Occasional report

Growth and transformation of the small intestinal mucosa – importance of connective tissue, gut associated lymphoid tissue and gastrointestinal regulatory peptides

E O RIECKEN, A STALLMACH, M ZEITZ, J D SCHULZKE, H MENGE, AND M GREGOR

(This article is one of a series linked with the Festschrift for Christopher Booth. See Gut Festschrift 1989; 30.)

Physiologically, the architecture of the small intestinal mucosa is maintained by a delicate balance between cell production in the crypt compartment, enterocyte migration along the villi, and extrusion of mature epithelial cells from the tip of the villi into the lumen.1 Migration and maturation of the lining cells are affected in response to various types of stress and under conditions of mucosal damage and repair. This is also true for the tissue components beneath the lining cells. On the other hand, although the equilibrium of the lining cells may be disturbed by a great variety of various types of stress, the response of the mucosa is rather uniform and restricted to three distinct mucosal patterns only – namely, hypertrophy, hypotrophy, and mucosal transformation of the hyperregenerative type (Fig. 1).2 In these mucosal patterns alterations of the absorptive cells and the zonation of villi and crypts have been studied extensively. The regulation of cell proliferation and differentiation in this system is, however, still not well understood although the importance of some factors such as luminal nutrition and the ornithine decarboxylase system have been recognised.3

Furthermore, the role of regulatory peptides has attracted much attention, though with few concrete results, and the implications of the interaction between the lining cells, the mesenchyme, and the gut associated lymphoid tissue with respect to cell proliferation and maturation have only recently gained interest.4

It is the purpose of this contribution to briefly describe the three principal mucosal responses to various types of stress and also to discuss some aspects of the connective tissue, the gut associated lymphoid tissue and the regulatory peptides in the control of intestinal mucosal growth and their perspectives in future research as far as the regulation of mucosal growth is concerned. It is not intended, however, to be complete within the frame given, but rather to focus on areas of our own interest in the subject.

One of the prerequisites of the striking small intestinal mucosal potential to change its structure and function is the fact that each villus possesses numerous crypts with an enormous proliferative potential. The average number of crypts per villus in upper human jejunum amounts to 8-3 per villus.5 In man, 23 of 33 cell positions of the lower part of the crypt cell column belong to the proliferative compartment, whereas the upper third represents the maturation area in which the crypt cells obtain their functional competence.3 The crypt is able to enlarge and to decrease its size in diameter and depth as a consequence of changes in its proliferative capacity.

In this respect, it is of interest that the crypts of

Fig. 1 Principal forms into which the small intestinal mucosa may be transformed.
the small intestine are surrounded by a prominent sheath of fibroblasts within the lamina propria of the bowel Fig. 2. It has been assumed that in the adult small intestine, this specialised mesenchymal cell type, the 'pericycral myofibroblast' undergoes a rapid turnover similar to that of the epithelial cell. Synchronous division and parallel migration of epithelial and subepithelial mesenchymal cells may form a 'unit' of both cell populations. Zajicek has proposed the concept of an 'intestinal proliferon', consisting of epithelial, mesenchymal, neural, and vascular elements, in which a turnover of the different cell populations is closely coordinated. Other findings, however, indicate that the division rate of pericryptal myofibroblasts is lower than the division rate of epithelial cells, some 'H-thymidine labelled myofibroblasts remain at the basis of the crypt for a long time and also become polyploid.  

**Mucosal hypertrophy**

Mucosal hypertrophy with hyperplasia of the cells is one condition in which the crypt cell compartment has altered its geometry. The model in which this mucosal pattern can be studied best is the ileal remnant after proximal small intestinal resection in the animal. The mitotic activity increases first and is followed by an increase in crypt depth and in crypt diameter, whereby the number of crypts per mucosal area is reduced. Concomitantly, there is a proportional increase in villus size, which again leads to a reduction in the number of villi per unit area. Thus, there is a largely proportional enlargement of villi and crypts which contain an increased number of lining cells, which may undergo distinct changes in size and function. Thus, the number of cells per unit length may increase, and microvilli of absorptive cells after intestinal resection are shortened. In addition, specific brush border enzyme activities have been shown to be decreased and *in vitro* absorption of non-electrolytes in the hyperplastic mucosa is reduced. These findings have led to the concept that there are functionally immature enterocytes after intestinal resection, which is in accordance with the fact that cell migration time after intestinal resection is increased and the cell life span of the enterocytes is shortened. None the less, Menge *et al* could show that in the hypertrophic mucosa brush border vesicle transport was not altered after proximal resection, as judged by studies of the accumulation of D-glucose. Furthermore, when using more sophisticated enzymatic *in situ* studies, a differential enzyme response has been observed at the cellular level: a significant decrease in both V<sub>max</sub> and K<sub>m</sub> values of neutral α-glucosidase were already expressed four days after resection and

---

**Fig. 2**  *Scheme of migration and differentiation of epithelial cells and subepithelial myofibroblasts along the crypt-villus axis in the adult small intestine. Left panel shows postulated interrelationship between stem cells and connective tissues into different surface cells.*
confined to the apical villus while no changes in the kinetics of the lactase/β-glucosidase occurred. Similarly, Chavez et al showed selective increments in enzyme expression in ileal enterocytes after proximal small bowel resection. Therefore, a complex pattern of cellular enzymatic and morphological adaptation to proximal resection ensues in the otherwise rather uniform pattern of hyperplastic ileal mucosa with increased zonation of villi and crypts.

Because in the hypertrophic mucosa the absorptive capacity is mainly restricted to the upper third of the villi as in the normal intestine the main increase in function per centimetre intestine is achieved by an increase in diameter of the small intestine. This has been experimentally proven: a 45% proximal small intestinal resection leads to an increase in villus height, mucosal circumference and glucose absorption in vivo when the substrate disappearance rate is expressed in terms of unit serosal length. A 70% resection induces a further increase in mucosal circumference and glucose absorption, while villus height remains unchanged.

Intestinal mucosal hyperplasia in man largely corresponds to the findings in the experimental animal, although it has been investigated less systematically and less completely. Booth and coworkers had already shown in 1961 that the functional reserve after proximal intestinal resection is so effective that malabsorption is not a problem even if 250 cm of intestine have been removed. In this context two additional phenomena have been recognised to be important in man. First, the intestinal remnant may also grow in length after resection and thereby enforce function of mucosal hyperplasia. Second, the full adaptive response may require a long period to develop, according to studies in jejunoileal bypass up to 12 months.

Mucosal atrophy

Mucosal atrophy is the second pattern of the small intestine in which the geometry of the mucosa is distinctly altered and which has been thoroughly explored in Thiry-Vella fistulae to bypass the passage of ingesta and in selfempting intestinal blind loops. Its main features are a reduction of villus height, crypt depth, mitotic activity, and diameter of the small intestine while the single enterocyte does not usually exhibit features of regression. Hence, the fine structure of the absorptive cells is not altered, brush border enzyme activities are not reduced, and reduced absorptive function correlates roughly with the reduction in absorptive surface. Brush border vesicle transport of absorptive cells in the atrophic intestine as judged by sodium dependent D-glucose accumulation studies also remains unaltered. Thus, in mucosal atrophy as in mucosal hypertrophy, the initial step of non-electrolyte absorption is not dependent on the proliferative state of the mucosa, thereby signalling that the absorptive function of the individual enterocyte remains unaltered.

Similar changes of the mucosa as in the bypassed intestine do occur in longterm parenteral nutrition and in the starved animal in which diameter and depth of crypts decrease, as does eventually the number of villi and crypts. Mucosal atrophy may be altered further in a number of conditions such as cytostatic treatment and irradiation, the damage thereby depending on dose, time course, and action of the drug.

Mucosal transformation

The third mucosal pattern which represents the most striking alteration of the mucosal geometry is 'mucosal transformation of the hyper-regenerative type'. It is characterised by hyperplasia and mucosal growth as is the case in mucosal hypertrophy. It differs, however, from mucosal hypertrophy in that it is characterised by the transformation of the normal zonation with reduced villi and enlarged crypts, the reason why we have chosen this term. In addition, it is associated with damage to the surface cells and the underlying connective tissue. A suitable model to study this form of mucosal response to stress is the self-filling small intestinal blind loop in the experimental animal in which stasis, bacterial overgrowth and increased concentrations of toxic deconjugated bile acids are the stressors to the surface epithelium. In response to these damaging agents increased exfoliation of damaged surface cells occurs which is followed by hyperproliferation of crypt cells in the germinative zone. In longterm lactic acid perfusion studies we have shown that the initial step of this mucosal transformation corresponds largely to mucosal hypertrophy in which there is a proportional enlargement of villi and crypts with increased mitotic activity. As damage continues and cell exfoliation surmounts cell proliferation, mucosal transformation is taking place. The villi become shorter and the crypts become longer. In this process the totally flattened surface with complete loss of villi, maximal elongation of crypts and high numbers of mitotic cells in the germinative compartment represents an endpoint of mucosal transformation.

In contrast with mucosal hypertrophy and hypotrophy, the surface cells in the severely transformed mucosa exhibit clear cut features of damage: they are flattened, the fine structure is severely altered with irregular and sparse microvilli, increased lysosomal bodies and activated endoplasmatic reticulum.
Functionally, there is a reduction of brush border enzyme activities and sodium dependent D-glucose accumulation in brush border vesicles is distinctly decreased. According to kinetic analysis in short term incubation experiments, this decrease is the result of a reduction in the $V_{\text{max}}$, indicating that the number of transport proteins in the brush border of absorptive cells in the transformed mucosa is reduced.

Electrical measurements in the experimental blind loop of rat jejunum provided an increased epithelial resistance, which was paralleled on freeze fracture electron microscopy by an increase in total tight junctional depth (more pronounced in cells of the crypt than in the villus), and by an increase in tight junctional strand counts in one of four regions along the villus axis, which showed the most leaky junction, namely in the villus tip. These changes have been interpreted as an adaptive response to reduce back leakage, and absorb electrolytes across the tight junction. In biopsies from patients with untreated coeliac sprue, lateral aberrant strands appeared in surface cells, a change which resembles that seen in the experimental blind loop syndrome.

In addition, strand discontinuities and a more leaky tight junction (decreased strand counts) were observed in coeliac sprue. Both alterations, however, may be attributed to the higher degree of hyper-regenerative transformation, as blind loops showed only a small reduction in villus height, while coeliac sprue patients display flat mucosa. In spite of this, the structural and functional impairments of the absorptive cells glucose absorption correlates closely with the decrease in mucosal surface measured by segmental perfusion studies in man using the triple lumen tube technique.

The clinical significance of mucosal transformation and the hyper-regenerative type is considerable, and there are many conditions in which it is seen, its most pronounced example being coeliac sprue in which most of the studies in man have been performed. Wright et al have calculated from a stathmokinetic model that the cell production rate in the flat jejunum of coeliaics is increased up to 155 cells per crypt and hour, which is six times higher than normal while duration of mitosis remains constant and cell cycle time is reduced from 50 to 20 hours. These figures show the enormous proliferative potential of the mucosa and also the importance of its regulation.

**Regulation of mucosal growth in relation to connective tissue**

Although the connective tissue has not attracted much attention in this respect there is increasing evidence that it is important for proliferation and differentiation of intestinal epithelial cells. Thus, electronmicroscopical analysis of the developing gut in the rat documents that the process of maturation of the epithelium starts after the development of close cell-cell contacts between surface and subepithelial cells of the mesenchyme. In addition, experiments with recombined tissues grafted under the kidney capsule of adult rats have confirmed the existence of strong inductive influences of the intestinal mesenchyme on the epithelium of the esophagus and stomach, which lead to a differentiated intestinal epithelium with regular microvilli and typical intestinal brush border enzymes. Further, myofibroblasts of the duodenal mucosa of sucklings rats can induce ‘intestinalisation’ of undifferentiated gastric epithelial cells. These results are of particular interest in view of the known instability of the gastric epithelial phenotype in intestinal metaplasia. Previous *in vitro* results from our own laboratory also suggested a strong organ specific potential of the fetal intestinal mesenchyme in inducing intestinal epithelial cytodifferentiation.

In the intestinal tract itself the mesenchyme exerts a strong morphogenetic induction on intestinal epithelial cytodifferentiation, including cell lineage into absorptive, goblet, endocrine, and Paneth cells. Using mouse aggregation chimaeras as an experimental model, Ponder et al demonstrated immunohistologically that the epithelium of individual crypts in the small intestine is derived from a single progenitor cell. This single progenitor cell may be divided into several stem cells responsible for cell renewal and differentiation. Intestinal epithelial cell lines, established from fetal rat intestine and resembling in some features progenitor crypt cells, do not show self differentiation *in vitro*. Neither hormones, cytokines, regulatory peptides nor luminal factors induce features of differentiation of these cells. When combined with fetal intestinal mesenchyme, however, these primitive cells will actually form villous structures as well as mucus, endocrine, and Paneth cells.

To gain further insight into the role of the intestinal subepithelial mesenchyme in the instruction of intestinal progenitor cells by distinct mesenchymal cell populations, coculture studies with purified and well characterised mesenchymal cells, that is, myofibroblasts, neural and endocrine cells, vascular cells, cells of the mucosal immune system, and primary fetal intestinal epithelial cells are necessary.

In addition to diffusible molecules and the transmission of signals through cell-cell contacts the possible role of the extracellular matrix in the epithelial-mesenchymal interactions has been stressed by Grobstein. The epithelial mesenchymal interface in the intestinal mucosa implies the
epithelium, the subepithelial basement membrane, and adjacent reticular lamina propria. Basement membranes are ubiquitous extracellular matrices formed from a unique set of macromolecules, including laminin, collagen type IV, nidogen, heparan sulfate proteoglycan, and fibronectin. During intestinal morphogenesis, the deposition of specific extracellular matrix components at the epithelial-mesenchymal interface and the formation of the intact basement membrane have been found to be associated with the cytodifferentiation of the intestinal epithelium. It has recently been documented that the individual basement membrane components have different modifying effects on intestinal epithelial cell adhesion, cell proliferation, and cytodifferentiation. Laminin and nidogen especially promoted cytodifferentiation of fetal intestinal epithelial cells, whereas cell adhesion of intestinal epithelial cells was strongly promoted by fibronectin (own results, submitted for publication).

In addition to results on the importance of interactions between the mesenchyme, the extracellular matrix and the intestinal epithelium in epithelial cell differentiation during organogenesis, some data indicate that the extracellular matrix plays a crucial role in intestinal disease. The restitution of epithelial defects during healing of erosions and ulcers in the gastrointestinal tract is bound to an intact basement membrane on which non-injured, viable epithelial cells proliferate and migrate from the border of the lesions.

Proliferation and adhesion of malignant intestinal epithelial cells may be influenced by cell-surface receptors to basement membrane components (own results, submitted for publication). These cell-surface receptors mediate specific interactions of neoplastic cells with the extracellular matrix and may facilitate tumour invasion and metastasis.

Preliminary results on the immunohistological characterisation of the basement membrane in patients with untreated coeliac disease with mucosal transformation of the hyper-regenerative type did not reveal, however, a pathological distribution and organisation of basement membrane components as compared with the mucosa of normal intestine (U Hahn, personal communication).

The process of intestinal epithelial proliferation and cell migration along the crypt-villus axis which includes maturation and differentiation of the epithelium has been shown to follow distinct regulations and we are only beginning to understand some of its molecular aspects. Of importance in this respect are detailed investigations to identify extracellular matrix protein producing cells, to analyse their matrix protein metabolism and to characterise matrix protein receptors on intestinal epithelial cells along the crypt-villus axis. This includes also the analysis of their receptor gene expression in the normal small intestine and in the different forms of mucosal transformation in response to stress and damage.

**Mucosal growth and its relation to the gut associated lymphoid system**

The possible role of the gut associated lymphoid tissue in the regulation of mucosal growth is derived mainly from two pieces of evidence. First, several immune mediated diseases of the gastrointestional tract are accompanied by changes in the architecture of the intestinal mucosa (reviewed in 46). It is therefore likely that local immune reactions might directly influence intestinal epithelial growth hereby leading to mucosal transformation. Second, recent observations suggest that cytokines derived from lymphoid cells are also important regulatory factors for the growth and differentiation of other cells of either epithelial or mesenchymal origin (reviewed in 47). These factors therefore may be of importance in the interaction between lymphoid cells and other cell types in the mucosa. With respect to these observations we will first consider some characteristics of gut associated lymphocytes which may be of importance for their interaction with intestinal epithelial cells. Second, recent studies will be discussed which indicate that mucosal transformation is at least in part mediated by immune mechanisms.

**Characteristics of mucosal lymphocytes**

T cells in the lamina propria, the effector compartment of the intestinal mucosa, have certain characteristics, which distinguish them from other lymphocyte populations in the body. Lamina propria T lymphocytes are predominantly of the helper phenotype and lack the CD45R antigen on their cell surface and therefore have the phenotype of memory T cells. They express certain activation antigens and are able to synthesise high amounts of interleukin 2. After stimulation with antigen, these T cells do not proliferate, but provide helper function for B cells. These T cells therefore can be characterised as differentiated effector cells which respond to antigen stimulation with the production of certain cytokines. Consequently, this population of T cells may be of special importance in the interaction with epithelial cells and therefore in the maintenance of the mucosal structure.

**Immune mediated mucosal transformation**

Protoypes of experimental models, in which mucosal transformation is thought to be primarily
mediated by immunological mechanisms, are small intestinal allograft rejection and graft-versus-host reaction (GvHR) of the small bowel.6-8 The rejection of grafts of fetal intestine transplanted heterotopically under the kidney capsule of mice is characterised first by infiltration of the mucosa by lymphocytes and increased mitotic activity in the epithelial cells in the crypts, later by flattening of the villi and exfoliation of surface enterocytes, and finally by destruction of the mucosa.9 Several experiments have shown that intestinal allograft rejection represents a local cell mediated immune reaction.10 By using a GvHR model in mice Ferguson and coworkers further investigated the hypothesis that mucosal transformation occurs by direct effect of T cells on the crypts rather than through damage of the villi. The results of these experiments showed that crypt length and crypt cell production rate increased parallel to the development of GvHR; villus length was not reduced, and the number of IEL increased significantly.11-13 Additional investigations by this group14 and by Elson and coworkers15 provided indirect evidence that the effects on the crypts in GvHR were caused by soluble T cell products rather than by direct T cell cytotoxicity.

The characteristic mucosal collagen deposition in chronic GvH disease of the intestine may also be mediated by T cell derived cytokines; supernatants from clones of T cells from mice with acute GvHR caused fibroblasts to proliferate in vitro, and supernatant of chronic GvH disease clones stimulated an increase in collagen production per fibroblast.16-18 These experiments strengthen the hypothesis that T cell derived cytokines play a central role in the interaction between the different cellular components in mucosal transformation.

The mucosal transformation seen in allograft rejection and intestinal GvHR resembles hyper-regenerative transformation. As discussed above mucosal transformation of the hyper-regenerative type is a hallmark of coeliac disease and the question if coeliac disease is caused by direct damage of the mucosal epithelium by gliadin or by immune mediated mechanisms is not yet clarified. Strong support for this hypothesis is provided by organ culture studies of intestinal biopsies from coeliac disease patients: biopsies from patients with active disease under a gliadin containing diet and biopsies from patients with ‘inactive’ disease – that is, normal appearing mucosa – under a gluten free diet were cultured in vitro with and without gliadin. A toxic effect of gliadin on mucosal morphology and brush border enzyme activity was only observed on biopsies of patients with active disease.19-22 These studies indicate that tissue of coeliac disease patients does not manifest gliadin induced tissue injury in vitro unless tissue is pre-exposed to gliadin in vivo. The activation of an endogeneous mechanism of toxicity thus has to be postulated before gliadin may exert its damaging effect. This endogeneous mechanism most likely consists of an immunological reaction. Coeliac disease therefore most likely is another example of immune mediated mucosal transformation of the hyper-regenerative type.

Of importance in this context is the finding that patients with coeliac disease may become refractory to a gluten free diet after developing an intestinal lymphoma. Intestinal lymphomas in coeliac disease have recently been shown to be T cell lymphomas most likely arising from IEL.23,24 In addition, these lymphomas express certain activation antigens. The activated lymphoma T cells therefore may mediate the toxic effect on the intestinal epithelium by producing certain cytokins leading to a refractory sprue syndrome.

More direct evidence that activated mucosal T cells are able to induce mucosal transformation of the sprue type is given in a recent study by MacDonald and Spencer.25 These authors used explants from human fetal intestine in organ culture. These intestinal explants were then cultured with certain substances (pokeweed mitogen and anti-CD3 monoclonal antibody) to activate mucosal T cells. Parallel to the appearance of activated T cells in the lamina propria (measured by the expression of interleukin-2 receptors) an increase in proliferating crypt epithelial cells and a reduction in villus height occurred. Addition of cyclosporin A, a potent inhibitor of T cell activation, abrogated the effects on mucosal transformation. These experiments show that activation of lamina propria T cells induces hyper-regenerative transformation of the small intestinal mucosa.

In the acquired immunodeficiency syndrome (AIDS) gastrointestinal manifestations are very frequent and clinical studies suggest the occurrence of small intestinal dysfunction.26 As mentioned above, intestinal lamina propria T cells are predominantly of the helper phenotype and are more activated than T cells in other sites. These characteristics render these cells highly susceptible to HIV-infection. In fact, HIV-infected lamina propria mononuclear cells have been shown. In addition, there are indications of small intestinal atrophy with reduced mitotic rate in the crypts in patients with AIDS, especially in patients with mucosal HIV-infected mononuclear cells (own results, submitted for publication). It is possible that infection of regulatory mucosal T cells by HIV with concurrent lymphocyte dysfunction is responsible for the atrophic mucosal changes. Mucosal HIV-infection might therefore be an example for an immune mediated alteration which
leads to mucosal atrophy, although the proof for this hypothesis is lacking.

When summarising these different findings it can be stated that, in spite of the lack of systematic studies on the different patterns of intestinal mucosal transformation, several experimental models and clinical findings indicate an influence of the local lymphoid tissue on mucosal structure and function. Specialised regulatory T cells in the intestinal lamina propria may influence epithelial growth and differentiation as well as connective tissue metabolism by the production of cytokines. So far the evidence for this interaction is indirect, however, and to dissect the mechanisms of immune mediated mucosal transformation, better experimental models are needed. Special emphasis should be given to the characterisation of cytokines produced by intestinal T cells which may be responsible for mucosal transformation.

Regulation of mucosal growth in relation to gastrointestinal regulatory peptides

In vitro and in vivo studies have provided considerable information on the possible physiological function of circulating gastrointestinal hormones as well as locally acting regulatory peptides in the multifactorial control of adaptive gastrointestinal epithelial cell proliferation and cell renewal.\textsuperscript{16-71} At the present time these studies suggest that gastrin may act as a growth promoting factor in the stomach and possibly also, although less likely, in the small intestine and colon.\textsuperscript{72-77} Furthermore, it has been shown that epidermal growth factor (EGF), mainly produced in the salivary glands and Brunner’s glands of the duodenum of man and the rat, may have local and systemic proliferative effects on the intestinal epithelium.\textsuperscript{78} Whereas bombesin and growth hormone releasing factor (somatocrin) have been reported to stimulate intestinal cell proliferation, somatostatin may act as a growth inhibiting factor in the gastrointestinal tract.\textsuperscript{79,84}

Enteroglucagon (EG) (gut glucagonlike immunoreactivity I; gut GLI-I) is a peptide of 69 amino acids which has been shown to be part of a larger pre-glucagon molecule being produced by so-called L-cells located predominantly in the distal small intestine and colon.\textsuperscript{85-90} It contains the entire sequence of pancreatic glucagon linked by two pairs of basic dipeptides, lysin-arginin, at its N-terminus to a glicentin-related pancreatic peptide of 30 amino acids (GRPP) and at its C-terminal end to a hexapeptide.\textsuperscript{91-95} The physiological role of gut GLI-I has not yet been fully elucidated. It has been suggested by circumstantial evidences that gut GLI-I may act as a trophic factor on the intestinal mucosa which may account for adaptive changes of the small intestine after various stimuli.\textsuperscript{96-98} An enteroglucagon secreting tumour was associated with increased height of the villi and increased mucosal thickness which was reversed after tumour removal.\textsuperscript{99,100} Subsequently, raised circulating concentrations of enteroglucagon have been reported in various clinical conditions and experimental animal models being associated with an increase in the intestinal epithelial cell turnover which all had in common that they were characterised by an increased food supply to the intestine.\textsuperscript{101} Especially the adaptive hyperplastic response of ileal remnants after proximal bowel resection are paralleled by an increase in plasma and tissue concentrations of enteroglucagon both showing a close relationship to the extent of the small intestine being resected.\textsuperscript{102}

In order to gain additional insight into the putative role gut GLI-I of acting as an enterothrophic regulatory peptide we investigated two different experimental conditions which are known to induce intestinal hyperplasia not being associated with an increase in the nutritional load of the intestine: (a) germ free rats were conventionalised with a thermoduric flora and were held thereafter as open conventional animals. Four weeks after conventionalisation the ileal mucosa exhibited a significant increase of mitotic activity and crypt length accompanied by unchanged basal EG plasma concentrations and decreased tissue levels of EG in the contaminated group. (b) Jejunal self-filling blind loops were surgically created in germ free rats. Three weeks postoperatively there was a significant increase in mitotic activity, crypt length and villus surface area in the jejunal self-filling blind loops when compared to unoperated germ free control rats. Again the basal plasma EG concentrations remained unchanged (submitted for publication).

These two particular experiments show that there are situations in which intestinal hyperplasia does not correspond to an increase in the concentrations of EG in plasma or intestinal mucosa. This implies that in conditions which are not or only to a limited extent accompanied by an increase in luminal nutrition other factors than EG have to be responsible for the regulation of the hyperplastic adaptation of the small intestinal mucosa.

In a further experiment we investigated the effects of longterm in vivo immunonutralisation of endogenous circulating gut GLI-I by intravenous and continuous intraperitoneal administration of newly developed monoclonal antibodies to gut GLI-I on the hyperplastic ileal response of the ileal remnant after a 70% proximal small bowel resection.\textsuperscript{103,104} A group of rats was given either antibody free plasmocytes ascites or undiluted hybridoma ascites for 14 days after the operation. The hybridoma ascites was
prepared from the clone 23.6B4 synthesising a monoclonal antibody directed toward the N-terminal to the central region of the glucagon molecule which represents the immunoreactant common to all gut GLIs and glucagon in different species such as man, rat, and pig. The three dimensional architecture and the proliferative activity of the ileal remnant were evaluated two weeks postoperatively. Despite a continuous immunounneutralisation of circulating endogenous gut GLI-I by monoclonal antibodies there was an adaptive response of the ileal remnants which was of the same magnitude as in the control group but was even greater considering the increased number of mitoses per crypt. These data did not support the hypothesis of gut GLI-I being a circulating enterotrophic regulatory peptide.

An in vitro model was used to investigate the effect of highly purified rat G-GLI I on the proliferative response of primary small intestinal epithelial cells of fetal rats (submitted for publication). Gut GLI I was purified from rat small intestines by gel filtration, high capacity immunoaffinity chromatography using monoclonal antibodies and reverse phase HPLC. Fetal rat intestinal epithelial cells were dissected from the surrounding mesenchyma after collagenase treatment and seeded onto petri dishes previously coated with a complex basement membrane extract (submitted for publication). After the initial attachment phase, the proliferation rate was assessed planimetrically, correlating with the mitotic index, before and after the addition of highly purified G-GLI I in various concentrations as well as EGF. Whereas there was a well known growth promoting action of EGF the proliferation of rat fetal intestinal epithelial cells was inhibited by the addition of purified gut GLI-I.

These results indicate that gut GLI-I does not act as an enterotrophic factor but provide the first direct evidence consistent with an antitrophic role of gut GLI-I in the small intestine. They do not, however, rule out a paracrine enterotrophic action of gut GLI-I in vivo controlling cell proliferation by a synergistic interaction among gut GLI-I and other still unknown growth factors. Confirmation of our studies must await the isolation and purification of sufficient quantities of gut GLI-I for in vivo infusion experiments.

Abteilung für Innere Medizin, mit Schwerpunkt Gastroenterologie, Klinikum Sieglitz der Freien Universität, Hindenburgdamm 30, D-1000 Berlin-45, Fed Rep Germany

References
2 Riecken EO. Derangements of mucosal growth and differentiation in small-intestinal diseases. Scand J Gastroenterol 1988; 23 [suppl 151]: 86-93
8 Parker FG, Barnes KGJ. The pericryptal fibroblast sheath. IV. Replication migration and differentiation of the subepithelial fibroblasts of the crypts and villi of the rabbit jejunum. Gastroenterology 1974; 67: 622-35
12 Weser E, Hernandez MH. Studies of small bowel adaptation after intestinal resection in the rat. Gastroenterology 1971; 60: 69-75
14 Menge H, Murer H, Robinson JW, Glucose transport by brush-border membrane vesicles after proximal resection or ileo-jejuninal transposition in the rat. J Physiol (Lond) 1978; 274: 9-16
16 Chaves M, Smith MW, Williamson RCN. Increased activity of digestive enzymes in ileal enterocytes
39 Stallmach A, Hahn U, Merker HJ, Hahn EG, Riecken EO. Differentiation of rat intestinal epithelial cells is induced by organotypic mesenchymal cells in vitro. Gut (In press)
48 Zeitz M, James SP. The role of IL-2, IL-2 receptors and...


50 Zeitz M, Greene WC, Peffer NJ, James SP. Lymphocytes isolated from the intestinal lamina propria of normal nonhuman primates have increased expression of genes associated with T cell activation. Gastroenterology 1988; **94**: 647–55.

51 Zeitz M, Quinn TC, Graeff AS, James SP. Mucosal T cells provide helper function but do not proliferate when stimulated by specific antigen in Lymphogranuloma venereum proctitis in nonhuman primates. Gastroenterology 1988; **94**: 553–66.


55 Mowat AMcL, Ferguson A. Hypersensitivity reactions in the small intestinal mucosa of the mouse. 6. Pathogenesis of the graft-versus-host reaction in the small bowel mucosa. Transplantation 1981; **32**: 238–43.


66 Laplace JP. Intestinal resorption in chronically blood-crossed twin pigs: blood carried factor(s). Digestion 1974; **10**: 229–32.


70 Williamson RCN, Bucholtz TW, Malt RA. Humoral stimulation of cell proliferation in small bowel after transplantation and resection in rats. Gastroenterology 1978; **75**: 249–54.


72 Crean GP, Gunn AA, Rumsceym RDE. Parietal cell Hyperplasia of the gastric mucosa induced by the administration of pentagastrin to rats. Gastroenterology 1969; **57**: 147–55.


75 Mayston DD, Barrowman JA, Dowling RH. Effect of pentagastrin on small bowel structure and function in the rats. Digestion 1975; **12**: 78–84.


80 Lehy T, Dubrasquet M, Bonfils S. Effect of somato-


