AN ELECTRON-AUTORADIOGRAPHIC METHOD OF INVESTIGATING RNA SYNTHESIS IN GASTRIC GLAND CELLS

A. A. Pal'tsyn, R. M. Filimonov, and N. D. Grafskaya

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An electron-autoradiographic method of investigating pieces of gastric mucosa obtained during clinical biopsy, and incubated with uridine-5-^{3}H is suggested. It was concluded from a comparison of sections with electron-microscopic autoradiographs prepared after injection of uridine-5-^{3}H into the intact animal and with the results of control sections treated with ribonuclease that the suggested method reflects RNA synthesis in the gastric gland cells.

KEY WORDS: gastric biopsy; gastric gland cells; RNA synthesis.

With the development of methods of tissue culture in vitro it has become possible to study the state of the fundamental biosynthetic processes in such tissues by autoradiography [5, 8-11]. In clinical practice this modification has been used to investigate, with the aid of thymidine-^{3}H, the proliferative activity (the number of cells synthesizing DNA) in certain human tumors: carcinoma of the cervix uteri [3], neuroglial tumors [1, 2], and carcinoma of the rectum [4]. The complexity of the cell structure of the gastric mucosa and the wide variety of pathological processes which develop in it have necessitated the use of very delicate methods of investigation in order to study their pathogenesis, including autoradiography of samples of tissue removed during life. Modern endoscopic instruments have led to the development of autoradiography of gastric biopsy material, whereby not only can such biopsy be carried out without risk to the patient's health, but autoradiographic data can also be compared with the structure of the mucosa as revealed by gastroscopy. In the course of clinical gastric biopsy the proliferative activity of the glandular epithelium has been studied by the use of thymidine-^{3}H and light-optical autoradiography [12]. Until recently autoradiographic investigation of biopsy material from the gastrointestinal tract has been regarded as a method of determining the rate of regeneration of the epithelium [7]. In order to study the gastric mucosa, it is evident that information must be obtained not only on the number of cells synthesizing DNA, but also on the character of other biosynthetic processes, including the finer details of metabolism in the ultrastructures of the cell. Such information can be given by electron-microscopic autoradiography, but as yet it has not been used for this purpose.

This paper describes a technique of electron-autoradiographic investigation of RNA synthesis in pieces of mucosa obtained during diagnostic gastric biopsy, developed by the writers.

Material for electron-autoradiographic investigation was obtained from patients between the ages of 14 and 76 years during gastric biopsy by means of an Olympus apparatus. The reason for gastroscopy and biopsy of the mucosa was a disturbance of gastric function associated with hypo- and hyperchlorhydric gastritis, or burns. Biopsy was carried out in the region of the fundus of the stomach.

Immediately after removal the specimen of mucosa was immersed in medium 199, cooled to 2°C, to which 10% bovine serum had been added. After 20-60 min the specimen was cut into smaller pieces ($0.5 \times 0.5 \times 1.0$ mm) suitable for electron microscopic embedding. The pieces were transferred in that form to flasks, from under penicillin, containing 5.5 ml of original medium and 500 μCi uridine-5-^{3}H (specific activity 24 Ci/mmole). The flasks were incubated at 37°C, and an injection needle, connected by a tube to an oxygen cylinder, was lowered into each flask. Oxygen was bubbled slowly through the solution during incubation. Incubation continued for 1.5 h. At the end of this period the pieces were quickly washed with cold medium (one change) and then with phosphate buffer, pH 7.4 (three changes), after which they were fixed for 1.5 h in 2.5% glutaraldehyde solution in phosphate buffer. After fixation with glutaraldehyde the pieces were left overnight in phosphate buffer with 10 changes of the solution in order to remove precursor not incorporated into RNA. The material was then postfixed with 1% osmium tetroxide solution and embedded in Epon in the usual way.

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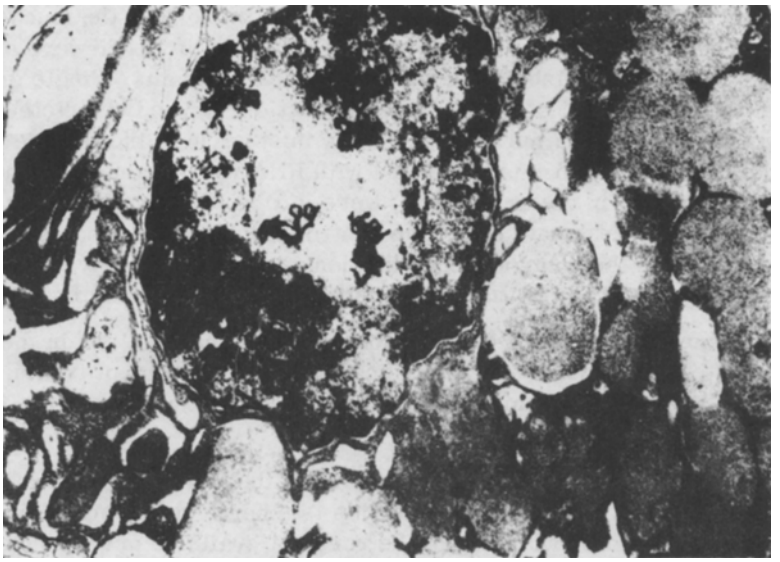

Fig. 1. Electron-microscopic autoradiograph of a chief cell of a gastric gland incubated with uridine-5-^{3}H. Grains of reduced silver were formed above the nucleolus, where their density was particularly high, and above the nucleoplasm.

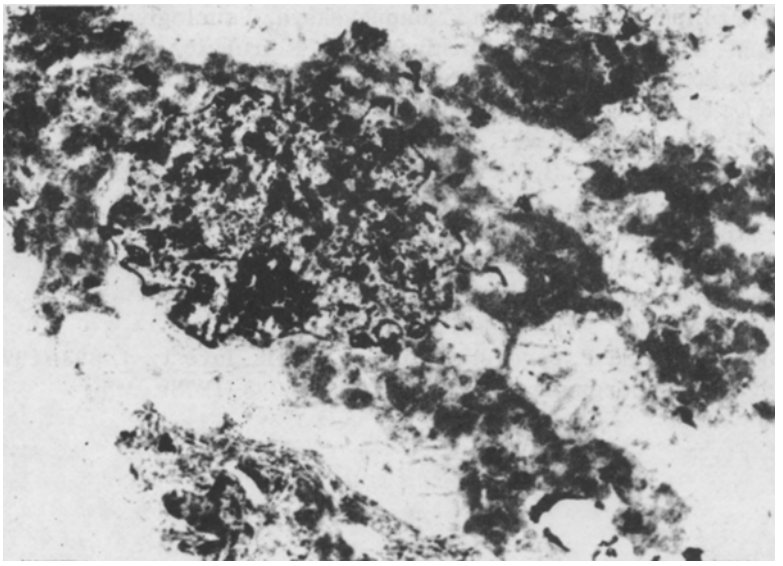

Fig. 2. Electron-microscopic autoradiograph of parietal cell of gastric gland incubated with uridine-5-^{3}H. Highest density of distribution of silver grains above nucleolus, lower density above nucleoplasm, and lower still above cytoplasm.

A large pyramid was first cut from each block from which semithin (1-2 μ) sections passing through the whole piece could be obtained. The arrangement of the piece of tissue in its capsule was taken into account when this was done. The semithin sections were placed on a slide, incubated at 80°C, and then immersed in liquid M emulsion and kept in a dark room. The sections were developed next day, stained with methylene blue and azure, and examined in the light microscope. Interesting areas of the gastric glands were chosen in the light-microscopic autoradiographs, and a small pyramid was cut out in the region of these areas for ultramicrotomy. M emulsion was applied to ultrathin sections by the method described by Sarkisov et al. [6]. The electron-microscopic autoradiographs were developed with D-19 developer 1-2 weeks after application of the emulsion.

Intensive labeling was found above all the main types of cells found in the mucosa of the gastric fundus (Figs. 1 and 2). After incubation for the chosen period (1.5 h) grains of silver were concentrated chiefly in the nuclei, where the highest density of label was found above the nucleolus. Single grains also were found above the cytoplasm. This arrangement of the label in the sections agreed completely with the character of distribution of newly synthesized RNA in the cells after administration of the isotope to the intact animal. Further evidence that the label recorded was connected with RNA was obtained in control experiments, in which several pieces (after fixation in glutaraldehyde) were incubated with ribonuclease in a concentration of 2 mg/ml for 3 h at 37°C. Subsequent treatment of the control pieces was the same as in the main experiments. In autoradiographs prepared from the control pieces the density of the grains was only very slightly higher than in the background and was much lower than the density in the main batch of autoradiographs.

It can be concluded from the pattern of distribution of the grains of silver in the electron-microscopic autoradiographs of cells of the gastric mucosa and from the results of control treatment that the recommended method does in fact reflect RNA synthesis in the cells that continue to survive in the incubation medium after isolation from the body.

Electron-autoradiographic investigation directly reflects the intravital state of the cells, for the method records the rate of biosynthesis in living structures. This is a particularly promising feature of this method. Its development must largely depend on widening of the range of available precursors. The wider this range, the greater the variety of metabolic processes which can be studied in cells from biopsy material. Another substantial advantage of electron-microscopic autoradiography of biopsy specimens compared with the ordinary electron-autoradiographic investigation is that the time taken for the investigation can be shortened from several weeks to a few days, because of the increased concentration of the precursor in the incubation medium.

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