ISSN- 0102-9010

EFFECTS OF SOMATOSTATIN, LHRH AND TGF-α ON EPITHELIAL CELL PROLIFERATION IN FETAL STOMACH MAINTAINED IN ORGAN CULTURE

Eliane Maria Goldfeder* and Eliana Parisi Alvares

Department of Histology and Embryology, Institute of Biomedical Sciences,
University of São Paulo (USP), São Paulo, SP, Brazil.

ABSTRACT

An organ culture technique was used to examine the effects of somatostatin, luteinizing hormone releasing hormone (LHRH) and transforming growth factor (TGF-α) on epithelial cell proliferation in rat fetal stomach. The explants were obtained from 20-day rat fetuses and were maintained in organ culture for 24 h. Half of the culture dishes were supplemented with 10% fetal bovine serum (FBS). Cell proliferation was assessed using the metaphasic index. Light and electron microscopy showed that the explants could be maintained in good condition, independent of the FBS or hormone treatment. Re-epithelialization occurred at the edges of the fragments. The addition of 10% FBS was not advantageous for evaluating cell proliferation in this organ culture system. The low metaphasic index showed that somatostatin and LHRH significantly inhibited cell proliferation after 24 h of treatment. In contrast, TGFα had a mitogenic effect on fetal gastric mucosa and prevented glandular degeneration. These results corroborate our previous studies *in vivo* and provide direct evidence of the influence of hormonal and growth factors on gastric mucosa during fetal development.

Key words: Cell proliferation, growth factor, hormones, organ culture, stomach

INTRODUCTION

Cell proliferation and differentiation in the rat gastrointestinal tract during pre- and postnatal development involve interactions between hormones, growth factors and feeding schedule [20]. The modulatory role of these elements *in vivo* is influenced by endogenous factors.

The fetal-maternal relationship during the perinatal period precludes many studies *in vitro* and organ culture has been used instead since it allows the maintenance of the tridimentional aspect of the tissues as well as the direct study of the regulation of gastric cell proliferation [2,41]. Rapid differentiation processes occur in the gastric mucosa during the last five days of gestation in the rat [1], and different factors can influence the morphogenesis and differentiation of the glandular components.

Correspondence to: Dr. Eliana Parisi Alvares
Departamento de Histologia e Embriologia, Instituto de Ciências Biomédicas (ICB), Universidade de São Paulo (USP), Av. Prof. Lineu Prestes, 1524, CEP 05508-900, São Paulo, SP, Brasil. Tel: (55) (11) 3818-7303, Fax: (55) (11) 3818-7402, E-mail: elipar@usp.br
*Present address: Departamento de Morfologia, Universidade Federal de São Paulo (UNIFESP), São Paulo, SP, Brasil.

Transforming growth factor-α (TGF-α) and epidermal growth factor (EGF) are polypeptides that share the same receptor. The mitogenic effect of TGF-α on isolated gastric epithelial cells maintained *in vitro* has been demonstrated [6,31,34], however the role of this factor in proliferation and differentiation during the late fetal period is unknown. Other growth factors or hormones, such as insulin-like growth factor, EGF and thyroxin, are involved in the regulation of growth in the gastrointestinal tract, especially in the developing intestine [33]. We have been previously concerned about the growth regulation of the developing stomach and the effects of hormones like somatostatin and luteinizing hormone releasing hormone (LHRH) upon it [13]. Somatostatin is a well-known tetradecapeptide hormone distributed in the digestive tract and its role in gastrointestinal cell proliferation during development has been reported [32]. The administration of somatostatin to young rats decreases the labeling, and the mitotic and metaphasic indices of previously stimulated gastric epithelial cells [13,23]. Gama and Alvares [13] were the first to show the inhibitory effect of LHRH on the proliferation of the gastric epithelium in rats

during postnatal development. Since studying the effects of hormones and growth factors *in vivo* can be difficult because of the large number of uncontrollable variables, we have used a culture system for fetal rat stomach to evaluate the proliferation and morphology of differentiating fetal gastric mucosa in organ culture and to examine the quality of tissue preservation during the period of incubation. We also compared the possible direct effects of TGFα, somatostatin and LHRH on cell proliferation, with previous results obtained *in vivo*.

MATERIAL AND METHODS

Animals and explant preparation

Adult Wistar rats from the colony maintained by the Department of Histology and Embryology were used. Females were housed with males overnight (2 or 3 females per male). Day 0 of gestation was designated when spermatozoa were identified in vaginal smears. The rats were kept under natural light from about 6:00 a.m. to 6:00 p.m. Food and water were available *ad libitum*.

All the experiments were performed around 11:00 a.m. Timed pregnant Wistar rats at 20 days of gestation were anesthetized with ketamine (Ketalar, Aché Laboratories) and xylamine (Rompum, Bayer Laboratories) (1:1, 0.5 ml/100 g b.wt) and the fetuses were removed by laparotomy under sterile conditions. Fetal stomachs were taken from 7-8 fetuses per female and the proximal and distal thirds were discarded. Six explants (n = 42-48 explants) of 2-3 mm long could be removed from the presumptive corpus region of each fetal stomach, and washed with PBS-A and culture medium. The fragments were placed on strips of Millipore filter with the mucosal side up and then slightly immersed in 50mm x 10 mm organ culture dishes (Costar, USA). Each culture dish contained 3-4 explants.

Organ culture

The explants were cultured in F12/Dulbecco's modified Eagle's medium (DMEM-F12, Gibco, USA) containing 50 µg ampicillin/ml (Wyeth, BR) and buffered with 10 mM HEPES (Sigma, USA) and 1.2 mg of natrium bicarbonate/ml (Mallinckrodt, USA). Half of the dishes were supplemented with 10% FBS (Cultilab, USA). Some of the explants were treated for 24 h with 40 ng of somatostatin/ml (Sigma, USA) (Moyer *et al*., 1986), 50 ng of LHRH/ml (Relisorm-Serono, SWL) or 0.5 ng of TGFα/ml (Gibco, BRL) [32] which were added to the medium. Control fragments were maintained in medium for 2 h or 24 h without treatment. Dishes were incubated for 2, 24, 48 or 72 h in a humidified atmosphere of 5% CO_2 in air at 37°C.

Morphological studies

For light microscopy, the explants were fixed in Bouin's solution for 4 h, dehydrated and then embedded in Paraplast®. Four-micron sections were mounted and stained with hematoxylin-eosin or periodic acid Schiff (PAS). For electron microscopy, the fragments were fixed in 2.5% glutaraldehyde buffered with 0.1 M sodium cacodylate, postfixed in 1% O_sO_4, dehydrated and embedded in Araldite resin. Ultrathin sections were examined with a Jeol 100 CXII transmission electron microscope.

Metaphasic index and statistical analyses

All dishes (n = 10-12 for each experiment) were treated for the last 2 h of culture with 40 µl of vincristine sulphate/ml (Oncovin, Lilly, Brazil) to block mitosis. Since metaphasic cells were seen in the entire epithelia in this period of fetal development, two different counts of the proliferative compartment were performed, only in the glandular epithelium alone or in the glandular and superficial lining epithelia. Non-epithelial nuclei were not considered. Only one observer counted the cells. Each experiment was repeated five times in order to provide 5-10 dishes per treatment with each dish containing three explants. An average of four random optical fields (x100) per explant were counted. Finally, 20 to 40 random fields were counted per treatment. The Metaphasic index was calculated as the percentage of metaphasic cells relative to the total number of cells counted. The results are reported as the means ± S.D. and the statistical comparisons were done using the Mann-Withney test with $p<0.05$ indicating significance.

RESULTS

Morphological studies

Explants of the gastric corpus region from 20-day fetuses maintained for 2 h in organ culture consisted of shallow pit-glands (Fig. 1A) lined by a simple columnar epithelium containing some differentiated cells, mainly mucous (PAS+) and parietal cells, typically acidophilic. This epithelium overlays a mesenchyma rich in cells and a muscle layer.

There were no morphological differences between the explants maintained in serum-free medium and those kept in medium supplemented with 10% FBS. After 24 h in culture, 78 dishes (66%) contained well-preserved fragments. The morphology of the glands and components of the epithelia, connective tissue and smooth muscle layer was similar to that of control fragments. Some explants cultured for 48 h or 72 h were also well preserved (Fig. 1B), but the loss caused by degeneration was greater than after 24 h. Somatostatin, LHRH and TGF-α did not modify the morphology of the gastric mucosa *in vitro*, since the glands remained intact and showed well differentiated components. The morphology was greatly improved when the explants were cultured for 24 h in TGF-α-supplemented medium (Fig. 2).

In a few explants, the pit/gland structure disappeared, although the columnar superficial epithelial cells remained high and differentiated (Fig. 3A). These cells were PAS+ and were proliferating. We particularly named it as a continuous epithelium. This modification was never observed in 2 h-control or TGF-α-treated explants.

Re-epithelialization was observed as a layer of simple squamous or cubic epithelium at the sectioned edges of the explants (Fig. 3B).

Some apoptotic cells, distinguishable by the condensation and marginalization of the chromatin or by the presence of apoptotic bodies containing clumps of dense chromatin, were seen mainly in the lumen but also in the mucosa (Fig. 4A). Ultrastructural observations confirmed the cell shrinkage and chromatin condensation at the margins of the nucleus, as well as the fragmentation of the nucleus and cell (Fig. 4B).

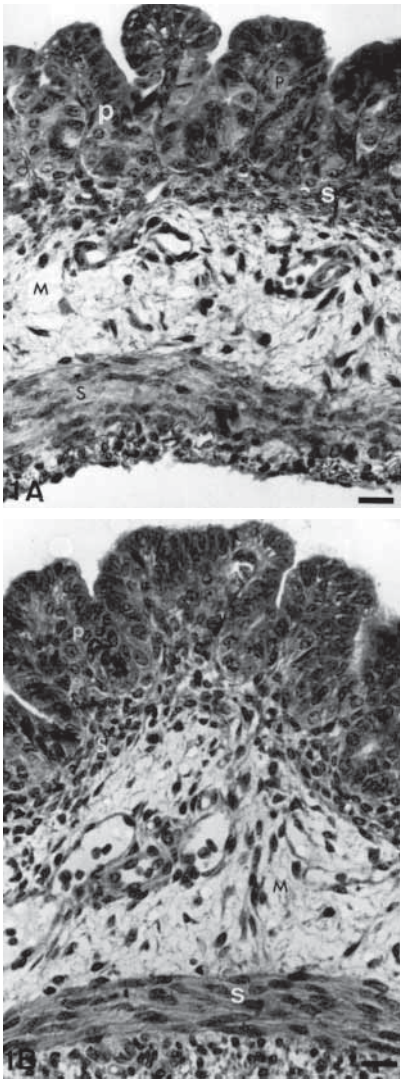

Figure 1. Light micrographs of transversal sections of fetal gastric mucosa explants (controls) cultured for 2 h (**A**) or 48 h (**B**) in serum-free medium. P = parietal cells in the glands, M = mesenchyma, s = smooth muscle layer. Bars = 2 μm.

The ultrastructural analysis after 24 h in culture showed that mucous cells retained their morphological features, including a cytoplasm containing many secretory granules (Fig. 5); the parietal cells presented many mitochondria and the intracellular canaliculi (Fig. 6A). Many coated pits and coated vesicles were seen in epithelial cells near the basal lamina (Fig. 6B), suggesting that epithelial-mesenchymal interactions were maintained.

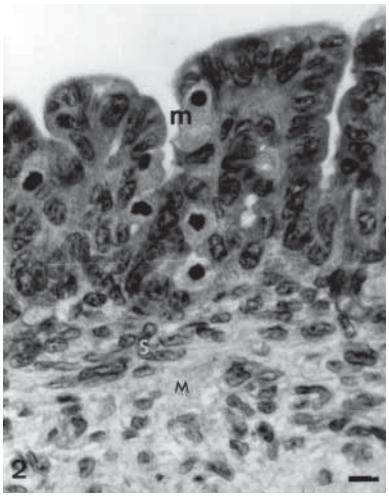

Figure 2. Light micrograph showing many metaphasic cells (m) in a fetal gastric explant incubated for 24 h in TGF-α-supplemented medium. s = smooth muscle layer, M = mesenchyma. Bar = 1 μm.

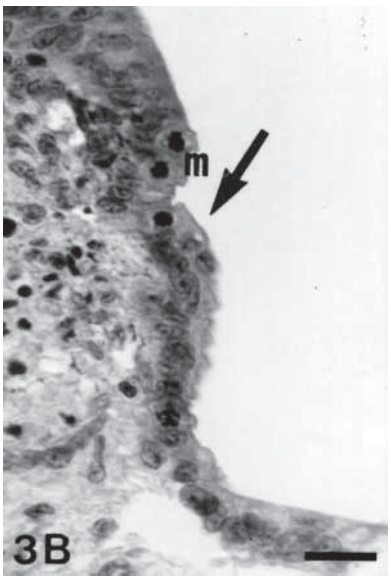

Figure 3. Light micrographs of transversal sections of fetal gastric mucosa explants: **A**- "Continuous epithelium" with high columnar cells (72 h with 10% FBS). Note the apoptotic and degenerating cells (arrows) in the connective tissue. **B**- Area of re-epithelialization (arrow) at the edge of a control fragment (24 h in serum-free medium). m = metaphasic cells. Bar = 2 μm (**A**) and 1 μm (**B**).

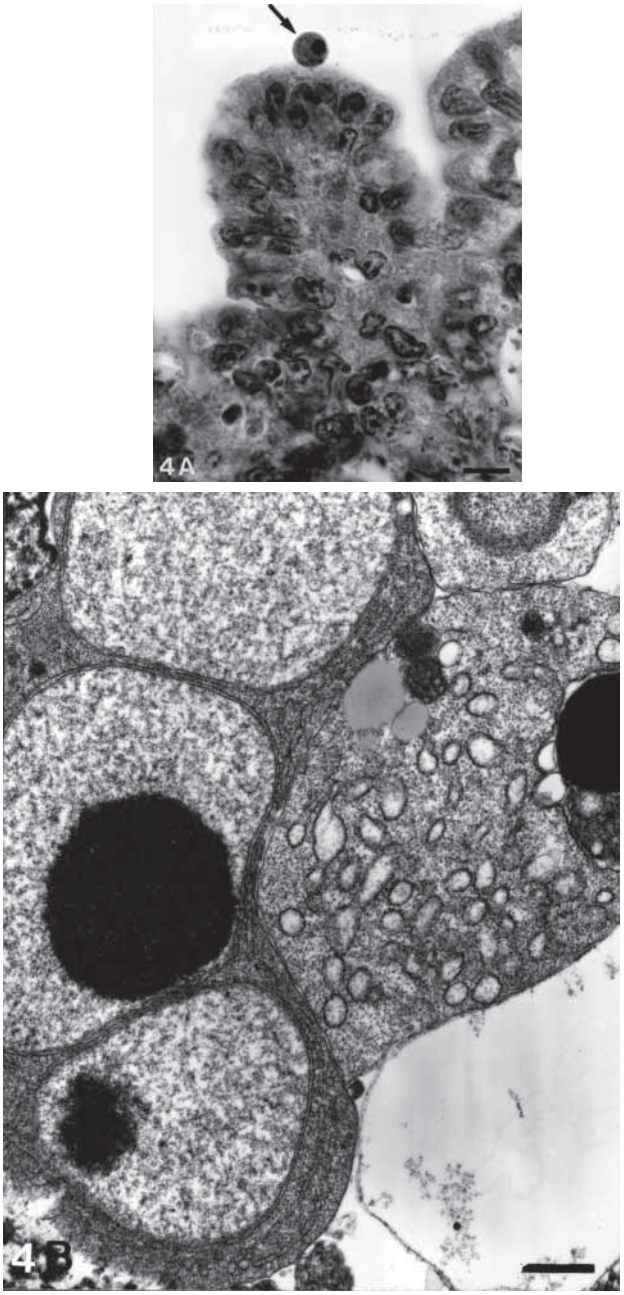

Figure 4. Apoptotic cells in the lumen of fetal gastric mucosa. **A-** Twenty-four hours in serum-free medium. Note the apoptotic cell in the lumen (arrow); **B**- Electronmicrograph of apoptotic bodies after 24 h of treatment with LHRH. Bar = 1 µm (**A**) and 0.1 µm (**B**).

Metaphasic index

For this analysis, the data were pooled from several experiments and do not represent equal numbers of fetuses in each case. Only well-preserved explants were used for counting.

Metaphasic indices of explants cultured with serum were lower than those of serum-free explants; however no significant differences were found between these groups (Fig. 7). The quantitative analysis showed that the organ culture conditions and the use of 10% FBS promoted no significant differences in cell proliferation in control groups of 24h without treatment, compared to 2 h-control group (Fig. 7). Somatostatin and LHRH significantly inhibited cell proliferation in fetal gastric epithelium when compared to the corresponding 24 h control. In contrast, TGF-α significantly increased the proliferation relative to 24 h controls, with or without 10% FBS. The metaphasic indices for TGF-α were also higher than in the 2 h controls, showing its mitogenic effect on gastric epithelial cells (Fig. 7).

DISCUSSION

The present study evaluated the morphological preservation in differentiating fetal stomach after 24 h in organ culture and also demonstrated the inhibitory effect of somatostatin and LHRH, as well as the mitogenic action of TGF-α on epithelial cell proliferation.

Primary explants are usually maintained in culture until the outgrowth of fibroblasts and epithelial cells. Miller *et al*. [28] used this method in human and adult rat gastric tissue to study the effect of pentagastrin on fibroblast and epithelial cell proliferation and differentiation. More recently, TØmmerÅs *et al*. [36] achieved cell differentiation in tissue culture of fetal stomach. The disadvantage of this method is that the epithelial architecture is not preserved. The gastric mucosa is not easily maintained in organ culture, and although the antral mucosa of adult rats has been maintained successfully for 24 h [16,24,40], the fundic mucosa showed degenerative changes after 6 h in culture [38]. Other authors have studied undifferentiated fetal stomach in organ culture [12,27,37,41]. Yeomans *et al*. [41] reported success using 18-day-old-rat fetuses, with a preservation rate of 71%. However, maturation occurred in only 14% of the explants. In our study, the rate of preservation was 67%, even in stomachs with differentiated cells. The glands and connective tissue of these fragments were well preserved, and proliferating cells were seen in all layers. Ménard and coworkers [27,37] also reported successful morphological and physiological maintenance of fragments obtained

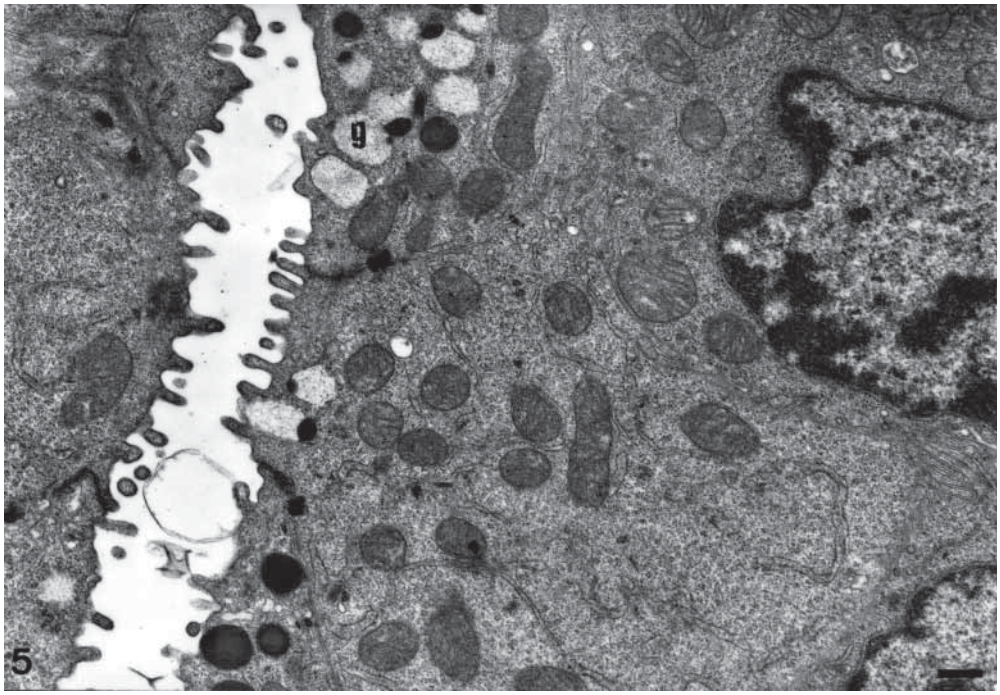

Figure 5. Electronmicrograph of an explant of fetal gastric mucosa maintained for 24 h in untreated medium. Note the epithelial cells with secretory granules (g). Bar = 0.5 μm.

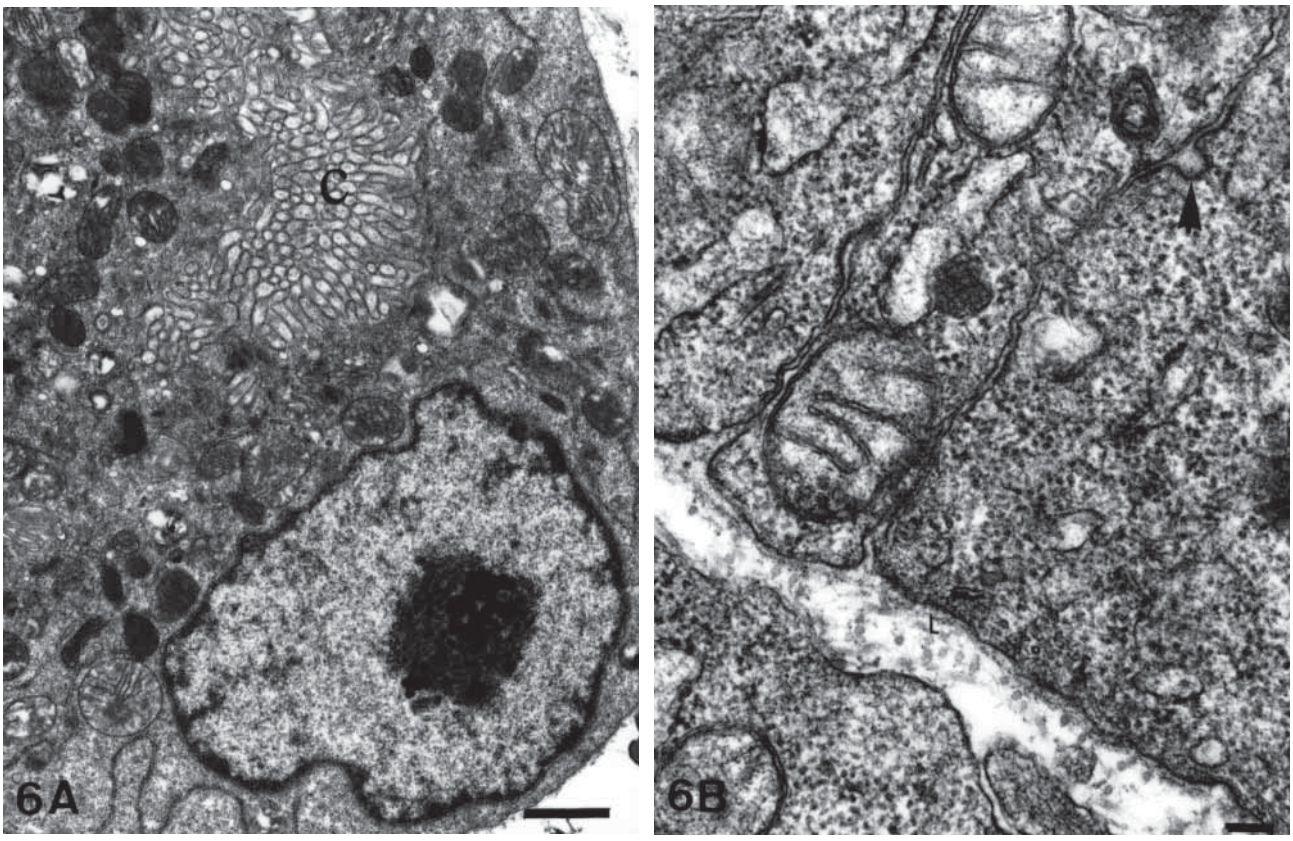

Figure 6. Electronmicrographs of fetal gastric mucosa fragments maintained for 24 h in medium treated with TGFα. **A-** Parietal cell with the intracellular canaliculus (C) filled with microvilli.; **B-** Basal region of gastric cell with underlying basal lamina (L) and coated pits (arrow head). Bar = 0.1 μm (**A**) and 0.2 μm (**B**).

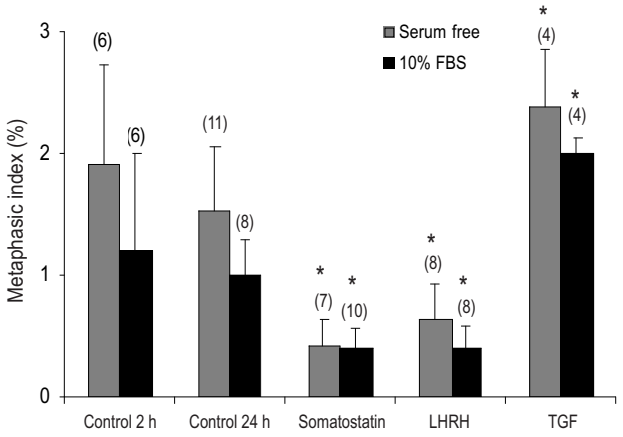

Figure 7. Effects of somatostatin, LHRH and TGF-α on the metaphasic index of fetal gastric glands after 24 h of culture in serum-free medium or in medium supplemented with 10% FBS. The values for a 2 h incubation are shown for comparison. Each bar represents the mean of (n) dishes + 1S.D. *p<0.05 compared to the corresponding 24 h controls.

from the human fetal stomach 12 to 17 weeks after fertilization. The above differences in the length of preservation may partly reflect differences in the ages of the fetuses. Explants of 20 day-old fetus have differentiated cells that may be more demanding than those younger cells.

FBS is a useful supplement *in vitro* and contains growth factors and hormones which are important for the maintenance of the explants. Some authors have used 10% FBS in culture, even when studying the effects of growth factors or hormones [10,36]. In our experiments, the addition of 10% FBS did not alter the morphology of the explants or their preservation, but cell proliferation was inhibited in both control and hormone-treated explants when compared with those maintained in serum-free medium. These results agree with Malo *et al*. [25] and confirm that FBS can interfere with studies involving hormonal effects.

Re-epithelialization is the ability of mucosal layer epithelial cells to cover the edges of explants. This process was described by De Ritis *et al*. [8] and others [5,9,17] in cultures of gastrointestinal organs but there is no consensus as to whether it involves cell migration or proliferation. Our observations suggest both possibilities, because the edge cells changed from cylindrical to a cubic or plain morphology; methaphasic cells were also seen in some fragments.

The occurrence of the continuous lining epithelium, where the glands disappeared, may be indirect evidence of the relevant role for growth factors. The overriding importance of epithelial tissue was suggested in an epithelial cell default-phenotype hypothesis [11], in which other cell types would not be maintained without the presence of specific factors and, as a result, these cells would assume the epithelial default program. Extending this hypothesis, in the case of some of the explants without TGFα, in which the glands could not be maintained, the lining epithelium remained unchanged, perhaps because of entry into the default program. In the absence of mesenchyma, fetal glandular stomach epithelial cells differentiate only into surface mucous cells and not into zymogenic cells [12]. This finding agrees with our results and supports the need for factors from epithelial-mesenchymal interaction to maintain differentiated epithelial cells in the stomach. Interactions between the epithelium and extracellular matrix are a requirement for normal epithelial ontogenesis. Recently, Tsukada *et al*. [39] reinforced the importance of the epithelial-mesenchymal interaction for the proliferation and differentiation of fetal stomach gastric cells maintained in culture, in the presence of glucocorticoids. Clathrin-coated pits and vesicles, as shown in the current study, characterize this route of uptake at the plasma membrane of basal epithelial cells. We have previously shown such vesicles near the basal membrane of fetal gastric epithelium in association with a network of lysosomes [1]. Thus, the presence of these structures support our conclusion that epithelial-mesenchymal interactions were maintained in organ culture.

The presence of some apoptotic cells may reflect the stress caused by the culture conditions [7]. The change from *in vivo* to *in vitro* certainly produces physiological disturbances during the initial period of culture, but these modifications were not sufficiently aggressive to lead to necrosis. Although apoptotic cells were not scored here, data from our laboratory showed that the percentage of these cells was less than 10%. However, we have observed a 3-fold increase in apoptotic cells during treatment with glucocorticoids (15), which strongly inhibit gastric epithelium proliferation.

A 24 h incubation was short to show other modifications in gastric mucosa as morphological differentiation, but was enough to demonstrate that

organ culture with differentiated cells is useful for studying the action of hormones and growth factors on gastric cell proliferation.

The administration of somatostatin *in vivo* affects gastric cell proliferation, but the mechanism of inhibition is not well understood [13,23]. The addition of somatostatin to the medium of normal and malignant isolated gastric mucosa cells showed that this hormone inhibits the growth of normal cells at high concentrations [30]. In our study, somatostatin decreased the proliferation of fetal gastric epithelium, probably through its interaction with cell membrane receptors and subsequent cAMP modulation [30]. A paracrine action on other cells that have a trophic effect on the gastric epithelium could also be involved [22,32].

LHRH is the hypothalamic regulator of pituitary LH release. LHRH has also been identified in extra hypothalamic sites, including immune tissues, and an immunomodulatory role for LHRH has been suggested [4]. LHRH inhibited cell proliferation in the gastric epithelium *in vitro*, in agreement with results obtained *in vivo* [13]. Binding sites for LHRH receptors were recently described [14] suggesting that the hormone can have a direct effect in the organ. It was already shown that this hormone could act in other organs besides the genital tract. Opposite responses were reported, including the stimulation of T cell proliferation [3,26] and the inhibition of pancreatic tumours [35].

Transforming growth factor-alpha (TGF-α) and its receptor, the epidermal growth factor receptor (EGFR) play important roles in cell proliferation in normal and regenerative gastric mucosa. Montaner *et al*. [29] demonstrated the expression of TGF-α in the cytoplasm and basolateral and apical membranes of surface and gastric pit cells whereas EGFR was detected in the supranuclear region of cells lining the gland. TGF-α and its receptor are also expressed in exocrine and endocrine cells of all gastrointestinal and pancreatic tissues during human fetal development [18]. The mitogenic action of TGF-α has been shown in epithelial cells of normal digestive tissues *in vivo* [19] and during ulcer healing [21], as well as in isolated gastric epithelial cells [6,31,34]. We observed a similar effect in gastric mucosa in organ culture in which this growth factor significantly increased the metaphasic index of fetal epithelium compared to the 2 h and 24 h controls. The immunolocalization of TGF-α and EGFR verified by others supports the hypothesis of a paracrine/autocrine function *in vivo* and *in vitro* and this could directly involve epithelial cells.

In conclusion, organ culture proved useful in demonstrating the inhibitory action of somatostatin and LHRH and the stimulatory effect of TGFα on epithelial gastric cell proliferation, thus corroborating our previous results obtained *in vivo* [13].

ACKNOWLEDGMENTS

The authors thank Cruz A. M. Rigonati for technical assistance and Dr. Patricia Gama for helpful criticisms on the manuscript. This study was supported by Fundação de Amparo à Pesquisa do Estado de São Paulo (FAPESP) and Conselho Nacional de Desenvolvimento Científico e Tecnológico (CNPq), Brazil.

REFERENCES

1. Alvares EP (1994) Extensive networks of TMPase positive basal lysosomes are present in fetal rat gastric epithelium before overt differentiation. *J. Submicrosc. Cytol. Pathol.* **26**, 515-523.
2. Arsenault P, Ménard D (1985) Influence of hydrocortisone on human fetal small intestine in organ culture. *J. Pediatr. Gastr. Nutr*. **4**, 893-901.
3. Azad N, Emanuele NV, Duffner L, Kirsteins L, Lawrence AM (1990) Further studies on lymphocyte luteinizing hormone releasing hormone (LHRH). In: *72nd Annual Meeting of the Endocrine Society,* Atlanta, GA, USA 1990. Abstracts p. 361.
4. Azad N, LaPaglia N, Agrawal L. Steiner J, Uddin S, Williams DW, Lawrence AM Emanuele NV (1998) The role of gonadectomy and testosterone replacement on thymic luteinizing hormone-releasing hormone production. *J. Endocrinol.* **158**, 229-235.
5. Calvert R, Micheletti PA (1981) Selection of a chemically defined medium for culturing fetal mouse small intestine. *In Vitro* **17,** 331-344.
6. Chen MC, Lee AT, Karnes WE, Avedian D, Martin M, Sorvillo JM, Soll AH (1993) Paracrine control of gastric epithelial cell growth in culture by transforming growth factor-α. *Am. J. Physiol.* **264,** G390-G396.
7. Cotter TG, Al-Rubeai M (1995) Cell death (apoptosis) in cell culture systems. *Trends Biotechnol.* **13**, 150-155.
8. De Ritis G, Falchuk ZM, Trier JS (1975) Differentiation and maturation of cultured fetal rat jejunum. *Develop. Biol.* **45,** 304-317.
9. Ferland S, Hugon JS (1979) Organ culture of adult mouse intestine. *In Vitro* **15**, 288-293.
10. Finney KJ, Ince P, Appleton DR, Sunter JP, Watson AJ (1987) A trophic effect of epidermal growth factor (EGF) on rat colonic mucosa in organ culture. *Cell Tissue Kinet.* **20,** 43-56.

11. Frisch SM (1997) The epithelial cell default-phenotype hypothesis and its implications for cancer. *BioEssays* **19**, 705-709.
12. Fukamachi H, Ichinese M, Ishihama S, Tsukada S, Yasugi S, Shiokawa K, Furihata C, Yonezawa S, Miki K (1994) Fetal rat glandular stomach epithelial cells differentiate into surface mucous cells which express cathepsin E in the absence of mesenchymal cells in primary culture. *Differentiation* **56,** 83-89.
13. Gama P, Alvares EP (1996) LHRH and somatostatin effects on the cell proliferation of the gastric epithelium of suckling and weaning rats. *Regul. Pept.* **63,** 73-78.
14. Gama P, Alvares EP (2001) Localization of luteinizing-hormone releasing hormone binding sites in the gastric mucosa of suckling rats. *Anat. Rec*. **264,** 43-50.
15. Gama P, Goldfeder EM, Bertacini de Moraes JC, Alvares EP (2000) Cell proliferation and death in the gastric epithelium of developing rats after glucocorticoid treatments. *Anat. Rec*. **260**, 213-221.
16. Harty RF, van der Vijver JC, McGuigan JE (1977) Stimulation of gastrin secretion and synthesis in antral organ culture. *J. Clin. Invest.* **60,** 51-60.
17. Hayashi T, Papla B, Stemmermann GN (1975) Gastric organ culture. *Am. J. Pathol.* **78,** 23-32.
18. Hormi K, Lehy T (1994) Developmental expression of transforming growth factor-alpha and epithelial growth factor receptor proteins in the human pancreas and digestive tract. *Cell Tissue Res.* **280,** 439-450.
19. Hormi K, Lehy T (1996) Transforming growth factor-alpha *in vivo* stimulated epithelial cell proliferation in digestive tissues of suckling rats. *Gut* **39,** 532-538.
20. Johnson LR (1985) Functional development of the stomach. *Ann. Rev. Physiol.* **47,** 199-215.
21. Konturek PC, Brzozowski T, Konturek SJ, Ernst H, Drozdowicz D, Padjo R, Hahn, EG (1997) Expression of epidermal growth factor and transforming growth factor alpha during ulcer healing. Time sequence study. *Scand. J. Gastroenterol.* **32**, 6-15.
22. Larsson LI, Goltermann N, Magistris L, Rehfeld JF, Schartz TW (1979) Somatostatin cell processes as pathways for paracrine secretion. *Science* **205**, 1393-1394.
23. Lehy T, Dubrasquet M, Bonfils S (1979) Effect of somatostatin on normal and gastrin-stimulated cell proliferation in the gastric and intestinal mucosae of the rat. *Digestion* **19,** 99-109.
24. Lichtenberger LM, Shorey JM, Trier JS (1978) Organ culture studies of rat antrum: evidence for an antral inhibitor of gastrin release. *Am. J. Physiol.* **4,** E410-E415.
25. Malo C, Arsenault P, Ménard D (1983) Organ culture of the small intestine of the suckling mouse in a serum-free medium. *Cell Tissue Res.* **228**, 75-84
26. Marchetti B, Guarcello V, Morale MC, Bartoloni G, Raeti F, Palumbo G Jr, Farinella Z, Cordado S, Scapagnini U (1989) LHRH agonist restoration of age associated decline of thymus weight, thymic LHRH receptors, and thymocyte proliferative capacity. *Endocrinology* **125,** 1037-1045.
27. Ménard D, Arsenault P, Monfils S (1993) Maturation of human fetal stomach in organ culture. *Gastroenterology* **104,** 492-501.
28. Miller LR, Jacobson ED, Johnson LR (1973) Effect of pentagastrin on gastric mucosal cells grown in tissue culture. *Gastroenterology* **64,** 254-267.
29. Montaner B, Asbert M, Perez -Tomás R. (1999) Immunolocalization of transforming growth factor-alpha and epidermal growth factor receptor in the rat gastroduodenal area. *Dig. Dis. Sci.* **44,** 1408-1416.
30. Moyer MP, Armstrong A, Aust JB, Levine BA, Sirinek KR (1986) Effects of gastrin, glutamine and somatostatin on the *in vitro* growth of normal and malignant human gastric mucosal cells. *Arch. Jap. Chir.* **42,** 293-314.
31. Nakajima N, Kuwayama H (1995) Stimulatory effect of transforming growth factor-alpha on gastric epithelial cell migration through proliferation. *J. Clin. Gastroenterol.* **21,** S45-S49.
32. Onolfo JP, Lehy T (1987) Comparative development of gastrin and somatostatin cell populations in the pancreas, stomach and duodenum of the rat during the perinatal period. *Anat. Rec.* **218,** 416-425.
33. Polk DB, Barnard JA (1999) Hormones and growth factors in intestinal development. In: *Development of the Gastrointestinal Tract*. (Sanderson IR, Walker WA, eds). pp. 37-55. BC Decker Inc.: Hamilton, Ontario.
34. Rutten MJ, Dempsey PJ, Solomon TE, Coffey RJ (1993) TGFα is a potent mitogen for primary cultures of guinea pig gastric mucous epithelial cells. *Am. J. Physiol.* **265,** G361-G369.
35. Szende B, Srkalovic G, Timar J, Mulchahey JJ, Neill JD, Lapis K, Csikos A, Szepeshazi K, Schally AV (1991) Localization of receptors for luteinizing hormone-releasing hormone in pancreatic and mammary cancer cells. *Proc. Natl. Acad. Sci. USA* **88,** 4153-4156.
36. TØmmerÅs K, Chen Y, Rhedin M, Cabero JL, MÅrdh S (1997) Proliferation and differentiation of cells from explants of fetal rat stomach. *Acta Physiol. Scand.* **159,** 155-161.
37. Tremblay E, Chailler P, Ménard D (2001) Coordinated control of fetal gastric epithelial functions by insulin-like growth factors and their binding proteins. *Endocrinology* **142**: 1795-1803.
38. Trier JS (1976) Organ-culture methods in the study of gastrointestinal-mucosal function and development. *N. Engl. J. Med.* **295**, 150-155.
39. Tsukada S, Ichinose M, Yahagi N, Matsubara Y, Yonezawa S, Shiokawa K, Furihata C, Miki K, Fukamachi H (1998) Induction of precocious pepsinogen synthesis by glucocorticoids in fetal rat gastric epithelium in organ culture: importance of mesenchyme for epithelial differentiation. *Differentiation* **62**, 239-247.
40. Yeomans ND, Millar SJ (1980) Synthesis and secretion of glycoproteins by mucosa of rat gastric antrum in organ culture. *Dig. Dis. Sci.* **25**, 295-301.
41. Yeomans ND, Trier JS, Moxey PC, Markezin ET (1976) Maturation and differentiation of cultured fetal stomach. *Gastroenterology* **71**, 770-777.

Received: May 7, 2001
Accepted: September 24, 2001