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# Morphological changes in the neonatal murine gut induced by SCF and EGF in organ culture — electron microscopy study

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The aim of our study was to determine the developmental effect on the murine small intestine in organ culture in presence of EGF and SCF. Morphological changes in connection with the effect of both growth factors were observed under transmission and scanning electron microscopes. Organ culture preparation was done by the methods of Playford et al. Specimens were subject to autoradiography and electron microscopy observation.

Using autoradiography approach we found that the number of dividing nuclei was increased both for the samples treated with EGF and SCF. Transmission electron microscopy and scanning electron microscopy showed well shaped enterocytes with typical microvilli for the organ explants treated with EGF and SCF. We found correlation in results obtained from different investigation methods-autoradiography and electron microscopy. Both growth factors had beneficial effect and affected maturation and development of the neonatal murine small intestine.

*Key words*: SCF, EGF, colostrum, gut development, morphology.

## Introduction

Colostrum and milk are essential for the development and growth of mammals. Postnatal development and maturation of their gastrointestinal tract is influenced by supply of maternal milk. Among the substances present in colostrum and milk epidermal growth factor (EGF) has major role in gut maturation and development along with stem cell factor (SCF). Many studies prove that EGF directly or indirectly regulates growth, function and maintenance in epithelial tissues and postnatal somatic and bone growth. It was proven that EGF can suppress adipocyte differentiation and maturation. Systemic treatment with EGF in rats reduced amounts of adipose tissue, decreasing muscle and fat mass [1]. Stem cell factor and its interaction with c-kit are considered to be important for the homeostasis of epithelial barrier function in the intestinal tract [2].

The aim of our study was to determine the effect of the latter growth factors on the development of murine small intestine in organ culture.

Morphological changes in connection with the effect of both growth factors were observed under transmission and scanning electron microscopes. Quantitative analysis of the number of dividing nuclei, stimulated or non stimulated with both factors was done.

## Materials and Methods

*Organ culture preparation:*

Organ cultures were prepared from 5-day-old Balb/c mice from both sexes. A short segment of small intestine extending distally from the pylorus was removed from each mouse. The segment was cut into 3 parts — duodenum, jejunum and ileum. Samples were taken according to intestinal length by Playford et al. [3]. They were opened so that the medium could wash the villi inside. The explants were incubated in culture medium RPMI 1640 containing 10 % fetal calf serum with 50 ng/ml rm EGF or 20 ng/ml rm SCF /Immunotools /Germany/ for 24, 48 and 72 hours at 37° C, 5% $CO_2$ at 100 % humidity.

*Autoradiography:*

For the purpose of autoradiography investigation we cultured bowel specimens as described above and we added 5 µl of $^3$H-Thymidine/Amersham, UK/ to both SCF and EGF treated cultures. Gut explants were put into Tissue Tek culture medium and frozen at — 25°C. Later they were sectioned and applied to glass slides. Autoradiographic emulsion/Hypercoat EM-1, Amersham, UK/ was used to incorporate at a dark room. When dry the slides were placed in a slide box over silicagel, wrapped in foil and placed in a refrigerator for two weeks. Autoradiographic emulsion was developed at dark room and finally slides were washed with distilled water. Counterstaining with hematoxylin showed the histological details of the tissue.

*Electron microscopy observation:*

Incubated explants were removed from the culture and washed three times in PBS buffer and fixed in 2.5% glutaraldehyde, containing 0.4 M Na-caccodilate. Post fixation was performed with 1% osmium tetra oxide and then specimens were dehydrated in graded ethanol solutions (50-100%) and embedded in resin. Ultra thin sections were contrasted with uranyl acetate and Reynold's lead citrate and examined with transmission electron microscope.

Preparation for scanning electron microscopy was performed as follows: specimens were fixed in 2.5% gluaraldehyde, washed twice in 0.1M caccodilate buffer and then dehydrated in graded ethanol solutions (50-100%). Critical point drying was performed and then specimens were coated with gold in a sputter coater and observed under scanning electron microscope.

## Results

Using autoradiography approach we measured number of dividing nuclei at the small gut wall. For that purpose we used StatMost for Windows. One-Way ANOVA Results were used to prepare graphs. For all days of incubation the number of dividing nuclei was increased for the samples treated with EGF (Fig. 1). The incorpora-

tion of $^3$H-thymidine was also stimulated at the samples treated with SCF (Fig. 2). Data are presented as the mean ± SEM. *P* value < 0.05 was considered statistically significant.

Transmission electron microscopy of the three parts of the small intestine stimulated with EGF showed well shaped enterocytes with typical microvilli, characteristic

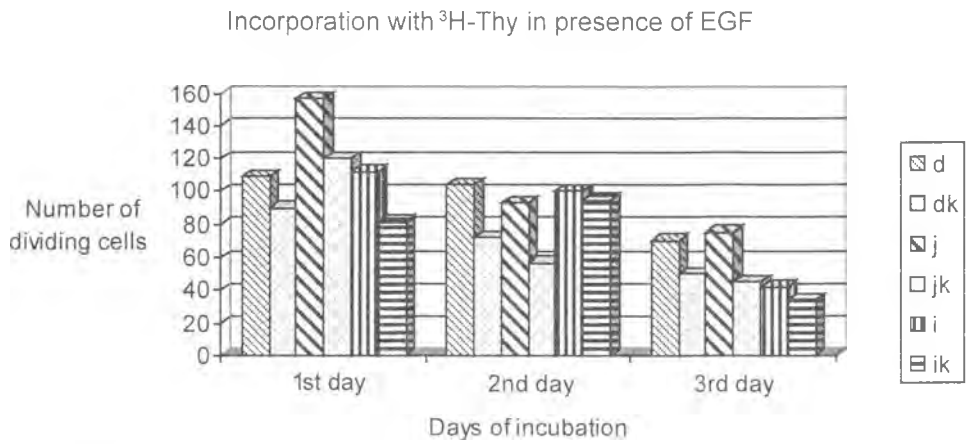


Fig. 1. Explants treated with EGF from all days of incubation. The increased number of dividing cells in presence of EGF is visible. Obvious is the increased incorporation with $^3$H-Thymidine ($^3$H-Thy) at the duodenum. P — value < 0.05; d — duodenum, dk — duodenum control, j — jejunum, jk — jejunum control, i — ileum, ik — ileum control

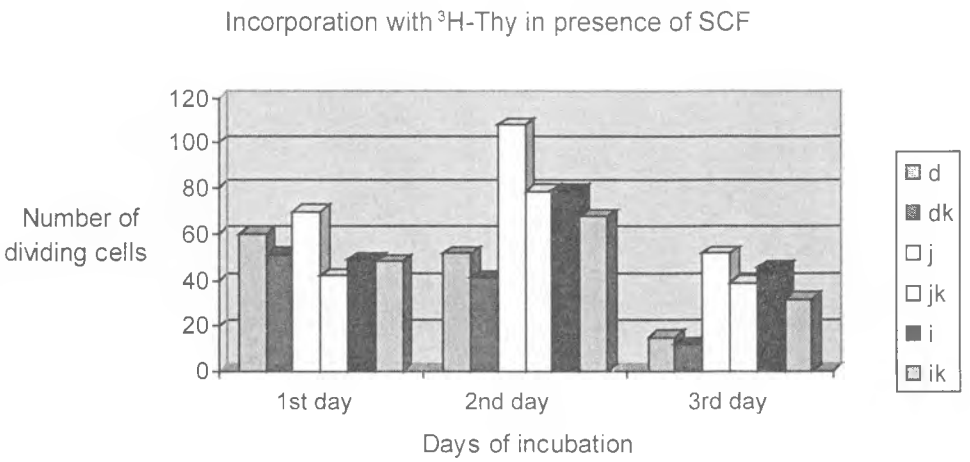


Fig. 2. Incorporation with $^3$H-Thymidine in presence of SCF. Increased number of dividing cells for all specimens treated with SCF. P — value < 0.05. d — duodenum, j — jejunuk, i — ileum; dk — duodenum control, jk — jejunum control, ik — ileum control

for the brush border (Fig. 3a). Tight junctions were also observed between the enterocytes. A great number of nuclei were activated. Untreated specimens showed less activated nuclei along with fewer amounts of microvilli covering the brush border region. Some regions showed lack of microvilli or if present they were thinner and irregular. Loose junctions between different enterocytes were observed, which we suppose were in connection with the absence of EGF in the culture medium (Fig. 3b).

Organ explants stimulated with SCF showed well shaped microvilli covering the brush border in comparison to the control specimens (Fig. 4a, b), where microvilli

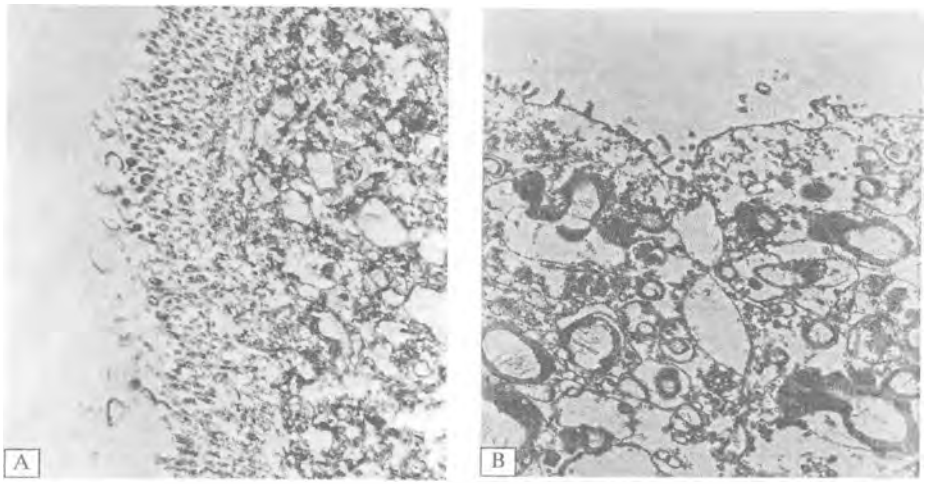


Fig. 3. Transmission electron micrograph of duodenum of 5-day-old mouse, treated with EGF-second day of incubation. In presence of EGF (A) microvilli are thicker. When cultured without EGF microvilli are fewer and loose junctions are seen between cells. Originally × 12000 for both micrographs

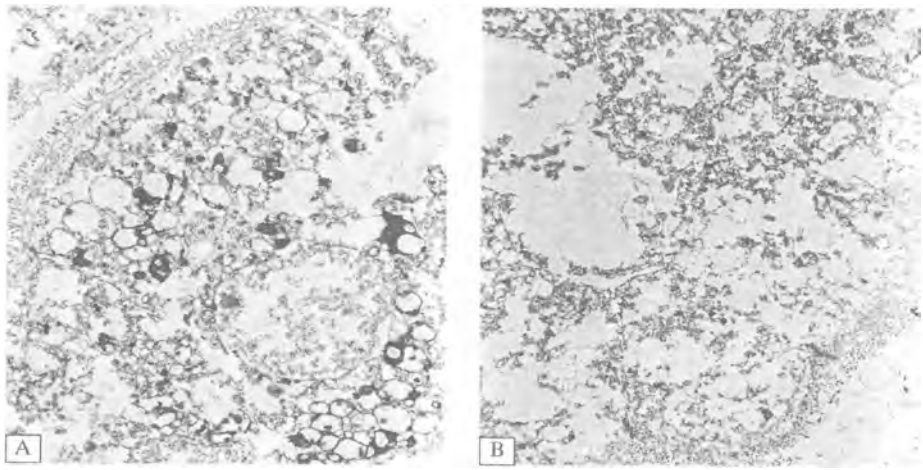


Fig. 4. Transmission electron micrograph of jejunum of 5-day-old mouse. Specimen treated with SCF (A) showed well shaped enterocytes with typical microvilli and activated nuclei. Originally × 4400. Cultured in absence (B) of the growth factor microvilli were fewer. Originally × 3000

were shorter and had more gaps in between. Both cases showed tightly situated cells and activated nuclei.

Scanning electron microscopy (SEM) provides vivid three-dimensional images which are easy to understand in comparison to TEM. For this point of view SEM is advantageous in morphological research and it can serve as adequate method to reveal intracellular structures [4].

Scanning electron microscopy of duodenum, jejunum and ileum cultured with EGF showed straight villi covering viscera (Fig. 5a). Controls observed were thicker and shorter (Fig. 5b). In most ileums not treated with EGF we found curved villi with loosely situated enterocytes.

Scanning electron microscopy of murine small gut stimulated with SCF revealed longer villi with more contrasting and tightly situated enterocytes (Fig. 6a). Controls had shorter and thicker villi and more space between them (Fig. 6b) Using

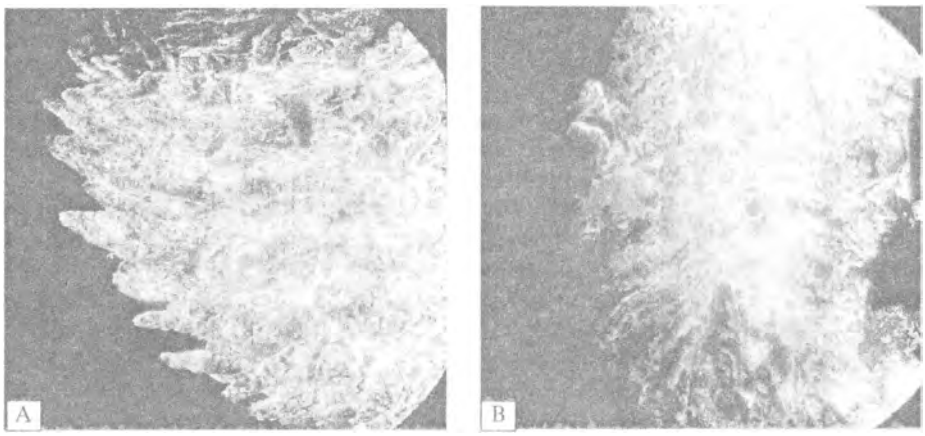


Fig. 5. Scanning electron micrograph of duodenum of 5-day-old mouse, second day of incubation. Treatment with EGF increases the number of villi covering viscera (A). In absence of EGF villi are fewer and curved (B). Originally × 180

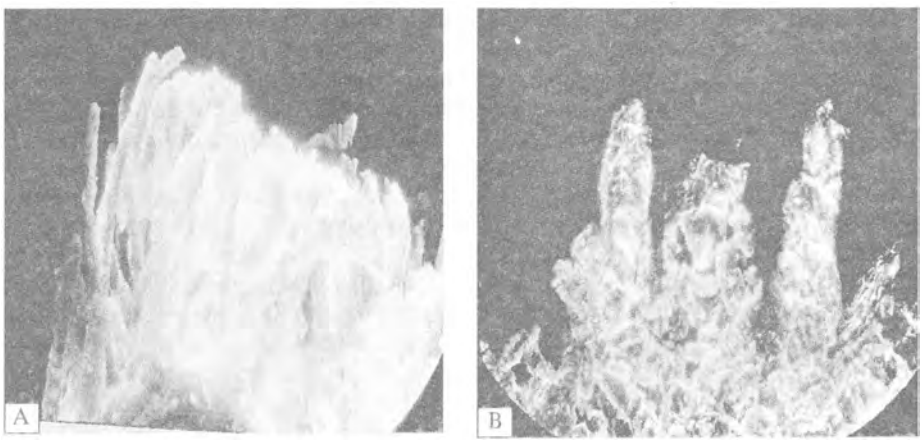


Fig. 6. Scanning electron micrograph of ileum, second day of incubation with SCF. Long finger-like villi are observed over treated viscera (A). Fewer irregular villi are observed in controls (B). Originally × 180

higher magnifications discrete details of villous morphology were revealed. Corrugations of the surface of the villi were obvious in all investigated samples. Those treated with SCF showed tight cell to cell location. Specimens that weren't treated with SCF had more gaps between enterocytes and cells were unequal.

## Discussion

EGF and SCF are present in colostrum and milk but data suggests different results about their influence on gastro-intestinal tract.

Epidermal growth factor (EGF) is a 53 amino acid peptide that is produced by the salivary glands, mammary glands, Brunner's glands of the duodenum and kidneys [5]. It is a potent stimulant of proliferation and healing of the gastrointestinal tract, acting as a cytoprotective agent and is also stabilizing cells against noxious agents such as indomethacin [6]. It was shown that EGF can prevent hepatic injury and multiorgan injury [7, 8] Clinical trials of EGF are presently under way for treatment of ulcerative conditions of the bowel. A rapid intervention with EGF may maintain organ viability [9]. Addition of EGF to organ culture had beneficial effect on gut mucosa, accelerating the maturation rate and proliferation of intestinal cells in early embryonic murine gut [10]. It was shown that enterocyte maturation was more sensitive to EGF than cell proliferation [11].

The autoradiography investigation gave us quantitative results abut those nuclei that were in the S phase of division during the exposure to $^{3}$H-Thymidine. The quantities of dividing nuclei at the gut wall were in correlation to our electron microscopy results. Enlarged number of dividing cells for the explants treated with EGF and for those treated with SCF showed the stimulating and maturating effects on enterocytes of both growth factors.

Stem cell factor (SCF) has the ability to stimulate growth of early hematopoetic progenitors capable of maturing to erythroid, megacaryocyte, granulocyte, lymphocyte, and macrophage cells. Mammals treated with SCF increase hematopoetic cells of myeloid and lymphoid lineages. [1] It also promotes the development of mast cells from CD 34+stem cells in vitro and in vivo [12]. The interaction of SCF and c-kit is considered to be an important signalling event for the homeostasis of the epithelial barrier function in the intestinal tract [13].

Scanning electron microscopy of the small bowel revealed the morphology of the stimulated explants. At birth the villi are finger-like and regular. Simultaneously the villi shape changes from finger- to leaf- or tongue-like and the number of dividing villi and villi with indentations increase remarkably in time [14-15]. Observations based on SEM analysis indicate very dynamic growth-related changes that occur in association with the growth factors added. It was shown that in the unsuckling piglets the villi were short and dense [16]. In our case in presence of EGF we found straight and thick situation of the villi. On the other hand, unstimmulated explants had shorter and denser villi. As visualized, small intestinal histological features were similar to those described in other species. [17]. Specimens treated with SCF were again dense and a finger-like shape was obvious. Explants that were not treated with SCF had shorter villi with finger-like form. The villi looked twitched. It was due to a reduction in basal vascular resistance simultaneous with dramatic increase in local intestinal blood flow and lymph formation [16]. Higher magnification graphs showed many corrugations between cells and weak cell to cell contacts.

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