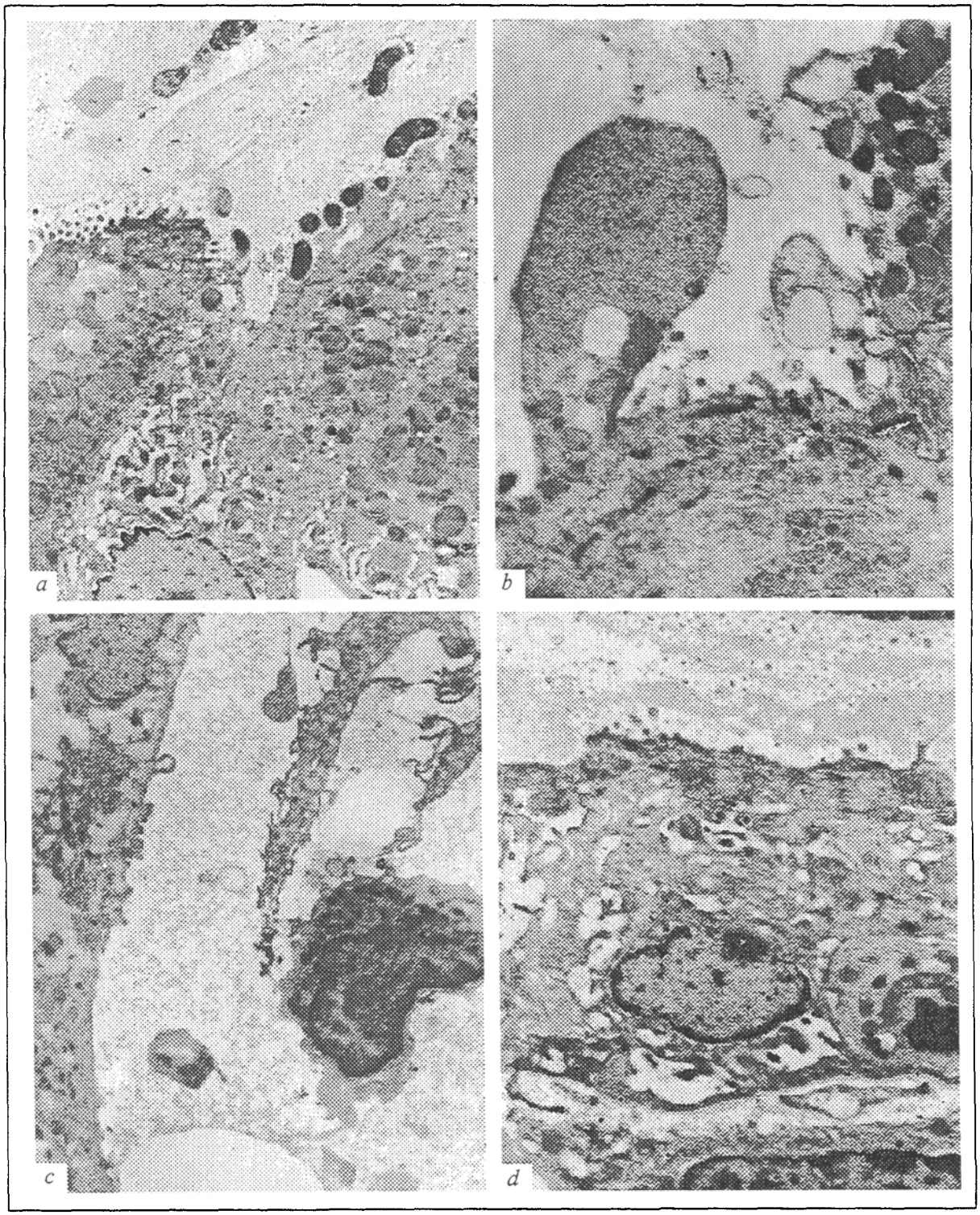


Fig. 2. Ultrastructural changes in stomach specimens in chronic active hepatitis. *a*) enlargement of intercellular spaces; *H. pylori* at surface of luminal epithelium, ×2000; *b*) cytoplasmic protrusion of epitheliocyte containing small granules, ×5000; *c*) granulocyte in an enlarged intercellular space, ×2700; *d*) luminal epitheliocyte with barely distinguishable ultrastructural organization and with the absence of secretory granules, ×3300.

analyze. In addition to their similarity in electron density, some of this cells, with respect to the structure of mucous granules, are identical to normal surface cells of the stomach; other cells resemble goblet enterocytes; the third group has a mixed morphological phenotype. Such a heterogeneity of the ultrastructure may be due to functional differences.

It should be mentioned that changes were observed in the vessels of the microcirculatory bed of the GM. The majority of the capillaries had a thinned endothelial lining; the structure of endotheliocytes was indicative of their low functional activity (no invaginations of the nuclear and cytoplasmic membranes or pinocytic vesicles); perivascular bundles of collagen fibers were frequently observed.

Thus, the complex morphological and clinical-endoscopic investigation demonstrated the involvement of the GM in the pathological process in all patients with chronic hepatitis. It is worthy of note that sclerosis is a predominant form of the process, its fundamental specificity being primary dystrophy and atrophy of the parenchymatous structures (luminal-foveal and glandular epithelium), as well as the reactive development of connective tissue.

In the majority of cases the morphogenesis of sclerotizing forms of gastritis in chronic hepatitis corresponds, with respect to the clinical and structural-functional characteristics, to primary-dystrophic gastritis resulting in the atrophic-sclerotic process [4] and probably reflects systemic manifestations of regenerative-plastic insufficiency [5]. A disturbance in the metabolic and plastic function of the liver, which regularly occurs after its damage, promotes the development of plastic insufficiency and underlies systemic manifestations of this phenomenon.

## REFERENCES

1. L. N. Aruin, P. Ya. Grigor'ev, V. A. Isakov, and E. P. Yakovenko, *Chronic Gastritis,* Amsterdam (1993).
2. L. I. Geller and A. L. Geller, *Klin. Med.,* № 12, 24-29 (1987).
3. A. K. Krylov, *Ter. Arkh.,* № 2, 139-141 (1991).
4. G. I. Nepomnyashchikh, V. V. Omigov, L. M. Nepomnyashchikh, *et al., Byull. Sib. Otdel. Akad. Med. Nauk SSSR,* № 4, 53-59 (1989).
5. G. I. Nepomnyashchikh and L. M. Nepomnyashchikh, *Arkh. Patol.,* № 6, 16-19 (1990).
6. D. S. Sarkisov, A. A. Pal'tsyn, and B. V. Vtyurin, in: *Electron-Microscopic Autoradiography of the Cell* [in Russian], Moscow (1980).
7. A. M. Ugolev, *The Evolution of Digestion and Fundamentals of the Evolution of Functions* [in Russian], Leningrad (1985).
8. V. M. Uspenskii, *Functional Morphology of the Gastric Mucosa* [in Russian], Leningrad (1986).
9. M. A. Yasinovskii, *Physiology, Pathology, and Clinical Treatment of the Mucous Membranes* [in Russian], Kharkov-Kiev (1931).
10. M. J. Blaser, *Gastroenterology,* **93**, № 2, 371-383 (1987).
11. D. Y. Graham, *Ibid.,* **96**, 615-625.
12. W. L. Peterson, *New Engl. J. Med.,* **324**, № 15, 1043-1048 (1991).