The increase in the aging population has led to a growing interest in achieving a better understanding of the aging process and of diseases that are predominantly expressed during advancing age. Since the structural and, in turn, the functional integrity of the mucosa of the gastrointestinal tract (GI) are maintained by constant renewal of cells, a detailed knowledge of the events that initiate and regulate mucosal proliferative processes is essential for a better understanding of the normal aging process as well as age-associated dysfunctions, including malignancy that represent disorders of tissue growth. In Fischer-344 rats, aging is associated with increased mucosal proliferative activity in much of the GI tract. On the other hand, the functional properties are either decreased or remain unchanged during advancing age. Basal gastric acid and pepsin output decline during aging, as is gastrin secretion. In contrast, antral gastrin levels increase during this period, as is mucosal histidine decarboxylase activity. The age-related decline in gastrin secretion could partly be attributed to a higher ratio of somatostatin (D) to gastrin (G) cells in the antral mucosa. The age-related rise in GI mucosal proliferative activity could not be attributed to the trophic action of either gastrin or bombesin, since they caused no significant change in mucosal proliferation in aged rats. On the other hand, EGF and TGF-α appear to be involved in regulating mucosal proliferation during aging. Aging is associated with increased activation of EGF-receptor (EGFR), the common receptor for EGF and TGF-α. This could be due to (a) increased levels of membrane-bound precursor form(s) of TGF-α resulting in increased activation EGFR signaling processes through an autocrine/paracrine mechanism, (b) heightened sensitivity of mucosal EGFR to EGF and TGF-α such that comparatively lower levels of these peptides are required to activate EGFR in aged than in young animals and/or (c) loss of EGFR regulatory factor(s) such as ERRP (EGFR Related Protein), a "negative regulator" of EGFR.

Key words: Aging, mucosal proliferation, gastrin, bombesin, EGF, TGF-α, malignancy
Aging has been defined as the progressive accumulation of changes with time which are associated with or are responsible for ever-increasing susceptibility to disease and death, the final event of age. However, different tissues do not age at the same rate. Whereas the growth many organs ceases with adolescence, mucosa of the gastrointestinal (GI) tract is maintained by continuous cell renewal. Any deviation in these replicative processes may result in a loss of not only structural but also functional integrity. A detailed knowledge of mucosal cell proliferation and kinetics should increase our understanding of the normal aging process as well as many gastrointestinal diseases that represent disorders of tissue growth (2-8). In general, the incidence of many gastrointestinal dysfunctions, including malignancy increases with advancing age, which in itself is associated with alterations in the structural and functional integrity of the gastrointestinal tract (9, 10).

**Maintenance of Mucosal Structural Integrity:** Since the structural and, in turn, the functional integrity of the mucosa of various parts of the gastrointestinal tract are maintained by constant renewal of cells, a number of laboratories, including our own, have studied the age-related changes in gastrointestinal mucosal cell proliferation and the regulation of this process at different stages of life. We have reported that gastric mucosal proliferative activity in rats remains elevated during the first two weeks of postnatal life then decreases dramatically over the next 2-3 weeks (13). Although earlier observations in the mouse suggest that proliferative activity of the small intestine either decreases (14,15) or remains unchanged (16) with aging, recent morphologic as well as biochemical studies from this and other laboratories indicate that in Fischer-344 rats, aging is associated with increased gastric, small and large intestinal mucosal proliferative activity (11,16-23). Although the regulatory mechanisms have not been fully elucidated, in both gastric and colonic mucosa, we have observed that the age-related rise in proliferation is partly the result of enhanced transition from G1 to S phase as well as progression through the S phase of the cell cycle. (24,25). In the gastric mucosa, aging is also associated with increased activation of ERKs and JNK1 MAP kinases and transcriptional activity of AP-1 and NF-κB (26), which are known to be involved in regulating the expression of a variety of genes that participate in growth related processes. However, these changes were not accompanied by increased growth of the organs. Therefore, the reported increased production of crypt cells in the intestine could not be explained by formation of more crypt, which suggests that in the small and large intestine of aged rats, DNA replication occurred without cytokinesis. A similar explanation could also be offered for our observation in the gastric mucosa in which increased mucosal proliferative was not accompanied by a concomitant rise in mucosal growth, but rather aging resulted in atrophy of the tissue, as evidenced by the decreased mucosal height as well as DNA and RNA content in 24-month-old Fischer-344 rats compared with their 4-month-old counterparts (21). Whether this is the result of increased cell loss or block in mitotic or other cell cycle regulatory
events remains to be determined. Interestingly, the age-related rise in gastric and colonic mucosal proliferative activity in Fischer-344 rats was not accompanied by an increase in apoptosis. In fact, the number of gastric and colonic mucosal cell undergoing apoptosis was found to be lower in older animals (27). In view of this, it is tempting to speculate that aberrant survival of a small group of cells or a single cell in the gastric and colonic mucosa during aging could promote accumulation of secondary genetic changes and thereby enhance susceptibility of the tissue to certain carcinogen(s) or tumor promoter(s).

**Regulation of Mucosal Growth:** Gastrointestinal mucosal cell proliferation is known to be under the regulation of a number of nutritional and hormonal factors. In general, deprivation of food results in decreased cell proliferation and refeeding reverses the situation (28). Holt and Yeh (18,20) have compared the effect of fasting and refeeding on small and large intestinal proliferative activity in young (3-4-month old) and aged (24-28-months old) Fischer-344 rats. In the mucosa of both small and large intestine, a 3-day fasting resulted in 40-60% reduction in proliferative activity in young rats, whereas in old rats it was decreased by only 10-20%, when compared with their corresponding fed controls (18,20). Although in both age groups refeeding increased small intestinal proliferative activity, in aged rats this increase was associated with broadening of the proliferative zone (18,20). Moreover, in fasted aged rats, proliferative responsiveness of the large intestine to food was blunted (18-20). The latter observation suggests that nutritional modulation of mucosal cell proliferation is affected by aging.

Age-associated changes in gastrointestinal mucosal cell proliferation could be secondary to alterations in hormonal influences. Over the past 3 decades considerable evidence has appeared to show that a number of gastrointestinal hormones/growth factors, specifically gastrin, bombesin (an amphibian peptide which structurally and functionally analogous to gastrin releasing peptide GRP) and epidermal growth factor (EGF) family of peptides regulate mucosal proliferation in much of the gastrointestinal tract, including the stomach (29,30). However, responsiveness of the gastric mucosa to these peptides changes at different stages of life. We have earlier reported that in rats, the gastric mucosa becomes responsive to the growth-promoting action of gastrin around the 3rd postnatal week of life (13). At this time the parietal cells also become sensitive to gastrin and secrete acid in response to the hormone, when gastrin receptor also appear (31). On the other hand, the functional properties are either decreased or remain unchanged during advancing age. For example, basal gastric acid and pepsin output decline during aging, as is gastrin secretion (23,32). In contrast, antral gastrin levels increase during this period, as is mucosal histidine decarboxylase activity (23,32). The age-related decline in gastrin secretion could partly be attributed to a higher ratio of somatostatin (D) to gastrin (G) cells in the antral mucosa (23). With aging there is also a progressive loss of gastric mucosal responsiveness to both acid secretory (32) and growth promoting (23) actions of
gastrin. Although the underlying mechanisms are unknown, one possibility could be the loss of functional receptors of gastrin. Singh et al. (33) reported that the number of gastrin binding sites in the gastric mucosa of 24-months old rats were lower than in their 3- and 6-months old counterparts. Taken together, the results suggest a relationship between gastrin receptor populations and gastric mucosal responsiveness to gastrin.

Like gastrin, bombesin has also been shown to stimulate gastric mucosal cell proliferation in young animals (34-36). To evaluate its role in gastrointestinal mucosal cell proliferation during aging, we examined changes in gastric mucosal DNA synthesis and ornithine decarboxylase (ODC) activity in young (4-months old) and aged (24 months old) rats following continuous infusion of bombesin (300 ng/kg/h) for 2 weeks. We observed that whereas in 4-months old rats bombesin infusion resulted in a significant 2-fold increase in gastric mucosal DNA synthesis and ODC activity, in aged rats the peptide had no effect (37). Whether the lack of response of aged gastric mucosa to the growth-promoting effect of bombesin could partly be the result of loss of receptors of the peptide is unknown.

EGF family of peptides, specifically EGF and TGF-α are known to regulate gastrointestinal mucosal cell proliferation (29). Feldman et al. (38) were the first to demonstrate that administration of EGF to suckling mice stimulates gastric and duodenal mucosal ODC activity. Subsequently, Malo and Menard (39) reported that EGF not only stimulates growth of the proximal but also of distal small intestine. Similar observations were also made us and others (40-43). Using an organ culture system, we have demonstrated that EGF stimulates protein and DNA synthesis in both pre- and neonatal rats (40) and ODC activity in the colonic mucosa of young mature rats (41). These observations not only indicate a direct growth-promoting effect of EGF on both small and large intestinal mucosa, but also shows that EGF may have a role in regulating mucosal growth from prenatal to adulthood. Gastric mucosa also responds to the growth-promoting action of EGF. Dembinski and Johnson (43) reported that in 10-day old suckling rats, repeated injections of EGF for 5 days result in a significant increase in gastric mucosal DNA, RNA and protein content. We have further demonstrated that in unweaned rats, daily injections of EGF at a dose of 20 µg/kg for 7 days significantly stimulate both gastric and small intestinal protein and nucleic acid content (23). Repeated injections of EGF for 2 days have also shown to stimulate gastric mucosal DNA synthesis in young mature rats (22,44). More recently, we examined the responsiveness of gastric mucosa of 4- and 24-months old Fischer-344 rats to TGF-α, the structural and functional analogue of EGF (44). We observed that pharmacological doses of TGF-α, which stimulated gastric mucosal proliferative activity in 4-months old rats, caused a marked inhibition of the same in their 24-months old counterparts (44). We postulated that this inhibition is in part be due to increased sensitivity of aged gastric mucosa to these peptides such that low doses of these peptides are stimulatory, whereas high doses inhibit
proliferative processes (45). In support of this postulation we have observed that the concentration of TGF-α needed to induce maximal stimulation in EGF-receptor tyrosine kinase activity in gastric mucosal membrane preparations from aged rats is considerably lower than that required for the same induction in young rats (45).

In evaluating the age-related rise in gastrointestinal mucosal proliferative activity, we have focused on the role of tyrosine kinases, which catalyze phosphorylation of tyrosine residues in proteins and play a crucial role in regulating proliferation, differentiation and transformation of cells (46,47). The notion that tyrosine kinases may play a role in regulating gastrointestinal mucosal cell proliferation comes from our earlier observation that the age-related rise in gastric mucosal proliferative activity is also accompanied by a parallel increase in overall mucosal tyrosine kinase activity and tyrosine phosphorylation of several membrane proteins (21,48).

However, tyrosine kinases are associated with the products of many protooncogenes as well as with receptors of several growth factors, including the EGF-receptor (EGFR), the common receptor for EGF and TGF-α (46,47), which is a 170 kDa transmembrane glycoprotein that spans the plasma membrane. It contains an amino-terminal extracellular ligand-binding domain, a single transmembrane-anchoring region, and a carboxyl-terminal intracellular domain that has tyrosine kinase activity (49). High affinity binding sites for EGF-family of peptides, specifically EGF and TGF-α have been detected in much of the gastrointestinal tract, with highest concentrations in the esophagus, stomach, and colon (50-53). Moreover, mRNAs for TGF-α as well as its receptor have been detected in gastric and colonic mucosal cells (54-56). Overexpression of EGFR with increased tyrosine kinase activity has been associated with many human malignancies, including gastric and colonic cancers (57-59). Results from this laboratory have demonstrated that activation of EGFR tyrosine kinase is associated with increased gastrointestinal mucosal proliferative activity. We have reported that increased mucosal proliferative activity in the colon in response to azoxymethane (a colonic carcinogen) and in the gastric mucosa after injury is accompanied by a concomitant rise in EGFR tyrosine kinase activity and that these increases can be greatly attenuated by prior administration of tyrphostin, an inhibitor of EGFR tyrosine kinase (60,61). These and other relevant observations suggest a role for EGFR tyrosine kinase in gastrointestinal mucosal cell proliferation.

We have reported that age-related rise in gastric and colonic mucosal cell proliferation is accompanied by a marked rise in expression and activation of several tyrosine kinases, including EGFR (62-64). Aging is also found to be associated with activation of EGFR in the gastric and colonic mucosa, as evidenced by increased tyrosine phosphorylation of EGFR (65). Ligand binding is one of the primary causes for activation of intrinsic tyrosine kinase activity of EGFR triggering a complex array of enzymatic and biological events leading to
stimulation in cell proliferation. There is considerable evidence, which show that TGF-α, which is synthesized in mucosa of much of the gastrointestinal tract including the stomach and colon, may play a key role in regulating EGFR tyrosine kinase activity in different patho-physiological conditions. For example, cell lines derived from adenocarcinomas of the colon express both TGF-α and EGFR (57-59). Our observation that the azoxymethane-induced colonic mucosal EGFR tyrosine kinase activity is accompanied by a parallel rise in synthesis and secretion of TGF-α (60) suggests that TGF-α may partly be responsible for regulating this process through an autocrine/paracrine mechanism. However, results from cell surface and biochemical studies have demonstrated that the presence of the transmembrane TGF-α is a normal consequence of TGF-α synthesis, and in most cases the peptide is present on the cell surface in its precursor form, which can also activate EGFR (66-68). We have observed that the age-related rise in EGFR tyrosine kinase in the gastric mucosa is accompanied by a marked rise in 16-20 kDa precursor forms of TGF-α in the mucosal membrane fraction, but not in the cytosol (64). The colonic mucosa also shows a similar phenomenon (65). In addition, our recent in vitro studies show that the membrane-bound precursor form(s) of TGF-α from the gastric and colonic mucosa of aged rats induces a greater stimulation in EGFR tyrosine kinase activity than those from young rats (65). This could partly be attributed to a greater amount of TGF-α bound to its receptor, the EGFR (65). However, since TGF-α, but not EGF, is synthesized in much of the gastrointestinal mucosa of normal adult rats (60,66,69), we postulate that TGF-α plays a key role in regulating gastric and colonic mucosa proliferation during aging, probably through an autocrine/paracrine mechanism. Recent results from our laboratory also suggest that aging enhances sensitivity of the gastrointestinal mucosa to both EGF and TGF-α such low doses of the peptide stimulate the EGFR signaling cascades leading to stimulation in proliferation, whereas high doses inhibit these processes. This inhibition is found to be due to enhanced internalization of the ligand-receptor complex (45). Likewise, responsiveness of the colonic mucosa to both EGF and TGF-α is also augmented after azoxymethane treatment (60,70).

Although the precise regulatory mechanisms for the age-related rise in EGFR activation in the mucosa of the gastrointestinal tract is unknown, our recent data suggest this could partly be the result of loss of ERRP (EGFR Related Peptide), a novel "negative regulator", which we have identified and characterized (71). ERRP, a ~55 kDa secretory protein, possesses approximately 90% homology to the external ligand binding domain of EGFR (71). Expression of ERRP is high in benign human colon, stomach and pancreas but low in the respective invasive adenocarcinomas (71,72). We reported that ERRP inhibits cellular growth by attenuating EGFR signaling processes (71,72). Overexpression of ERRP in colon cancer cell lines, HCT-116 and Caco2, or exposure of the cell lines to recombinant ERRP inhibits EGFR activation as well as proliferation (71,72). In addition, intratumoral or subcutaneous (away from the tumor site) injections of
recombinant ERRP cause regression of palpable tumors in SCID mice xenograft of HCT-116 colon cancer cells (72). Since aging as well as carcinogenesis of gastrointestinal tissues are associated with increased expression and activation of EGFR, we hypothesize that these increases could partly be the result of loss of ERRP, which we believe to be a suppressor of EGFR function. To test this hypothesis, we examined the relationship between age-related changes in EGFR activation and expression of ERRP in the gastric mucosa of rats. Indeed, we observed that in the gastric muosa of Fischer-344 rats, the age-related rise in EGFR activation was associated with a marked reduction ERRP levels (73). The same phenomenon is also now noted in the colon, where the levels of ERRP in both proximal and distal colon of the Fischer-344 rats are found to be lower in aged (21 months old) than in young (6 months old) animals (unpublished observation). Since ERRP possesses a substantial homology to the ligand binding extracellular domain of EGFR, we hypothesize that ERRP may compete with EGFR for the ligands. To test this hypothesis, we analyzed the amount of TGF-α bound to EGFR and ERRP in the gastric mucosa of 6- and 24-months old Fischer-344 rats. We observed the amount of TGF-α bound to ERRP was about 50% lower in the gastric mucosa of aged than in young rats (23). In contrast, the amount of TGF-α bound to EGFR in the gastric mucosa of aged rats was found to be about 70% higher in aged than in young rats (73). These changes were accompanied by parallel alterations in EGFR and ERRP levels (73). We suggested that the higher amount of TGF-α bound to EGFR in the gastric mucosa of aged rats could be the consequence of a greater availability of TGF-α resulting from decreased amount of TGF-α bound to ERRP. The age-related decline in ERRP levels together with a concomitant reduction in the amount of precursor forms of TGF-α bound to ERRP suggests that increased activation of EGFR, observed in the gastrointestinal mucosa of aged rats, could partly be due to a greater availability of TGF-α and possibly other ligands of EGFR resulting from the loss of ERRP.

Aging and Gastrointestinal Carcinogenesis: One of the most consistent pathological observations in senescent animals is increased incidence of many types of malignancies, including gastric and colorectal cancers. Gastric cancer rarely occurs before the age of 40 years, but its incidence increases subsequently with peak incidence occurring in the seventh decade. Many probable reasons including altered carcinogen metabolism and long-term exposure of cancer-causing agents have been offered for the age-dependent rise in malignancies (74).

Carcinogenesis, which is a multi-step process, results from the accumulation of mutations during progression from normal epithelium to carcinoma (75). Genetic changes that occur at different stages of epithelial cell carcinoma have been extensively studied by Vogelstein and his colleagues in human colon cancer (75). At least for colon cancer, it has been suggested that the loss or inactivation of the tumor suppressor gene APC (adenomatous polyposis coli) initiates genomic instability that may produce phenotypic appearance of an adenoma.
The advanced tumors, however, possesses mutations and/or deletion of a number of oncogenes and tumor-suppressor genes not seen in early adenoma (75). Although such detailed analysis of genetic alterations has not been performed for gastric cancer, inactivation of several tumor suppressor genes, including APC, p53 and DCC (deleted in colorectal cancer) has been observed in gastric cancer (76). However, little information is available whether aging, which is thought to predispose the gastrointestinal tract to carcinogenesis, is associated with increased inactivation of tumor suppressor genes. We have recently demonstrated that in humans, the incidence of mutations of several tumor suppressor genes, specifically APC, DCC and p53 in the gastric mucosa is higher in older subjects (77).

Hyperproliferation is considered central to the initiation of carcinogenesis in the gastrointestinal tract (78). Activation of certain growth factor receptors signal transduction pathways, including that of the EGFR, is also evident in both preneoplastic neoplastic lesions (60,70). In the gastrointestinal tract, a positive relationship between the hyperproliferative state and increased tyrosine kinase activity have been demonstrated in various premalignant and malignant lesions (79-81). We have observed that aging is not only associated with increased tyrosine kinase activity of EGF-R but also pp60c-src, a non-receptor tyrosine kinase, in the gastric mucosa of Fischer-344 rats (62). This, together with the observation of increased incidence of inactivation of a number of tumor suppressor genes and increased mucosal proliferative activity of the stomach in the aged suggests that aging may predispose the stomach to carcinogenesis. Moreover, the fact that the age-related rise in gastric and colonic mucosal proliferation is also accompanied by a concomitant reduction in apoptosis (27,82) raises the possibility whether aberrant survival of a small group of cells or a single cell could increase the susceptibility of the gastrointestinal mucosa to certain carcinogen(s) or tumor promoter(s) that may initiate the process of carcinogenesis of the gastrointestinal tract.

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