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## EFFECT OF HELIUM-NEON LASER IRRADIATION OF THE GASTRIC MUCOSA ON ITS EPITHELIAL CELLS

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Laser treatment of gastroduodenal ulcers has now been successfully introduced into clinical practice [3-5]. Helium-neon lasers (HNL) with an emission wavelength of 632.8 nm are most widely used for this purpose [4, 5]. However, the morphological basis of biological stimulation of ulcer healing has been inadequately studied [5]. There have been virtually no investigations of the action of low-intensity laser-radiation on the intact gastric mucosa (BM) in the case of endogastric irradiation.

This paper gives the results of a study of the action of HNL radiation on the microrelief, morphology, and proliferation of epitheliocytes in the gastric fundus during irradiation of GM.

### EXPERIMENTAL METHODS

Experiments were carried out on 50 male Wistar albino rats weighing not less than 140 g. The technique of irradiation of GM by means of a light guide, coupled with an LG-75 laser, and introduced perorally into the stomach with the aid of a special tube [6], developed previously, was used. Only a zone of the gastric fundus selected beforehand was irradiated in this method. The power of the radiation at the exit of the light guide was 8 mW, the diameter of the zone of irradiation was 3 mm, the duration of irradiation 1, 3, and 5 min, and the doses of irradiation given were 6.78, 20.34, and 33.9 J/cm<sup>2</sup> respectively. For the autoradiographic investigation <sup>3</sup>H-thymidine was injected intraperitoneally into the starving animals at 10 a.m. in a dose of 18.5 kBq/g body weight, and irradiation began 10 min later. The animals were killed by instant decapitation 1 h after the injection of <sup>3</sup>H-thymidine, and the stomach was removed and fixed by injection of 3-5 ml of 10% formalin into its cavity. Paraffin sections of circular fragments of the stomach, including the zone of irradiation (ZI) and the adjacent area (AA) were stained with hematoxylin and eosin, and by the PAS method, and sections for autoradiography were covered with type M emulsion. The index of labeled nuclei (ILN) of epitheliocytes of each type was determined by counting 1,000 cells in longitudinal sections through the fundal glands, and expressed as percentage. Statistical analysis was carried out by the Fischer-Student method. Fragments of ZI and AA for scanning and transmission electron microscopy were taken from the stomach immediately after irradiation and prepared for study by the usual method. Specimens were examined in H-600 and S-405 A

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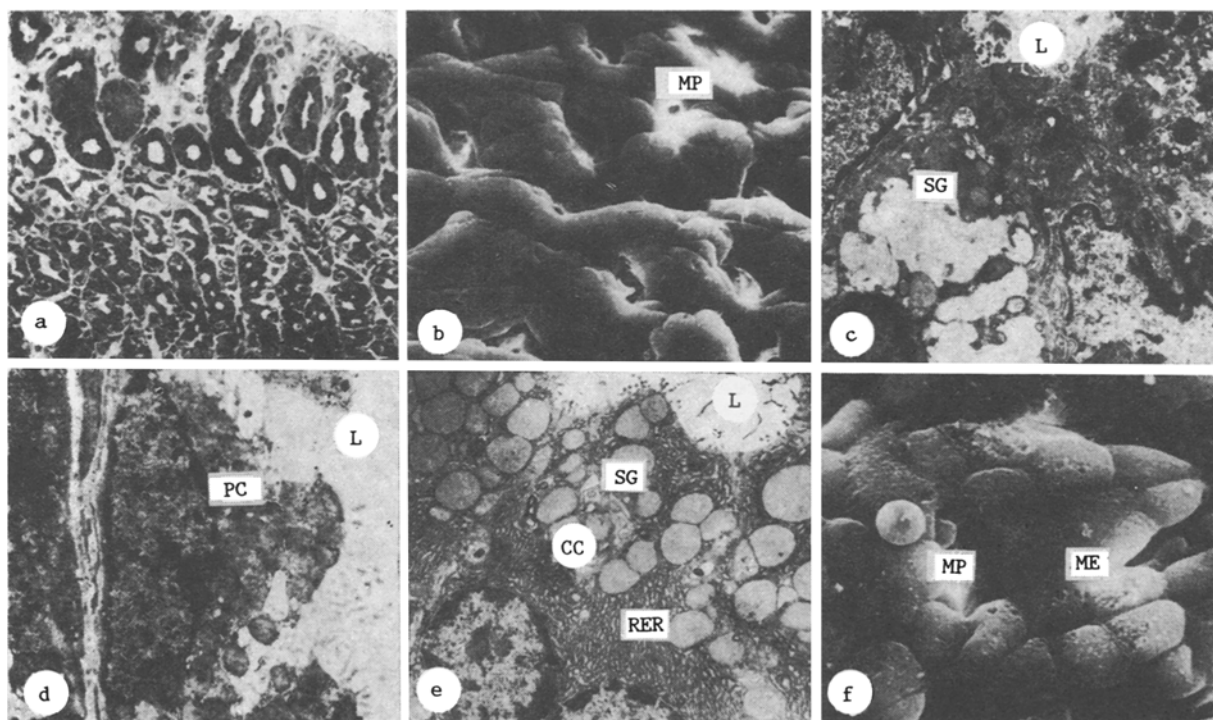


Fig. 1. Epithelial cells of GM after laser irradiation. a) Emptying of contents of surface epithelial cells (irradiation for 3 min). Semithin section. Methylene blue-fuschine. 100 $\times$ ; b) Smoothing of apical surface of cells of surface epithelium (irradiation for 3 min). 1500 $\times$ ; c) Fusion of secretory granules of accessory cells (irradiation for 3 min). 6,000 $\times$ ; d) Evagination of apical surface of parietal cell into lumen of gland (irradiation for 3 min). 5,000 $\times$ ; e) Evagination of apical surface of chief cell (CC) into lumen of gland (irradiation for 5 min). 5,000 $\times$ ; f) Microerosions of apical surface of surface epithelial cells (irradiation for 5 min). 2,000 $\times$ . PC) parietal cell; L) lumen of gland; MP) mouths of gastric pits; SG) secretory granules; LA) lamellar apparatus; RER) rough endoplasmic reticulum; ME) microerosions of apical surface of cells.

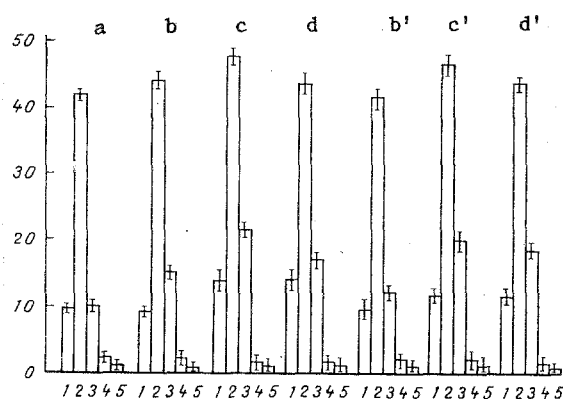


Fig. 2. Incorporation of  $^3\text{H}$ -thymidine into cells of GM after irradiation by HNL. 1) Surface epithelial pit cells; 2) epithelial cells at neck of pits; 3) accessory cells; 4) parietal cells; 5) chief cells. a) Control, b) irradiation for 1 min, c) for 3 min, d) for 5 min. b, c, d) ZI; b', c', d') AA.

electron microscopes (Hitachi, Japan). Semithin sections were stained with methylene blue and fuchsine.

#### EXPERIMENTAL RESULTS

Immediately after laser irradiation for 1 and 3 min emptying of the cytoplasm of the mucocytes lining the rugae of the stomach was observed. A few secretory granules were found, mainly in the apical part of the cells. At the same time, intensive accumulation and release of mucoid secretion by the epitheliocytes of the gastric pits was observed. An increase in the dose of irradiation intensified these processes at the expense of the deeply lying mucocytes (Fig. 1a). Many electron-dense secretory granules and widening of the profiles of the lamellar apparatus (LA) and rough endoplasmic reticulum (RER) were found in these cells. Clearing of the cytoplasm and a decrease in the number of free ribosomes were observed. The microrelief of GM acquired a distinctly rhythmic pattern as a result of swelling of the apical parts of the cells, which also induced folding of their surface (Fig. 1b.)

The greatest changes in the epithelium of the fundal glands were observed in the accessory cells. Hypertrophy LA and RER took place. Fusion of secretory granules was observed much more often than in the control (Fig. 1c). In the parietal cells intracellular secretory tubules were widened, the matrix of the mitochondria was clarified, and the area of the tubulovesicles was increased. In the chief cells vacuolation of LA, widening of the profiles of RER, and an increase in the number of secretory granules were observed. The size and electron density of the latter varied considerably. Active release of secretory material was observed. Evaginations of the apical part of the parietal and chief cells and a free distribution of fragments of their cytoplasm in the lumen of the glands were very often observed. This can perhaps be explained by "clasmatosis" (Fig. 1d).

The changes described above also were observed in AA of GM, but they were less marked. Increasing the dose of irradiation to 33.90 J/cm<sup>2</sup>, with an exposure of 5 min, aggravated these changes. Features reflecting the dystrophic character of this dose of irradiation were observed much more frequently. On the apical surface of the surface mucocytes of the pits numerous microerosions were observed, and in the chief cells there was stronger evidence of clasmatosis (Fig. 1e, f).

In AA of GM the action of laser irradiation was weaker. The effect of laser irradiation still persisted 50 min after its end in both ZI and AA. It was manifested to a greater degree in the mucus-forming cells after irradiation for 3 and 5 min. The structure of the parietal and chief cells was close to that normally observed throughout the area of the fundal glands (Fig. 1).

The autoradiographic investigation showed that laser irradiation affects the rate of proliferation of the epitheliocytes of GM. For instance, ILN of the accessory cells after irradiation for 1 min was  $15.0 \pm 0.48\%$  in ZI and  $12.5 \pm 0.54\%$  in AA, significantly higher than this parameter in the control ( $10.2 \pm 0.44\%$ ). A tendency for it to increase also was found in the surface pit cells, mainly at the expense of the epithelium of the pits. An increase in the dose of irradiation led to an even greater rise in the level of proliferation of these epitheliocytes. ILN of the accessory cells was  $22.7 \pm 0.74\%$  in ZI and  $20.5 \pm 0.64\%$  in AA. After irradiation for 5 min some decrease was observed in ILN of the accessory cells, and it was more marked in ZI (Fig. 2).

The effect of HNL radiation is thus determined by the dose of irradiation and it may also spread to areas of GM adjacent to the zone of irradiation. Doses of irradiation not causing pathological structural changes give rise to functional adjustments of the epitheliocytes, indicating more intensive formation of secretion in them, especially in the mucotypes, and also increased proliferative activity of the cells. The dystrophic character of the radiation is manifested primarily as changes in highly specialized epitheliocytes.

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