An ultrastructural investigation in stomach epithelial cells of mice during pregnancy and early lactation

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Abstract

In this study, stomach epithelial cells from adult nonpregnant, pregnant lactating mice were examined by histochemical, immunohistochemical and ultrastructural methods. Tissue samples taken from different groups were stained with haematoxylin-eosin and PAS-alcian blue and semithin sections were stained with toluidin blue for light microscopic examination. For ultrastructural examination ultrathin sections were stained with uranyl acetate and lead citrate. Additionally, by applying immunoperoxidase method, the distribution of EGF receptors of all groups was studied.

We concluded that in the pregnant mice, stomach surface had lower mucus than the nonpregnant group. The acid content was balanced in active and inactive parietal cells. Examination results of lactating mice were similar to the results obtained from nonpregnant and pregnant mice. (Acta gastroenterol. belg., **2003**, 66, **137-143**).

Key words : stomach, gestation, ultrastructure, immunhistochemistry, mice.

Introduction

It is diffucult to explain the effects of pregnancy and lactation period on the gastrointestinal system. In this period, normal hormonal effects change the motilities and secretion. In the first trimester of pregnancy, women complain from nausea and vomitting. In the second and third trimesters 60-80% of the women use antacid drugs because of heartburn complaints. Many hormonal studies indicate that prostaglandins stimulete the contraction of smooth muscle fibers. Relaxin stimulates myometrial contraction and inhibits the contraction of smooth muscle fibers in the gastrointestinal system. Also, pregnant women with high urine hCG levels coplain from nausea and vomiting. Furthermore, it has been reported that decreasing symptoms of stomach ulcer in pregnancy is related with the synthesis of progesterone and placental histaminase. Progesterone decreases stimulated acid secretion and basal acid levels and it causes the increased production of mucus in stomach epithelium.

So, it is believed that this decreased acid and increased mucus balance protects the gastrointestinal system by producing a protective sheath (1-7).

Moreover, in immunohistochemical studies, it has been shown that, EGF stimulates the synthesis and secretion of glycoproteins in stomach mucosa so, it has positive effects on protection of stomach mucosa by stimulating mucus production. Some of the several reports indicate that EGF-r is located only on the neck cells, whereas others claim that parietal cells have this receptor (8-12).

Several studies depending on biochemical methods have been reported on mucus secretion during pregnancy. Our aim was to add ultrastructural data to the literature about the topic. For this purpose, we examined the stomach epithelial cells from pregnant, nonpregnant and lactating mice by histochemical, immunohistochemical and ultrastructural methods.

Material and methods

Preparation of animals

Mice housed in groups of 4 in stainless-steel cages were divided into 3 groups as nonpregnant (control group), pregnant and lactating groups. 3 female and one male mice were put together overnight in four groups in order to have pregnant mice. The next morning, female mice were taken from cages and their vaginal smear and vaginal plug were controlled. Positive 4 female mice were chosen to form the pregnant group. The day after coitus was accepted as the first day of pregnancy. Other pregnancy positive four mice were reserved for realization for birth. Nonpregnant mice were chosen randomly. Female Swiss albino mice (Gazi University Medical Research Center, Ankara,Turkey) were fed on commercially available mice food and tap water during their pregnancy and lactation periods. Pregnant mice were killed on 9th day of pregnancy. Lactating mice were killed 3 days after birth and nonpregnant mice were killed immediately by decapitation.

Histochemical and immunohistochemical staining

After surgical removal, stomach tissues were fixed in 10% neutral formalin for about 72 hours for PAS-alcian blue and immunohistochemical staining. After this, each sample was parafin embedded for conventional histological study. Sections for histochemical methods were stained by a routine PAS-alcian blue technique. In immunohistochemical part of the study, on the other hand, endogenous peroxidase activity was blocked in 0.1% hydrogen peroxide (Fisher Scientific, Meliose

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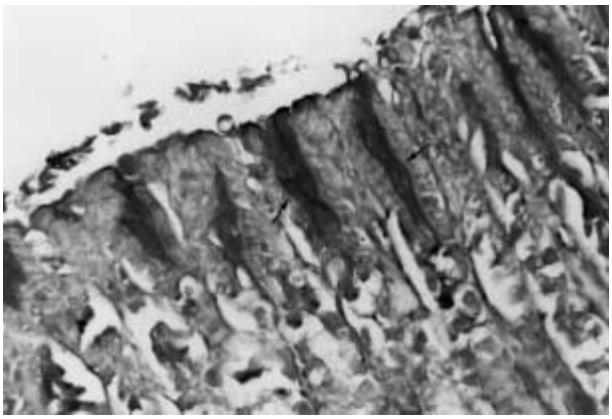

Fig. 1. — In nonpregnant group strong PAS positive reactivity was recognized especially on neck cells (thin arrows). PAS-alcian blue ×200.

Park.) for one minute, after deparafinization saponin **was** applied to enhance the staining. Nonspecific labelling was blocked in serum blocking solution. Sections were incubated with EGF rabbit polyclonal antibody (oncogene**,** IgG H11) diluted in 1 : 20 PBS for overnight at +4ºC. Biotinylated anti-rabbit IgG secondary antibody was applied for 30 min at room temperatur. The reaction was detected with peroxidase conjugated avidin-biotin complex (ABC) Diaminobenzidine (DAB) was used as chromogen and counterstaining was done with Mayer's haematoxylin.

The procedure control was done by omitting the primary antibody. Normal skin and breast carcinoma tissues which were included in the kit were used as positive controls.

Cytoplasmic staining were accepted as positive. Evaluation was made semiquantitatively as follows (+) weak, (++) mild, (+++) strong staining.

Ultrastructural methods

Moreover, stomach specimens from each group were prepared for transmission electron microscopy. Briefly, the corpus of the stomach were fixed at 4°C in 2.5% gluteraldehyde in 0.2 M phosphate buffer, pH 7.3 for 2 h and then post fixed in 2% osmium tetroxide in phosphate buffered saline (PBS) for 2 h at room temperature and dehydrated in ethanol at 4°C and embedded in araldite. Semithin sections satined with toluidin blue were examined under the light microscope, then ultrathin sections were stained with uranyl acetate and lead citrate ; and then examined under the EM 900 Carl Zeiss electron microscope.

Results

Histochemical results

In PAS-alcian blue stained slides of nonpregnant group, strong PAS positive reactivity was recognized especially on neck and surface cells. However, parietal cells located in the deep portion of the glands showed weak PAS positivity in higher magnification but other cells and lamellae had normal appearance (Fig. 1). In electronmicroscopic semithin sections, neck cells were definitely stained with toluidine blue (Fig. 2).

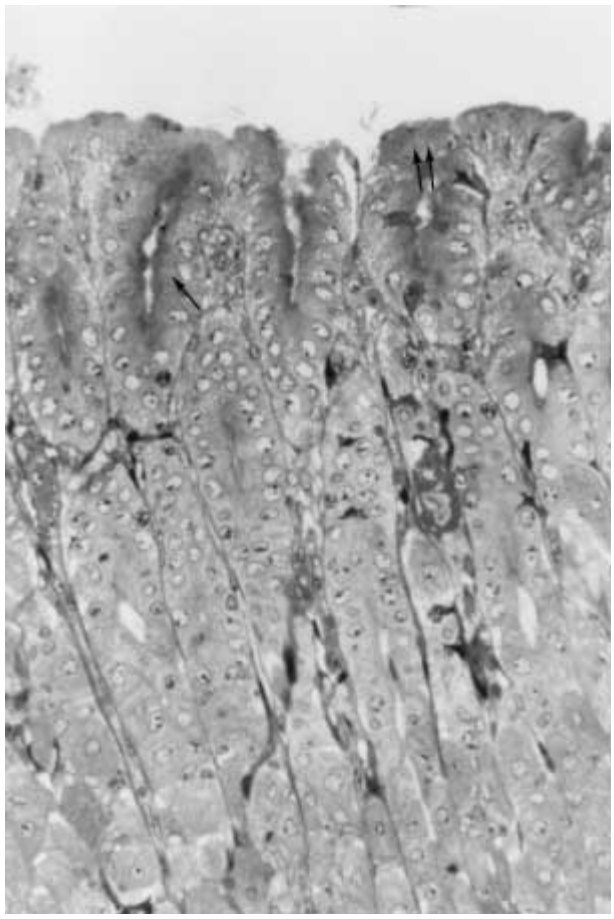

Fig. 2. — In the same group neck and superficial cells were definately stained with toluidine blue. (thin arrows), Toluidine blue ×200.

In PAS-alcian blue staining of pregnant group, weak PAS positivity was observed in superficial and neck parts of the glands (Fig. 3). Same areas were stained weaker than the control group with toluidine blue (Fig. 4).

On the other hand, it was observed that PAS –alcian blue and toluidine blue stains were stronger in tissues that were taken from lactating mice than that of the nonpregnant group (Fig. 5).

Immunohistochemical results

Expression of EGF-r was strongly positive on superficial cells but neck cells showed mild to weak immunreaction from apical to basal cytoplasm of the epithelium in nonpregnant group (Fig. 6), (Table 1).

Parietal, superficial and neck cells of pregnant group showed stronger immunoreactivity than that of the nonpregnant group (Fig. 7), (Table 1).

However, in lactating group, immunoreaction varying from mild to weak was observed (Fig. 8), (Table 1).

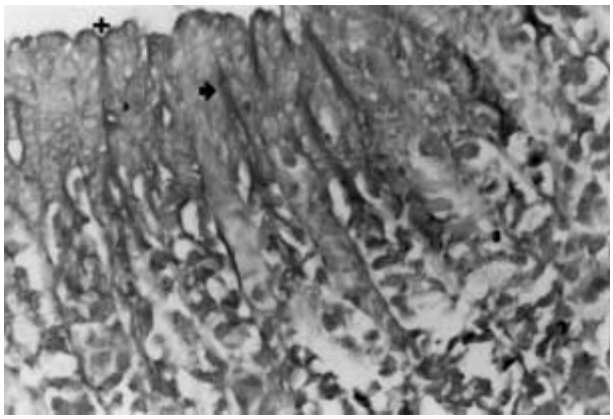

Fig. 3. — In pregnant group weak PAS positivie was seen in superficial (+) and neck parts of the glands.(thck arrows),PAS-alcian blue ×200.

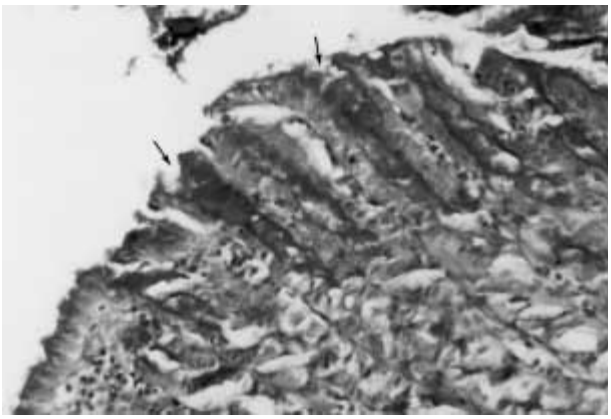

Fig. 5. — Mucus structure stained more PAS positive in lactating group than the nonpregnant and pregnant group (thin arrows) PAS- alcian blue ×200.

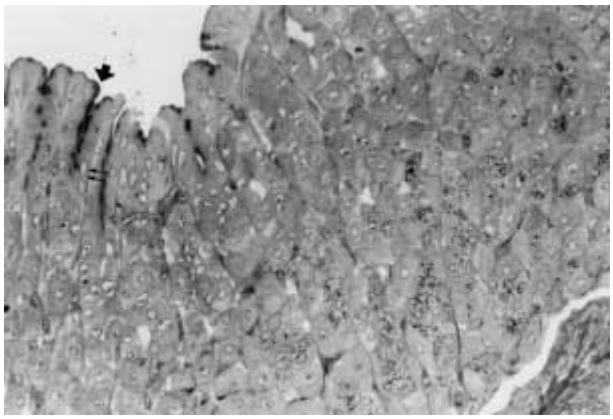

Fig. 4. — Superficial and neck parts of glands of pregnant group stained weak with toluidine blue (thin arrows), Toluidine blue ×200.

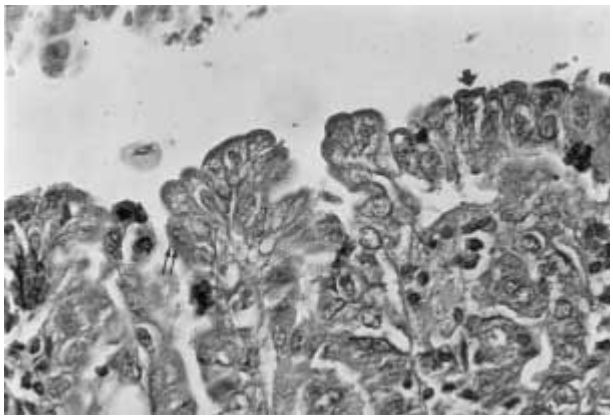

Fig. 6. — In the nonpregnant group. EGF-r was strongly positive on superficial cells (thick arrows) but neck cells showed mild to weak reaction from apicale to basale (thin arrows) Immunoperoxidase + hematoxylin ×200.

Table 1. — **Semiquantitative evaluation of cytoplasmic staining**

	Superficial mucus cell	Neck mucus cells apical	Neck mucus cells basal	Parietal cells
Nonpregnant	+++	++	+	+
Pregnant	+++	+++	++	+++
lactating	+	+	-	+

Ultrastructural results

In electron micrographs of the nonpregnant group stomach samples, there were pale mucus granules varying in size located in the apical cytoplasm of superficial and neck cells of epithelium (Fig. 9). Granules of these cells were fewer in number, denser and were located just under the apical cell membrane in the pregnant group. However, active appearance of their Golgi complexes was interesting (Fig. 10). Ultrastructural appearance of the lactating group was similar to the appearance of the pregnant group (Fig. 11).

A group of parietal cell intercellular canaliculi, a few cytoplasmic tubulovesicules and normal number and appearance of microvilli were like active cells (Fig. 12). In the other group of parietal cells, on the other hand, intracytoplasmic canaliculi were similar to the appearance of the lumen of the glands. Microvilli were fewer but intracytoplasmic vesicles were higher in number (Fig. 13).

Also, enterochromoffin-like cells were high in number in the pregnant group. In the control group, the number of these cells was low.

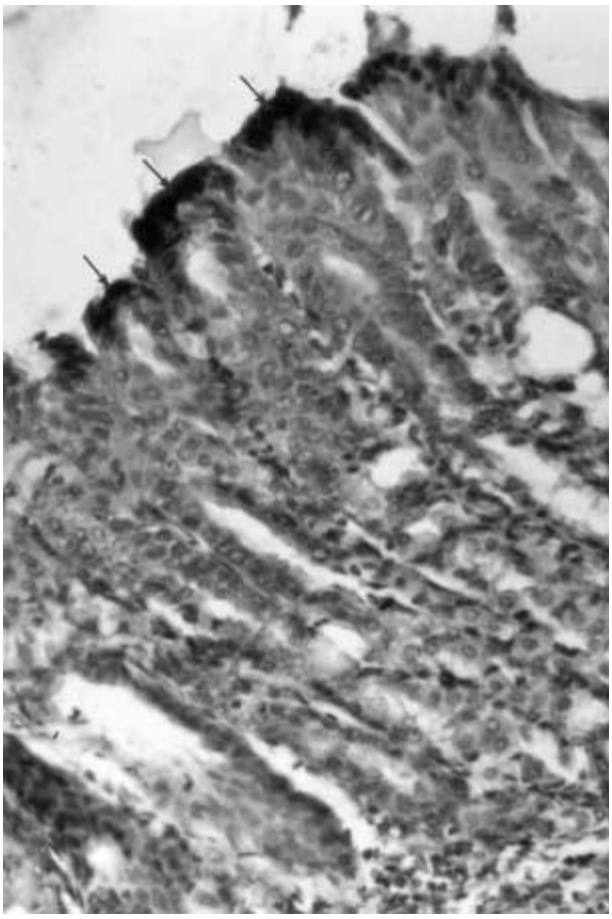

Fig. 7. — Superficial (thin arrow), neck cells (thick arrows) and parietal cells showed stronger immunreactivityin the pregnant group than nonpregnant group. Immunoperoxidase + hematoxylin ×400.

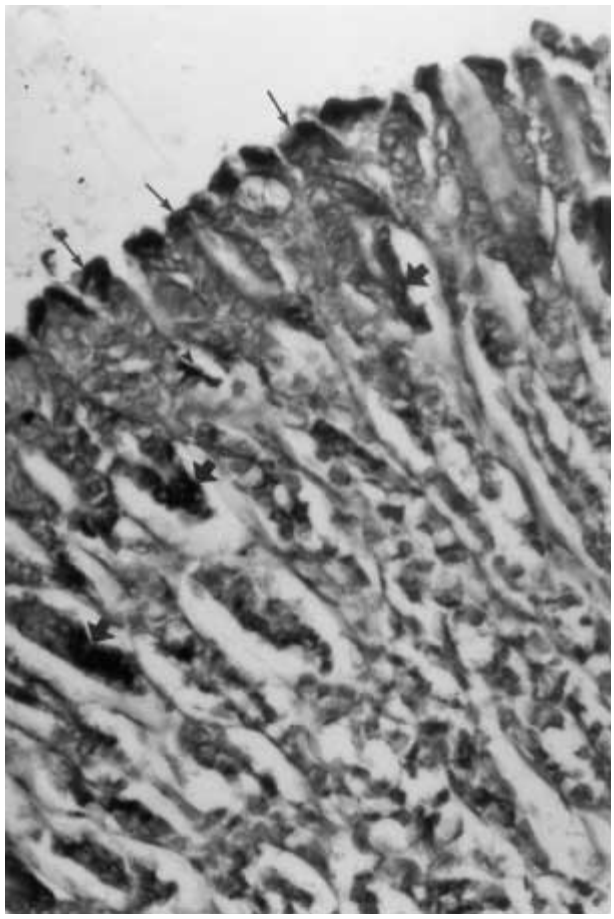

Fig. 8. — Immunreaction on superficial (thin arrows), neck (thick arrows) and parietal cells varying from mild to weak was significant on lactating group, Immunoperoxidase + hematoxylin ×400.

Discussion

Pregnancy affects the gastrointestinal system in several ways. Normal hormonal effects change motility and secretion during this period (2,5,13-15).

In the present study, we observed less clear and weak PAS+ reaction of the mucus in pregnant mice when compared with the control group. Similar situation was observed in semithin sections stained with toluidin blue. This difference was observed more clearly in the electron microscopic examination. Although, the whole apical part of stomach superficial cells are fulled with a large number of mucus granules in the nonpregnant mice, these granules were bigger, denser but fewer in number and located only under the apical cell membrane in the epitheliae cells of pregnant mice. This difference was also observed more evidently in the electron microscopy.

Honiotes *et al.* 1970, reported that female sex hormones during pregnancy, lower the risk of stomach ulcer by reducing the production of gastric acid secretion. They also indicated that estrogen lowers gastric acidity (16).

Hunt *et al.* 1958, reported that little decrease was observed in the gastric acid secretion during the first 30 weeks of pregnancy (17).

In another study it has been reported that the signs and symptoms of peptic ulcer disease (PUD) decreased in pregnancy.This indicates that the improvement of PUD in pregnancy was due to progesteron which lowers the levels of basal and stimulated acid secretions from stomach (7,18).

Chen *et al.* 1999, reported that ECL cells play a key role in the regulation of parietal cell activitiy by releasing histamine. They indicated that, in response to long-term gastrin stimulation, vacuoles and lipofuscin bodies develop in the ECL cells,forming part of a crinophagic pathway by which the ECL cells strive to eliminate superfluous secretory products (19).

Clark *et al.* 1954, reported that the decrease in the histamine caused a decrease in gastric acid production.The placenta is also a rich source of histaminase, which antagonizes the action of histamine at the parietal cell (6).

In our study we found enterochromaffin like cells were very high in number especially in the group of pregnant mice under electron microscopy investigation.

Under these hormonal effects, especially indirectly affected parietal cells from histamine in the group of pregnant mice showed that their amount of nuclear

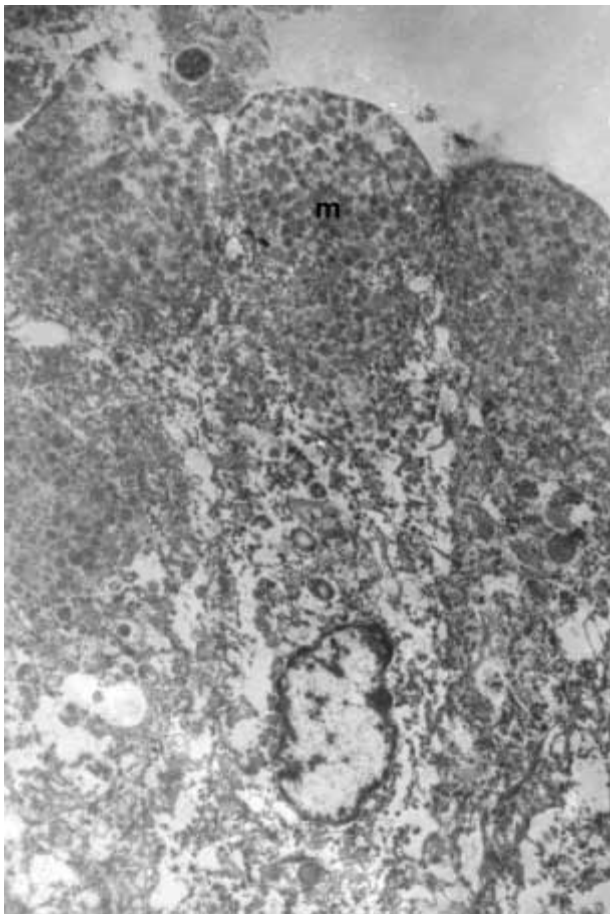

Fig. 9. — Ultrastructure of the observed nonpregnant group, pale mucus granules (m) of varying size located in apical cytoplasm in epithelial cells. Uranyl acetat-lead citrat ×9000.

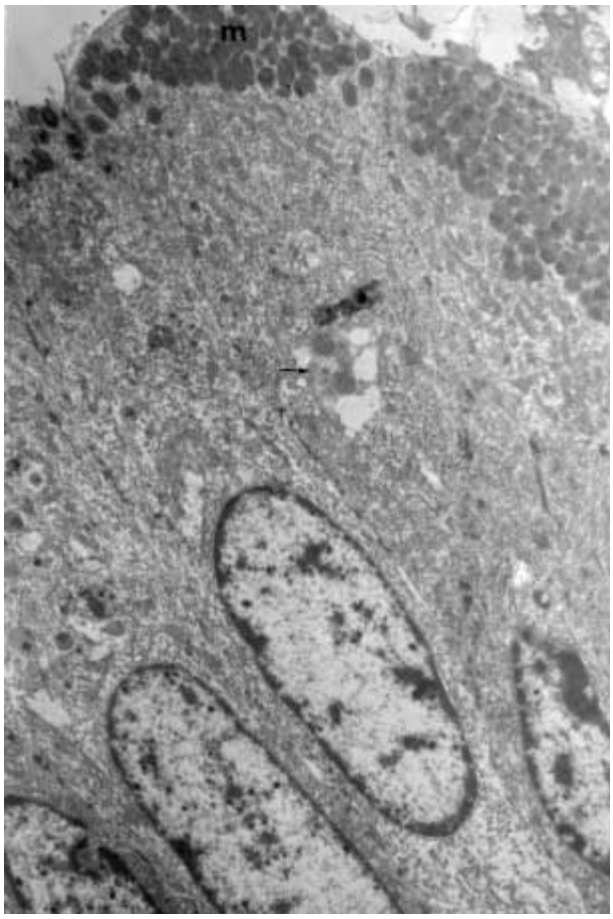

Fig. 10. — Granules of these cells were fewer in number, denser and were located just under the apical cell membrane and active Golgi complexes in pregnant group (m). Uranyl acetat-lead citrat ×9000.

chromatin and size decreased, although their number increased under the light microscope. These cells showed two different appearance when investigated with electron microscopy. The first group of the parietal cells structure similar to parietal cells of the control group. Their intracytoplasmic canalicules had great number of microvilli, but they had fewer number of intracytoplasmic tubulovesicules. The second group that we encountered often had wider intracytoplasmic canalicules. Structure of normal intracytoplasmic canalicules were similar to lumen of a gland. Cytoplasm was rich with tubulovesicules. These findings suggest that inactive cells were secreting hydrochloric acid.

In the present study, we also investigated the expression of EGF-r on nonpregnant, lactating and pregnant mice stomach immunohistochemically.

Strong positive immunoreactivity on surface epitheliae cells on adult mice stomach was noticable. Prevalent mild reaction was detected on the basal cytoplasm of the same cells. Changing reaction from mild to weak was recognized on the cytoplasm of the neck mucus cells. Prevalent reactivity was present in the parietal cells of the cytoplasm.

In the group of pregnant mice, superficial and neck mucus cells showed strong positive immunoreactivity. Immunoreactivity was very strong especially on the basolateral area of the pariatel cells of this group. Mucus structure of lactating mice, which was stained with PAS-alcian blue compound stain has been detected more evidently than the group of nonpregnant and pregnant mice. Moreover, evident secretional activity was detected on the oxyphyl and parietal cells of toluidine blue stained semithin slides of lactating group. Parietal cells were detected in normal structure with electron microscopic examination.

Lactating mice showed that, immunoreactivity of superficial epithelial cells in apical parts was weaker than that of the other two groups.

Moreover, our study showed an increased expression of EGF-r in superficial and neck mucus cells and parietal cells in the stomach of the pregnant mice group. This supports an important mitogenic role of EGF in protection of stomach mucosa during pregnancy.

It has been reported that EGF increases in the blood and body liquids and decreases in the last trimester but does not change during the lactation period (20).

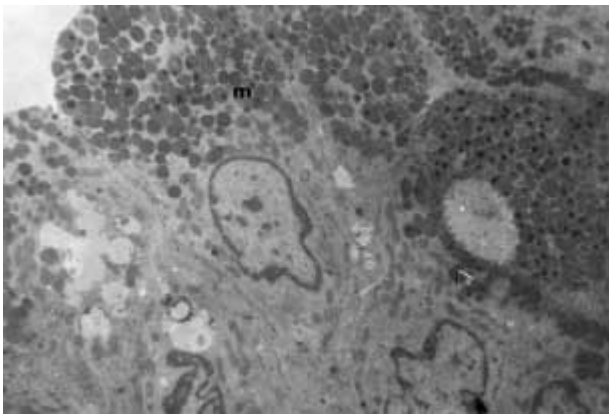

Fig. 11. — Ultrastructural appearance of the lactating group was similar to pregnant group (m). Uranyl acetat-lead citrat ×9000.

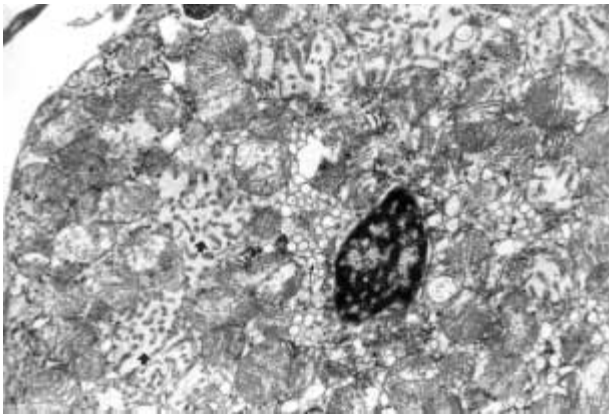

Fig. 12. — A group of parietal cells had normal appearence and number with their intracelluler canaliculi (thick arrows), a few tubulovesicules (thin arrows) and normal number and appearance of microvilli in pregnant group. Uranyl acetat- lead citrat ×9000.

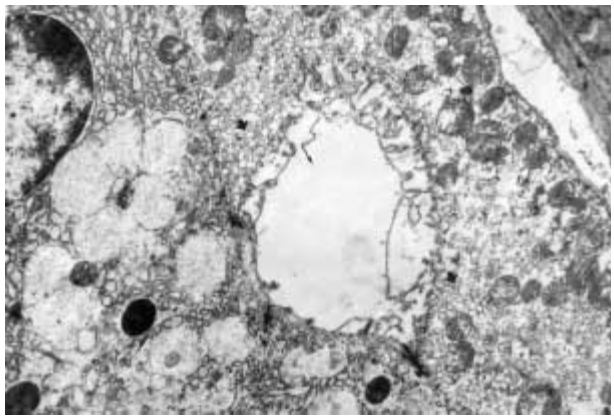

Fig. 13. — Other group of parietal cells in the pregnant group resembled inactive cells intracytoplasmic canaliculi which were similar to appearance of the lumen of glands (thin arrows). Microvilli were fewer in number but tubulovesicules increased (thck arrows). Uranyl acetat- lead citrat ×9000.

Burgess, 1989, indicated that EGF induces the synthesis of DNA on gastric mucosa of the rodents and EGF fastens the improvement of gastroduodenal ulcer lesions (1,21).

Konturek *et al.* 1997, investigated the rate of proliferation and the gastric secretion and gene expression of mRNA for EGF and TGF-α during ulcer healing is associated with enhanced proliferation of mucosal cells, gradual increase in the blood flow at the ulcer margin, and inhibition of gastric secration and increased plasma levels of gastrin. Moreover, they showed that EGF and TGF-α are equally over expressed at the ulcer margin and, therefore, may be equally important for the healing process (22).

Eastwood *et al.* 1990, also indicated that, luminal EGF protects the structure of gastrointestinal mucosa and induces the proliferation of mucosal cells physiolocically (8,23).

Tarnawski *et al.* 1992, investigated the expression of EGF-r on injury sign after the recovery of ulcer and gastric mucosa during the ulcer healing. EGF-r was found on some of proliferative zone as the parietal cells of normal gastric mucosa. It was seen that, EGF-r expression is extremelly increased in the mucosal cells of ulcer healing zone on rats during the healing process (12).

Orsini *et al.* 1993, investigated the expression of EGF and TGF-α receptors immunohistochemically on human gastric mucosa. Immunohistochemical results obtained by them showed that, strong EGF / TGF-α receptor immunoreactivity was located in the mucus-secreating cells of surface epithelium and pits and in mucus neck cells of the proliferative zone in normal gastric mucosa. Positive immunoreaction was particularly intense in the basal part of the cytoplasm and along the basolateral cell membranes. A definite linear staining decorated the basolateral membranes of parietal cells and mucus secreting pyloric gland cells, whereas the luminal surface membrane was not stained (11).

As a result, EGF supressed the secretion of HCl from the parietal cells under the influence of different kinds of sex and pregnancy hormones. In the present study, two different kinds of parietal cells were detected under the electronmicroscopy that were active and inactive cells on stomach mucosa of pregnant mice. Because of the fact that inactive cells decreased in number, we thought it is the ultrastructural sign of decreased HCl synthesis in this period of pregnancy.

We ultrastructurally and histochemically detected that mucus structure also decreases during pregnancy. So we thought that this phenomen explains protective property of stomach during this period. In many studies, EGF has been reported to be increased during pregnancy. Stomach of the pregnant mice produces similar symptoms to the stomach with ulcer, because, protection of stomach surface is partially broken down by the decreased amount of mucus structure, as increasing EGF and its receptor in stomach ulcer, especially in 3rd trimester of pregnancy. This situation was thought to be

the explanation of strongly positive expression of EGF-r in the stomach of pregnancy.

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