Quantitative and qualitative differences were thus found in the composition of membrane antigens of transformed and tumor Syrian hamster cells. These differences may evidently reflect the clonal nature of origin of the tumors, the stage of differentiation of the normal cell at which it was fixed by the transformation process, and the disturbance of synthesis of individual antigens by the cell during transformation and progression.

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ULTRACYTOCHEMICAL STUDY OF NUCLEAR NUCLEOSIDE PHOSPHATASE ACTIVITY IN EPITHELIAL CELLS OF THE NORMAL HUMAN GASTRIC MUCOSA AND GASTRIC CARCINOMA CELLS

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The role of adenosine triphosphatase (ATPase) in energy metabolism is well known. The ATP "pool" in the cell is maintained, in particular, by the enzyme inosine diphosphatase (IDPase). The function of this enzyme in the cells has not yet been fully explained. However, some workers [5, 8] consider that inorganic phosphate and inosine are liberated from inosine diphosphate under the influence of IDPase. During intracellular metabolism, adenosine is formed from inosine and is phosphorylated to form ATP.

Most workers [4, 7, 10, 14] describe the localization of the reaction product for ATPase at the light histochemical level entirely in the parietal cells. As regards the electron-histochemical study of ATPase and IDPase in cells of the gastric mucosa, there are only isolated reports [3, 11-13], the authors of which have not paid the necessary attention to the activity of these enzymes in the cell nuclei. Data on the electron-histochemical detection of these enzymes in nuclei of human gastric carcinoma cells are nowhere to be found.

Accordingly, the aim of the present investigation was to compare ATPase and IDPase activity in nuclei of normal epithelial cells of the human gastric mucosa and in gastric carcinoma cells.

EXPERIMENTAL METHOD

Pieces of tissue obtained by means of a fiberoptic gastroscope from tumors of the human stomach and from the gastric mucosa of persons without gastric tumors (control) were studied.* To investigate IDPase and ATPase activity, electron-histochemical methods were used.

*The gastroscopy and taking of the specimens were carried out by Cand. Med. Sci. E. A. Mikhailov (Department of Endoscopy, Oncologic Scientific Center, Academy of Medical Sciences of the USSR), to whom the writers are grateful.

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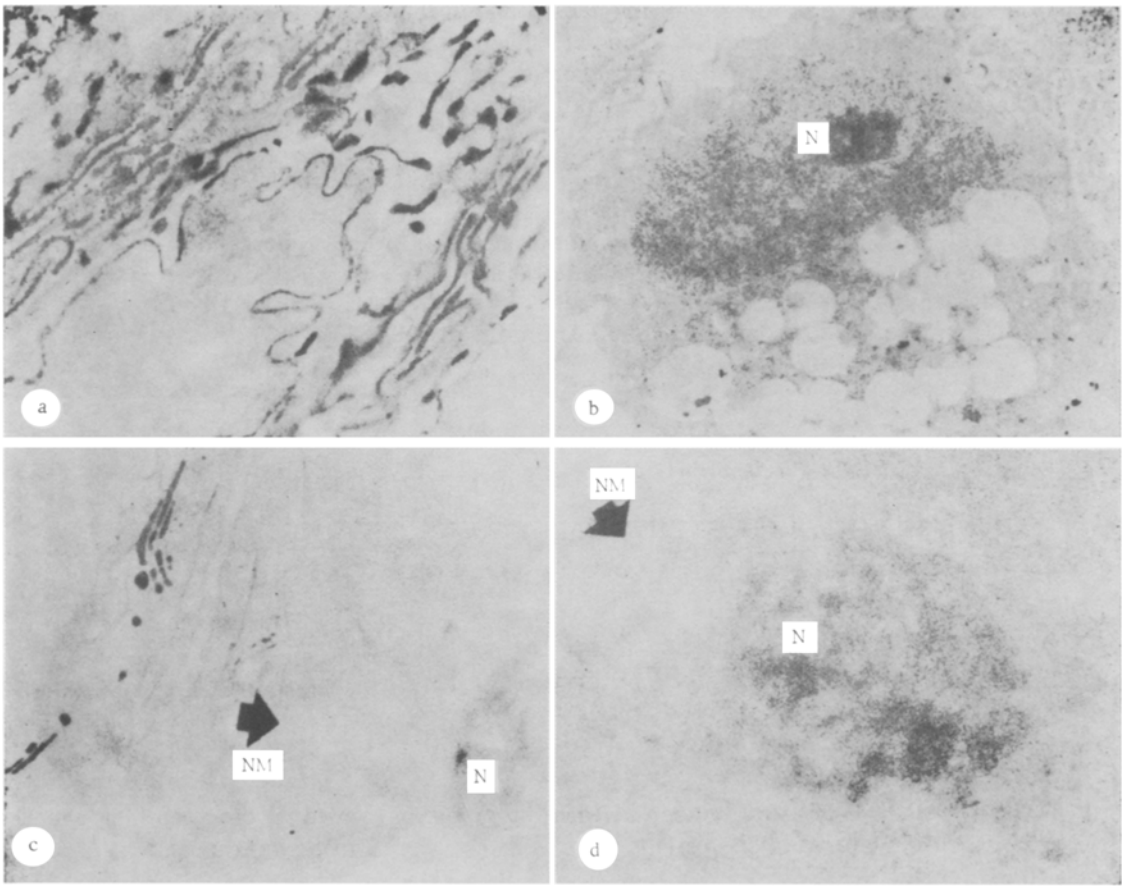

Fig. 1. IDPase activity in cells of gastric mucosa and gastric carcinoma cells; a) basal portion of cell in epithelium lining gastric pits; product of reaction for IDPase localized in cisterns of rough endoplasmic reticulum, nucleolemma, plasmalemma of developed interdigitations, and diffusely in nucleus; reaction product is absent in zone of nuclear pores (15,000×); b) accessory cell of gastric mucosa; product of reaction for IDPase distributed profusely in karyoplasm and nucleolus and also in plasmalemma. Enzyme activity in nucleolus is stronger than in karyoplasm; reaction product absent in nucleolemma (8300×); c) undifferentiated cancer cell; considerable weaking of IDPase activity in nucleus; reaction product absent in nucleolemma and plasmalemma of cell; high IDPase activity in Golgi lamellar complex (20,000×); d) nucleus of cancer cell of accessory type; marked weakening of IDPase activity in nucleoplasm and nucleolus; enzyme activity is stronger in nucleolus than in nucleoplasm; reaction product absent in nuclear membrane (25,000×). Here and in Fig. 2, sections unstained; N) nucleolus; NM) nuclear membrane.

Immediately after removal the fragments were prefixed in 2.5% glutaraldehyde solution in 0.05 M cacodylate buffer (pH 7.4) with 0.25 M sucrose for 1 h at 4°C. They were then washed in 0.25 M sucrose solution for 10, 20 and 90 min successively at 4°C. Sections were cut in a cryostat to a thickness of 45-50 μ, and then incubated on a water bath at 36-37°C for 45 min in medium for detection for IDPase, which contained 5 mM inosine diphosphate (from Reanal, Hungary), 80 mM Tris-maleate buffer, (pH 7.2-7.4), 30 mM magnesium acetate, and 3.6 mM lead acetate. The composition of the incubation medium for detection of ATPase and the subsequent processing were described by the writers previously [2]. To compare the results of detection of IDPase activity in the cells, parallel incubation of the sections was carried out in the medium of Novikoff and Goldfischer [9] and in the medium suggested above.

Semithin sections were cut from all the Epon blocks on an LKB Ultratome III. Some of the semithin sections, 1 μ thick, were stained with toluidine blue by Lynn's method [6], others 3-5 μ thick were treated with ammonium sulfide solution by a modified method of Lysenko [1]. After this procedure the semithin sections were studied in the light microscope, where the place for sharpening the block was selected. Ultrathin sections were studied in the JEM-100C electron microscope.

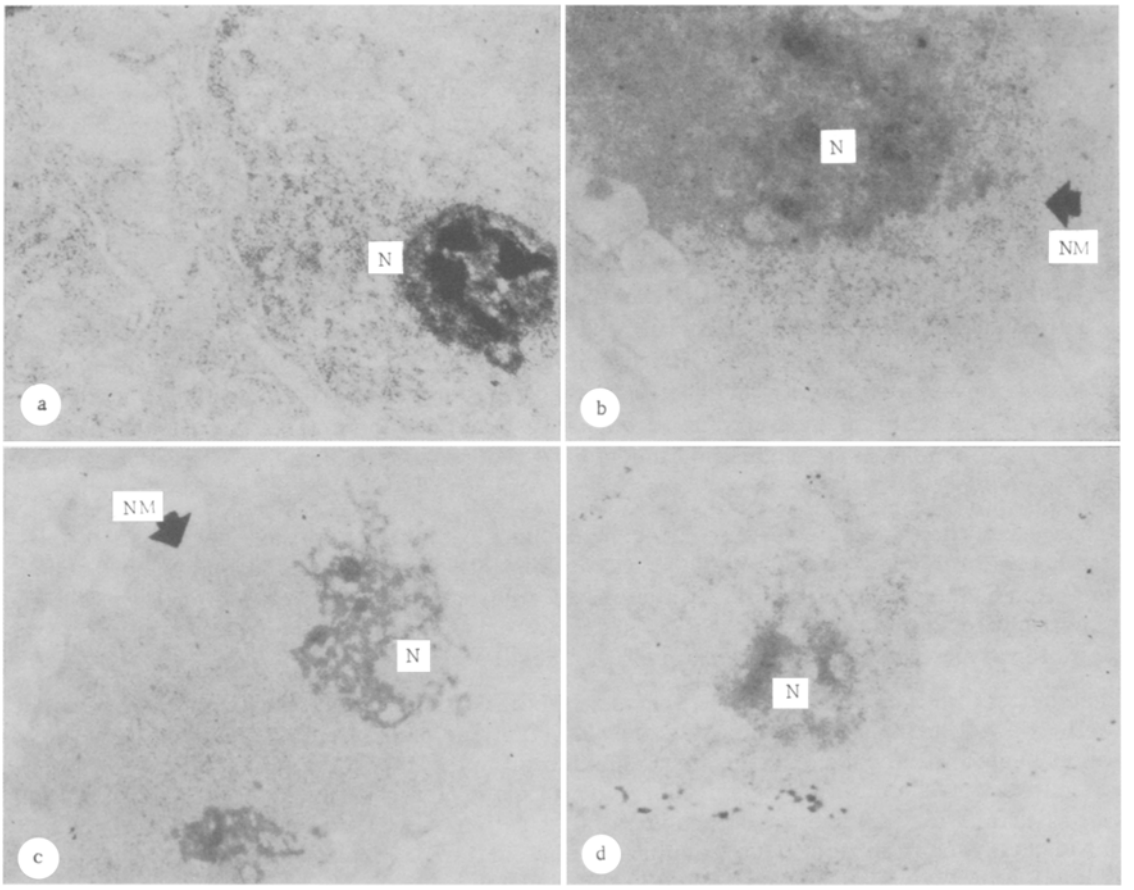

Fig. 2. ATPase activity in cells of normal human gastric mucosa and in gastric carcinoma cells; a) chief cell of gastric mucosa; product of reaction for ATPase localized on membranes of developed rough endoplasmic reticulum (seen from matrix of cytoplasm), and also in condensed chromatin, and in granular and fibrillary components of nucleolus (15,000×); b) accessory cell of gastric mucosa; products of histochemical reaction distributed uniformly throughout nucleoplasm with predominant localization in nucleolus; activity in nucleolemma is absent (15,000×); c) undifferentiated cancer cell; considerable weakening of ATPase activity in nucleus and nucleolus; reaction products more marked in nucleolus than in nucleoplasm; ATPase activity absent in nuclear membrane (7200×); d) nucleus of cancer cell of accessory type; ATPase activity connected with cell nucleus is weakened; product of reaction for ATPase located on plasmalemma of cell as well as in nucleus (15,000×).

EXPERIMENTAL RESULTS

When the incubation medium suggested above was used for electron-histochemical determination of IDPase, activity of this enzyme in the normal gastric mucosa was found to be associated with cisterns of the rough endoplasmic reticulum, the Golgi complex, plasmalemma, nuclear membrane, nucleoplasm, and nucleolus of the epithelial cells (Fig. 1a, b). The results confirm the observations of Rubin [11, 12], who described the IDPase activity of the parietal and endocrine cells of the gastric mucosa. However, unlike Rubin, we found that the reaction product was localized on the plasmalemma and inside the nucleus; in the latter case, moreover, the reaction product was located not only in the perinuclear space, but also distributed diffusely throughout the nucleoplasm. In the nucleolus, however, the density of the reaction product was much greater than in the nucleoplasm.

Cells with different levels of enzyme activity connected with the nucleus were encountered. For instance, in cells of the surface epithelium and the epithelium covering the pits, reaction product connected with the nucleolemma was found much more often (Fig. 1a) than in the accessory cells, in which they were often absent (Fig. 1b). If the reaction product was localized in the perinuclear space, the zone of the nuclear pores always remained inactive (Fig. 1a). Enzyme activity was distributed similarly in the nuclei of these cells also: ac-

cessory cells with well-defined reaction product, distributed diffusely in the nucleus and nucleolus, could be seen (Fig. 1b), whereas in other cells of the same type it was completely absent. Cells were found with differing (from low to high) enzyme activity in their nuclei. A similar picture also was observed in cells of the surface epithelium and lining the pits, and in the chief and parietal cells. This distribution of enzyme activity was evidently connected in particular with the physiological activity of the nuclei.

A comparative study of the results obtained by the use of Novikoff and Goldfischer's incubation medium [9] and the medium of the composition indicated above showed that during incubation of the section in the latter medium the intensity of formation of the reaction product for IDPase in the nuclei was much stronger than in the former. As a modification of Novikoff and Goldfischer's medium for determination of nuclear IDPase activity, the final concentration of the substrate in the medium was increased by 2.5 times and the concentration of bivalent activator-ions (stimulators) was increased sixfold – in the medium under discussion, this concerned magnesium ions. It must also be pointed out that instead of lead nitrate and manganese fluoride, which were present in Novikoff and Goldfischer's medium, we used lead acetate and magnesium acetate respectively. The presence of the same anions in the incubation medium as shown above is some protection against precipitation of a nonspecific residue.

All the gastric tumors studied were adenocarcinomas. A marked decrease in IDPase activity was observed in the nuclei of the cancer cells and as a rule the reaction product was absent in the perinuclear space. In undifferentiated cancer cells (Fig. 1c) and also in cells resembling accessory cells (Fig. 1d) or enterocytes in their ultrastructure, diffuse trace activity of this enzyme was found in the nuclear chromatin and in the nucleoli. Its intensity was greater in the latter structures than in the nucleoplasm.

ATPase activity in the normal gastric mucosa was localized in the nucleoplasm and nucleolus of the epithelial cells as well as in the rough endoplasmic reticulum and plasmalemma (Fig. 2a, b). The reaction product was connected with condensed chromatin and the granular and fibrillary components of the nucleolus; in the latter, moreover, the intensity was greater than in the granular components (Fig. 2a). ATPase activity, as the work of Rubin et al. [13] showed, depends on the duration of prefixation in glutaraldehyde and is inversely proportional to the prefixation time. For example, in the earlier publications of Rubin [11], when prefixation for 3 h was used, no ATPase activity was found in the parietal cells.

ATPase activity was much lower in the nuclei of the cancer cells and in some it was completely absent. Reaction product was distributed diffusely in the karyoplasm and nucleolus in undifferentiated cancer cells (Fig. 2c) and also in cells which preserved the ultrastructural features of accessory cells (Fig. 2d) or mucous cells of the surface epithelium of the gastric pits and parietal cells and enterocytes. The intensity of the reaction product in the nucleoli of the cancer cells in all cases was stronger than in the nucleoplasm.

The controls (incubation of sections without substrate, heating the sections before incubation for 5 min at 90°C, change of substrate to sodium β-glycerophosphate in an equimolar concentration) showed the specificity of these methods for electron-histochemical determination of IDPase and ATPase activity.

The results of this comparative electron-histochemical investigation of IDPase and ATPase in normal epithelial cells of the human gastric mucosa and in gastric carcinoma cells thus showed: first, the efficacy of the suggested incubation medium for use in the detection of IDPase in the nuclei and, second, disturbance of enzyme systems in human gastric carcinoma cell nuclei responsible for the regulation of energy metabolism and for maintenance of the ATP "pool" in the cell nucleus.

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INHIBITION OF TRANSPLACENTAL CARCINOGENIC EFFECT OF N-NITROSOMETHYLUREA IN RATS BY BUFORMIN

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An important but little studied aspect of the problem of transplacental carcinogenesis is the search for ways of preventing the onset of tumors in a progeny exposed to the action of chemical carcinogens during the period of embryogenesis [7]. In the writers' opinion, the approach based on the view that during the action of carcinogens in the body, long before clinical manifestation of the tumor, various hormonal-metabolic disturbances develop which, on the one hand, facilitate the development of malignant cells and on the other hand, depress the system of antitumor immunity [5], is very promising.

The aims of the present investigation were to detect some of these disturbances arising during transplacental carcinogenesis and to attempt to use antidiabetic agents to overcome them with a view to inhibiting tumor growth.

EXPERIMENTAL METHOD

On the 21st day of pregnancy female rats were given an intraperitoneal injection of N-nitrosomethylurea (NMU) in a dose of 20 mg/kg. All the F_1 offspring and also the intact rats used as a control were divided into three groups at the age of 3 months. In the rats of group 1, after starvation for 18 h, determinations were made of the blood sugar (by the o-toluidine method) immunoreactive insulin (IRI), cholesterol, triglycerides, and somatomedin, as described previously [3, 4]; The blood sugar, IRI, and somatomedin levels also were determined 30, 60, and 120 min after peroral administration of glucose in a dose of 3 g/kg. Hemicastration was performed on the female rats of group 2 and the ability of diethylstilbestrol propionate (DESP), injected subcutaneously in a dose of 0.57 μg over a period of one week, to depress compensatory hypertrophy of the ovary (CHO) was studied in these animals [2]. The remaining animals, exposed to the transplacental action of NMU and constituting group 3, were divided into subgroups, and until the end of life five times a week they were given perorally through a tube 1 ml of tap water or 5 mg buformin (N-butylbiguanide hydrochloride; "Adebit," from "Chinoin," Hungary), in the same volume of water. Neoplasms discovered were studied microscopically.

EXPERIMENTAL RESULTS

The ability of DESP to inhibit CHO induced by hemicastration was significantly depressed in rats exposed to the transplacental action of NMU (Fig. 1); this may be interpreted as a manifestation of a raised threshold of sensitivity of the hypothalamic-hypophyseal complex to inhibition by estrogen. This corresponds to observations made in experiments in which carcinogens were administered to sexually mature rats [2].

In three-month-old rats whose mothers were exposed to the action of NMU on the 21st day of pregnancy, a decrease in glucose utilization was observed after 1 and 2 h in the glucose tolerance test compared with the control (Fig. 2). The insulin level in the control and experimental animals did not differ significantly either before or after administration of glucose, whereas the somatomedin concentration was significantly depressed in rats exposed to transplacental action of NMU (Fig. 2). The insulin–glucose index in the control rats 2 h

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