REVIEW

Changes in the Luminal Environment of the Colonic Epithelial Cells and Physiopathological Consequences

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Evidence, mostly from experimental models, has accumulated, indicating that modifications of bacterial metabolite concentrations in the large intestine luminal content, notably after changes in the dietary composition, may have important beneficial or deleterious consequences for the colonic epithelial cell metabolism and physiology in terms of mitochondrial energy metabolism, reactive oxygen species production, gene expression, DNA integrity, proliferation, and viability. Recent data suggest that for some bacterial metabolites, like hydrogen sulfide and butyrate, the extent of their oxidation in colonocytes affects their capacity to modulate gene expression in these cells. Modifications of the luminal bacterial metabolite concentrations may, in addition, affect the colonic pH and osmolarity, which are known to affect colonocyte biology per se. Although the colonic epithelium appears able to face, up to some extent, changes in its luminal environment, notably by developing a metabolic adaptive response, some of these modifications may likely affect the homeostatic process of colonic epithelium renewal and the epithelial barrier function. The contribution of major changes in the colonocyte luminal environment in pathological processes, like mucosal inflammation, preneoplasia, and neoplasia, although suggested by several studies, remains to be precisely evaluated, particularly in a long-term perspective. (*Am J Pathol* 2017, 187: 476–486; <http://dx.doi.org/10.1016/j.ajpath.2016.11.015>)

The colonic epithelium is a dynamic structure with a rate of renewal among the fastest in the body. This renewal is made possible through a lifelong and highly coordinated sequence of events starting with mitosis of pluripotent stem cells¹ and ending with anoikis of fully mature cells in the colonic lumen.² The colonic epithelium, which is organized in crypts separated by a flat surface, is notably responsible for the physiological function of water and electrolyte absorption from the lumen to the bloodstream. This corresponds to the polarized structure of absorptive colonocytes, which are facing the luminal content. This latter is a complex and not fully characterized mixture of water, bacteria, undigested or partially digested dietary/endogenous compounds, and bacterial metabolites. The colonic epithelium can be viewed as a selective barrier in close proximity with this intestinal luminal content.³ Occasionally, the homeostatic processes

of colonic epithelial self-renewing and/or epithelium normal functions are disrupted; and such alterations may be associated with different colonic physiopathological states, including diarrhea,⁴ mucosal inflammation, and preneoplastic/neoplastic lesions.⁵ The objectives of this article are: i) to illustrate the impact of the macronutrient composition of the diet on the characteristics of the large intestine luminal contents using typical examples, ii) to give an overview on the effects of changes of the luminal environment on colonic epithelial cell physiology and metabolism, with a focus on the effects of bacterial metabolites, and iii) to examine to what extent such changes, above the

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adaptive capacity of colonocytes, may have significant consequences for the gut health.

Regarding the recognition of microbial ligands by intestinal epithelial cells, and the effects of bacterial toxins (eg, colibactin, *Bacteroides fragilis* toxin, and fragilysin) on colonic epithelial cells, the readers are invited to refer to recent reviews^{3,6} because these important aspects will not be presented herein.

Effects of the Diet on the Luminal Environment of the Large Intestine

In the colon, bacteria metabolize undigested or partially digested substrates available from endogenous (digestive secretions, exfoliated cells, and mucins) and dietary sources.⁷ Human dietary intervention studies revealed that the macronutrient composition of the diet can affect the amount of metabolites produced by the microbiota in the large intestine. In fact, dietary undigestible polysaccharides increase the fecal concentration of short-chain fatty acids,⁸ whereas high-fat diets increase bile acid secretion in the small intestine that leads to high fecal concentrations of microbiota-derived secondary bile acids, such as deoxycholic acid.⁹ High-protein diets increase fecal concentrations of amino acid–derived bacterial metabolites, such as branched-chain fatty acids, phenolic compounds, hydrogen sulfide, and ammonia.¹⁰

Colonic luminal pH may also be modified by dietary changes. However, this latter parameter is not easily accessible in humans, and most values are recovered from measurement of pH in animal models. For instance, a high-protein diet, in association with increased ammonia concentration, increases the pH in the rat colon content after 2 days.¹¹ In humans, most of the data referred to the effects of dietary changes on fecal pH, which reflects the pH in the distal part of the large intestine (ie, rectum). Ingestion of nondigestible carbohydrates decreases the fecal pH in adults,¹² whereas a high-protein and low carbohydrate diet will result in an increase.¹³

Collectively, these studies provide strong evidence that the human rectal environment (thus inferred from feces analyses) is dependent on the macronutrient composition of the diet.

Briefly, regarding the composition of the colonic microbiota, this complex parameter, although considered as globally stable at the individual level,¹⁴ can be rapidly affected by environmental modifications, notably from dietary origin.¹⁵ Concerning luminal substrate availability, there is evidence that this parameter plays a major role in determining the rate of production of bacterial metabolites.^{6,16,17}

Modified luminal bacterial metabolite composition in inflammatory bowel diseases has previously been reported.¹⁸ However, it is often difficult to determine whether such modifications are causes and/or consequences of these diseases. In the same line of thinking, although intestinal microbiota dysbiosis has been reported in colorectal cancers,¹⁹ and role of selected bacteria has been

anticipated,²⁰ it remains a hard task to determine what are the respective causal links between the bacterial composition/bacterial metabolic capacity/bacterial metabolite composition and the related pathophysiological situations.

In this overall complicated context, we review herein the effects of individual changes in the luminal environment (bacterial metabolite composition, pH, and osmolarity) on colonic epithelial cells and their potential implications in several digestive diseases.

Bacterial Metabolites and Colonic Epithelium Energy Metabolism

The gastrointestinal tract consumes as much as 20% of the whole body oxygen consumption, although it represents approximately 5% of total body weight.²¹ Postprandial hyperemia is concomitant with enhanced intestinal oxygen uptake from the arterial blood, presumably to fuel the digestive and absorptive processes.²² In contrast with the arterial partial pressure of oxygen, this latter parameter in the cecal luminal content is extremely low, not exceeding 1 mmHg.²³ However, oxygen from the host intestinal tissue can diffuse into the lumen, resulting in a radial gradient of oxygen-tolerant bacteria from the tissue to the lumen.

In the gastrointestinal tract, the intestinal epithelial cells are characterized by high energy demand, notably because of the rapid renewal of the epithelium and associated anabolic metabolism, and the activity of Na/K ATPase that allows sodium extrusion at the basolateral membranes.²¹

Modification of the partial pressure of oxygen inside colonocytes may affect their metabolism and physiology.²⁴ In active inflammatory bowel diseases, a metabolic shift toward hypoxia is observed, serving as an endogenous alarm signal in colonocytes.²⁵ Interestingly, when epithelial cells in the colon are challenged by hypoxia, up-regulation of the proto-oncogenes *c-fos* and *c-jun* is observed,²⁶ reinforcing the view that hypoxia acts in colonocytes on genes related to important physiopathological functions.²⁷

Absorptive colonic epithelial cells use fuel substrates from both luminal and blood origin. Fuels from arterial origin are mainly L-glutamine, L-glutamate, L-aspartate, and D-glucose. Short-chain fatty acids (namely, acetate, propionate, and butyrate) are major luminal fuels for colonocytes. They are produced by the microbiota from undigestible carbohydrates²⁸ and several amino acids originating from undigested proteins.²⁹ Among short-chain fatty acids, butyrate is characterized by its capacity to inhibit endogenous substrate oxidation.³⁰ Data showing that butyrate uptake is impaired in inflamed colonic mucosa suggest that the reduction of butyrate availability may decrease the physiological functions of this short-chain fatty acid in colonocytes.³¹ In addition to acting as a luminal fuel in colonocytes, butyrate also acts as a regulator of gene expression in colonocytes³² (Figure 1). Mitochondrial metabolism of butyrate in colonocytes is likely a way to

regulate the cytoplasmic concentration of this short-chain fatty acid, and then its inhibitory effect on histone deacetylase activity. Such inhibition is then responsible for increased histone acetylation, which affects gene expression in colonocytes. In addition, experiments with ^{14}C -labelled butyrate indicate that butyrate acts as an acetyl-CoA donor for histone acetylation.³² Then, it appears that butyrate metabolism in colonocytes and its effects on gene expression are tightly connected (Figure 1). As reviewed by Hamer et al,²⁸ the potential of butyrate to act, at least in experimental animal models, as a beneficial modulator in situations such as mucosal inflammation and colorectal carcinogenesis may rely partly on the butyrate ability to modulate gene expression in colonocytes and to affect key regulators of cell cycle, apoptosis, and associated signaling pathways.

Several other bacterial metabolites, including hydrogen sulfide, *p*-cresol, and ammonia, may interfere with colonocyte oxygen consumption in *in vitro* experiments. Hydrogen sulfide is produced by the microbiota from dietary and

endogenous S-containing substrates.³³ Hydrogen sulfide at excessive luminal concentrations inhibits markedly, although in a reversible way, the colonocyte oxygen consumption (Figure 2). This inhibition of cell respiration corresponds to the inhibition of the mitochondrial cytochrome *c* oxidase activity.³⁴ In addition, at concentrations >1 mmol/L, hydrogen sulfide dose dependently inhibits butyrate oxidation in human colonic epithelial cells,³⁴ likely as a consequence of the inhibition of the mitochondrial respiratory chain. Reduced colonocyte oxygen consumption, as observed under poisoning of colonocytes with hydrogen sulfide, may provoke an imbalance between ATP production and utilization in colonocytes. However, at micromolar extracellular concentrations, hydrogen sulfide has been shown to represent a mineral fuel for colonocytes,³⁵ the first one in this category. This is made possible by the oxidation of hydrogen sulfide by the mitochondrial sulfide oxidizing unit (Figure 2). Although hydrogen sulfide allows the generation of ATP in colonocytes, it is likely that in healthy colonocytes, hydrogen sulfide is not primarily

Figure 1 Schematic representation of the microbiota-derived butyrate metabolism and effects on colonocytes. Butyrate is produced from undigested carbohydrates and some protein-derived amino acids, diffusing through the mucus layers before being transported in its anionic form inside colonocytes through monocarboxylate transporter isoform 1 (MCT1) transporter, or diffusing in its neutral form in these cells. Butyrate is oxidized in the mitochondria, allowing ATP production. A part of cytosolic acetyl CoA produced from butyrate is used for lipid synthesis and histone acetylation, whereas unmetabolized butyrate inhibits histone deacetylase (HDAC) activity. Increased histone acetylation then modulates gene expression in colonocytes. The unmetabolized part of butyrate is then released in the bloodstream through the MCT transporters.

Figure 2 Schematic representation of the microbiota-derived hydrogen sulfide metabolism and effects on colonocytes. Hydrogen sulfide is produced from several dietary and endogenous sulfur-containing compounds, and diffuses through the mucus layers and colonocyte apical membrane. **Left panel:** When hydrogen sulfide concentration is low, mitochondria detoxicate it through oxidation into thiosulfate in the sulfide oxidizing unit [sulfide quinone reductase (SQR), dioxygenase ethylmalonic encephalopathy (Diox), and sulfur transferase (ST)] with ATP production. **Right panel:** When hydrogen sulfide is high, thus exceeding colonocyte detoxification capacity, it inhibits mitochondrial cytochrome (Cyt) c oxidase activity, leading to a decrease in oxygen consumption. Increased concentration of the intracytoplasmic hydrogen sulfide increases the expression of genes related to inflammation. iNOS, inducible nitric oxide synthase.

oxidized to increase the cellular level of ATP above a steady-state value, but rather to allow its detoxification, thus regulating its intracellular concentration.³⁶ At a concentration where hydrogen sulfide inhibits oxygen consumption in colonocytes, this gaseous compound increases the

expression of several inflammation-related genes¹⁷; this raises the view that above the capacity of colonocytes to detoxify hydrogen sulfide in the mitochondria, the cytoplasmic concentration of this agent can increase and then affect gene expression in the nuclei (Figure 2). Interestingly,

it has been shown that hydrogen sulfide produced endogenously maintains colon cancer bioenergetics, supporting colonic tumor growth.³⁷

Another bacterial metabolite is *p*-cresol, which is produced by the intestinal microbiota from L-tyrosine, and which is present at a low millimolar concentration in the human feces. It inhibits partially oxygen consumption in human colonocytes in acute *in vitro* experiments, while increasing after pretreatment proton leak through the inner mitochondrial membrane (and thus decreasing mitochondrial bioenergetics activity and ATP cell content) with concomitant increase of the net production of anion superoxide.³⁸ Anion superoxide can be generated within the mitochondrial respiratory chain, notably at two sites in complex I³⁹ and III,⁴⁰ particularly in situations of mitochondrial complex activity inhibition. Anion superoxide may affect cell physiology, notably by reacting with nitric oxide and forming the oxidant peroxynitrite.⁴¹

Ammonia (considered as the sum of NH_4^+ and NH_3) is produced by the bacterial microbiota from amino acid deamination and urea hydrolysis, and is present at millimolar concentrations in the colonic luminal content.²⁹ High millimolar concentrations of ammonia inhibit short-chain fatty acid oxidation⁴² and basal oxygen consumption in colonic epithelial cells.⁴³ However, colonocytes have the metabolic capacity to synthesize L-glutamine from ammonia and L-glutamate in the cytosol, and to synthesize carbamoylphosphate from ammonia and bicarbonate in the mitochondria. L-Citrulline is then synthesized from carbamoylphosphate and L-ornithine.^{11,44} These latter metabolic pathways allow presumably to regulate, up to a threshold level, the intracellular concentration of NH_3 , thus limiting its deleterious effect on colonocyte energy metabolism.

Then, it appears that several bacterial metabolites, when present at excessive concentrations, can be considered as luminal metabolic troublemakers, acting on the colonocyte mitochondria.³³

Bacterial Metabolites and Colonic Epithelial Cell DNA Integrity

Elevated mitochondrial reactive oxygen species production over the colonocyte capacity to detoxicate them, may damage and produce mutations in mitochondrial DNA, which is in vicinity with the electron transport chain, and which is not protected by histones.⁴⁵ Indeed, an example of mitochondrial DNA mutations observed in colon cancer are mutations in the cytochrome *c* oxidase (complex IV of the mitochondrial respiratory chain) with associated proton leak and thus decreased energy-conversion efficiency.⁴⁶ Incidentally, mitochondrial electron transport chain complex dysfunction has also been observed in the colonic mucosa from ulcerative colitis patients with both quiescent and active disease.⁴⁷

Hydrogen sulfide was previously reported to be able to alter genomic DNA integrity in intestinal colonic epithelial

cells.⁴⁸ However, these results were not confirmed in further experiments, maybe because of different experimental design. Indeed, using both *in vivo* colonic intraluminal instillation in rats and longer-term culture of human colonic epithelial cells with millimolar concentrations of sodium sulfide up to 3 mmol/L, no effect of this agent on DNA integrity was detected using the sensitive γ H2AX genotoxicity test.¹⁷

Using the same experimental design, *p*-cresol was found to dose dependently alter the DNA integrity in colonocytes without cytotoxic effects.³⁸ Phenol, which is produced by the intestinal microbiota from L-tyrosine, after reacting with nitrite, leads to the formation of the mutagenic compound *p*-diazoquinone.⁴⁹

Several other bacterial metabolites have been identified as able to alter DNA integrity in colonocytes. Indeed, fecal fecapentaenes, which are produced by the intestinal microbiota presumably from polyunsaturated ether phospholipids, represent potent mutagens toward colon epithelial cell DNA.⁵⁰ Deoxycholic acid produced in the colon by the intestinal microbiota can also act as a DNA-damaging agent.⁵¹ Although ethanol (which is found at millimolar concentrations in the rat colon¹⁶) is likely not genotoxic by itself for colonic epithelial cells, this compound can be converted to acetaldehyde by the gut microbiota. Acetaldehyde is considered as a potential carcinogenic compound in the rectum.⁵² Spermine, a polyamine produced by the intestinal microbiota and taken up by colonic epithelial cells,⁵³ can increase reactive oxygen species production and DNA damage in these cells through its increased catabolism by the spermine oxidase activity in response to enterotoxigenic *B. fragilis*.⁵⁴ Last, N-nitroso compounds, which are formed notably in the colonic luminal content, are viewed as compounds with potential genotoxic effects on colonocytes.⁵⁵ It is then conceivable that long-term exposure of colonic crypt stem cells to excessive DNA-damaging bacterial metabolites may increase the risk of unrepaired DNA lesions in these cells.⁵⁶

Bacterial Metabolites and Colonic Epithelium Proliferation

Several bacterial metabolites at high concentrations that can be measured in the colonic luminal content have been shown to inhibit the proliferation of human colonocytes. Most of these studies have been done using *in vitro* experiments with human colonocytes originating from colorectal cancer. Butyrate at millimolar concentrations strongly inhibits human colon adenocarcinoma HT-29 cell proliferation.⁵⁷ It has been recently proposed that differentiated colonocytes, by consuming butyrate, decrease the concentration of this short-chain fatty acid in the vicinity of stem/progenitor cells in the colonic crypts, protecting these cells from the inhibitory effect of butyrate on their proliferation.⁵⁸

Hydrogen sulfide at millimolar concentrations is able to inhibit dose dependently HT-29 Glc^{-/+} cell proliferation.³⁴

In response to pretreatment with the hydrogen sulfide donor NaHS, HT-29 Glc^{-/+} cells increase their glycolytic capacity, presumably as a way—together with reduced proliferation (and thus reduced associated ATP-consuming anabolic pathways)—to maintain their ATP cell content and thus cell viability.³⁴ The bacterial metabolite *p*-cresol is also able, in the range of physiological concentrations, to reduce human colonocyte proliferation by accumulating them in the S-cell cycle phase.³⁸ Deoxycholic acid is another bacterial metabolite that is able to inhibit intestinal epithelial proliferation at concentrations that are not cytotoxic.⁵⁹ Last, ammonia inhibits proliferation of colonocytes originating from colon cancer, characterized by their inability to metabolize this metabolite,⁶⁰ reinforcing the view that detoxification metabolism in colonocytes can avoid deleterious effects of some bacterial metabolites. Among bacterial metabolites acting on colonic epithelial cell proliferation, the polyamines putrescine and agmatine exert opposite effects, with the former being strictly necessary for colonocyte mitosis,⁵³ whereas the second displays an anti-mitotic effect.⁶¹

Dietary Compounds, Bacterial Metabolites, and Colonic Epithelial Cell Viability

Some dietary compounds, and bacterial metabolites derived from them, have been shown to affect colonocyte viability. Such loss of cell viability, independently from the physiological process of fully mature colonocyte apoptosis/exfoliation, may lead to the so-called process of compensatory hyperproliferation.⁶² An interesting example of a dietary compound that affects colonic epithelial cell viability and induces colon epithelial hyperproliferation is represented by the heme molecule. Heme in meat and fish is mainly present in hemoglobin and myoglobin. Heme is only partly absorbed in the small intestine, with part of it being recovered in the large intestine luminal content.⁶³ Heme in vicinity with the colonic surface epithelium exerts cytotoxic effects, resulting in epithelial damage and compensatory hyperproliferation, leading to hyperplasia in a gut microbiota-dependent manner.^{62,63} In addition, heme is genotoxic toward colonic epithelial cells.⁶⁴ These heme characteristics may explain the positive association between the heme intake and the risk of colorectal cancer risk.⁶⁵ However, not all epidemiological studies found correlation between dietary heme consumption and colorectal cancer incidence,⁶⁶ challenging this concept and suggesting that heme may synergize with other dietary components (eg, protein as precursors of amino acid-derived deleterious bacterial metabolites) for the colorectal carcinogenesis process.⁶⁷

At a low millimolar concentration, phenol, another bacterial metabolite, has been shown to significantly impair the viability of human colonocytes.⁶⁸ Last, deoxycholic acid has been reported to be cytotoxic on colonic epithelial cells in addition to its genotoxic effect.⁶⁹

Bacterial Metabolites and Colonic Epithelial Barrier Function

Some bacterial metabolites have been suspected to affect, either positively or negatively, the colonic epithelial barrier function. For instance, it has been proposed that hydrogen sulfide, by reducing disulfide bonds in mucus, would increase the permeability of the mucous layer to luminal compounds, including the heme compound.⁶²

Interestingly, evidence has been presented that perturbed mitochondrial function induced by uncoupling agent in colonic epithelial cells causes intestinal barrier dysfunction,⁷⁰ adding further evidence to the view that altered mitochondrial function participates in alteration of the epithelial integrity.

Conversely, indole, a bacterial metabolite produced from L-tryptophan, has been shown to be beneficial for the colonic epithelium by increasing epithelial cell tight-junction resistance.⁷¹ Last, butyrate is also recognized as a bacterial metabolite that enhances the intestinal barrier function.⁷²

Bacterial Metabolites and Luminal pH

Several bacterial metabolites, either acidic or alkaline, can affect the colonic luminal pH. Conversely, the luminal pH can affect the acid/base ratio of several bacterial metabolites.

The pH of the human cecal content is slightly acidic, ranging from 5.7 to 6.8, while ranging from 6.1 to 7.5 in the descending colon and rectum.⁷³ The pH at the colonic mucosal surface in healthy subjects is averaging 7.1 in cecum and ascending colon, whereas it ranges from 7.2 to 7.5 in the descending colon and rectum.⁷⁴ The luminal pH depends on the respective concentrations of a complex mixture of acids and bases in the large intestine content, among which hydrogen and bicarbonate secretion by the colonic mucosa represent important determinants.⁷³ Short-chain fatty acids also participate as organic acids, together with other microbiota-derived acidic metabolites (present, however, at much lower concentrations) like branched-chain fatty acids, organic acids (lactate, succinate, and phenylacetate), and hydrogen sulfide. Ammonia intervenes in the luminal pH as a weak base.

In turn, changes in the luminal pH may affect the uptake of luminal compounds by colonocytes and then their action on these cells; however, few studies have unfortunately been performed on that specific important topic. For instance, hydrogen sulfide dissociates in solution, yielding hydrosulfide anion with a pK_a value equal to 7.04.³³ Thus, in case of lower luminal pH, the hydrogen sulfide/hydrosulfide anion ratio in the large intestine will increase (Figure 2). Taking into account that hydrogen sulfide, unlike hydrosulfide anion, easily penetrates biological membrane,⁷⁵ one can predict that lower luminal pH will increase hydrogen

sulfide concentration, and thus amplify its deleterious effect on colonocyte respiration at excessive concentrations.³⁶ The same reasoning can be made regarding butyrate and ammonia. Butyrate, according to its pKa value (4.7), exists predominantly in the anionic dissociated form at the normal colonic pH; this anionic form being transported by the monocarboxylate transporter isoform 1, which is present in the colonocyte brush-border membranes^{28,76} (Figure 1). Then, at a more acidic luminal pH, the concentration of the anionic form will decrease, resulting in lower uptake of this compound through monocarboxylate transporter isoform 1 by colonocytes, and presumably different impact on these cells.^{32,77} Last, ammonia with a pKa equal to 9.02 is mainly present as NH_4^+ in the colonic luminal content and transported in differentiated colonocytes through dedicated transporters from the RhBG and RhCG families.⁷⁸ A more alkaline luminal pH will displace the equilibrium in favor of NH_3 , knowing that this compound is highly diffusible across colonocyte membranes.⁷⁹ Then, increased intracytoplasmic NH_3 may penetrate mitochondrial membrane to a higher level,⁸⁰ affecting basal mitochondrial oxygen consumption and butyrate/acetate oxidation in colonocytes.^{42,43}

Beyond the influence of luminal pH on the transport and action of bacterial metabolites inside colonocytes, the modifications of the luminal pH per se may affect colonic epithelial cell physiology. Interestingly, lower colonic luminal pH in patients with ulcerative colitis⁷³ has been observed. In a model of rodent with chemical induction of colon carcinogenesis, lower luminal pH is associated with higher tumor yield and increased epithelial cell proliferation.⁸¹ A low pH inhibits the synthesis by the intestinal microbiota of the DNA-damaging secondary bile acids⁸² and reduces the activity of luminal proteases.⁸³ In addition, acidic extracellular pH has been shown to shift colorectal cell death from apoptosis to necrosis on exposure to short-chain fatty acids.⁸⁴ Last, low external pH has been shown to dramatically increase the expression of the multidrug resistance protein in human colon carcinoma cell lines,⁸⁵ rendering these cells more resistant to chemotherapeutic agents. Thus, it appears that a more acidic colonic luminal pH is associated with both beneficial and deleterious effects on colonocytes; these effects are apparently different according to the status of colonocytes (healthy or neoplastic).

Dietary Compounds, Bacterial Metabolites, and Luminal Osmolarity

Carbohydrate malabsorption associated with osmotic diarrhea likely depends primarily on the balance between the osmotic force of the carbohydrate incriminated, on the capacity of the microbiota to metabolize this compound, and on the colonic absorptive capacity toward these compounds. The same reasoning can be made

regarding the bacterial metabolites, which, depending notably on the food/microbiota composition, and on the transport capacity of colonocytes, may accumulate in the colonic/rectal luminal content and modify luminal osmolarity.

Osmotic strength of the fecal stool in humans is in the range of 321 to 350 mOsm/L. The most common manifestation of carbohydrate malabsorption, that is lactose malabsorption, may provoke osmotic diarrhea.⁸⁶ Increased luminal osmolarity has been associated with several effects on colonic epithelial cells in relationship with the epithelial barrier function and mucosal inflammation. In *in vitro* experiments, hyperosmotic stress induces cell signaling associated with cell survival in colon cancer cells through the osmotic regulator NFAT5.⁸⁷ Hyperosmotic medium increases the expression of SPAK, a protein whose expression in colonocytes is associated with increased epithelial permeability.⁸⁸ In other *in vitro* experiments with colonic epithelial cells originating from human cancer, hyperosmolarity is able to stimulate the expression of the proinflammatory IL-8 through NF- κ B activation.⁸⁹ In the rat colon, hypertonic solution increases mucus output.⁹⁰ Hyperosmolarity is able to increase within 2 hours the expression of the cyclooxygenase-2 gene in human colonocytes,⁹¹ this enzymatic activity being increased in the colonic inflammation and colonic epithelial carcinogenesis processes.⁹² Last, it has been shown that, in the experimental model of colitis induced in rodents with dextran sulfate sodium, luminal hyperosmolarity in colon plays a major role for mucosal inflammation.⁹³ Thus, overall, hyperosmotic environment of colonic epithelial cells appears to be associated with adverse effects in terms of epithelial barrier function and events associated with inflammation. Interestingly, higher fecal values of osmotic strength have been observed in Crohn's disease patients when compared with healthy counterparts.^{94,95}

Adaptive Processes in Colonocytes toward Changes of Bacterial Metabolite Concentrations

Although, as presented above, there is little doubt from *in vitro* and *in vivo* experiments that changes in the bacterial metabolite concentrations in proximity to the colonic epithelial cells affect their metabolism, functions, and likely the homeostatic process of epithelium renewal, the predicting of the impact of such changes on the colonic epithelium is not an easy task. Indeed, these compounds, which have been in most cases tested individually, can exert opposite, additive, or synergistic effects on colonocytes; and their effects depend on their respective free and bound luminal concentrations, and on the capacity of colonocytes to metabolize them. The situation is further complicated if we consider the adaptive capacity of colonocytes toward changing luminal environment. For

Table 1 Some Typical Effects of Bacterial Metabolites on Colonic Epithelial Cells

Bacterial metabolites	Modified parameters	References
Hydrogen sulfide and <i>p</i> -cresol	Decreased oxygen consumption	34,38
Hydrogen sulfide and ammonia	Decreased SCFA oxidation	34,42
Phenol, <i>p</i> -cresol, acetaldehyde, fecapentanes, and deoxycholic acid	Affect DNA integrity	38,49–52
Phenol and deoxycholic acid	Decreased cell viability	67,68
Hydrogen sulfide, <i>p</i> -cresol, ammonia, and deoxycholic acid	Decreased cell proliferation	34,38,58,59
Indole and butyrate	Increased barrier function	70,71
Hydrogen sulfide	Decreased barrier function	61

SCFA, short-chain fatty acid.

instance, an increased water content in the colonic lumen is likely to represent, at least in rodent models, a way to avoid sharp increase of bacterial metabolite concentrations in case of increased net production. In fact, in the rat model, high-protein consumption increases the colonic content of short-chain fatty acids¹⁶ and hydrogen sulfide¹⁷ when compared with animals consuming a normoproteic isocaloric diet. However, nearly proportional increased water content in the colon of animals fed with the high-protein diet⁴³ attenuates greatly the increased concentrations of these metabolites. Whether this increased water content simply results from increased osmolarity of the colonic content, and/or implies modified water and electrolyte transport, remains to be determined.

Increased detoxification pathways in colonocytes may also allow us to control the intracellular concentrations of deleterious bacterial metabolites like hydrogen sulfide and ammonia. Regarding hydrogen sulfide, it has been shown in the rat model that when the animals are fed for 2 weeks with a high-protein diet, a modest but significant increase of sulfide quinone reductase gene (*Sqr*) (Figure 2) expression is recorded in colonocytes when compared with normoproteic control animals.¹⁷ Interestingly, impaired hydrogen sulfide detoxification in intestinal mucosa is associated with Crohn's disease.⁹⁶ Last, a study indicates that the increased concentration of ammonia recorded in the colonic content of rats after high-protein diet consumption is associated with an increased catalytic activity of glutamine synthetase, in colonocytes, presumably allowing increased ammonia detoxification.⁴⁴

Conclusion and Future Prospects

Mostly from experimental studies with animal models and cell physiology experiments, the idea that changes in the luminal environment of colonic epithelial cells—and notably high concentrations of several bacterial metabolites—affect major aspects of their physiology and metabolism, like energy metabolism, DNA integrity, proliferation, and viability, affecting presumably the process of epithelial renewal, appears to represent a robust proof of concept (Table 1).

One main crucial unresolved question, regarding the effects of genotoxic bacterial metabolites, is related to the concentrations of these compounds in the vicinity of the colonic crypt stem cells, a parameter not easily measurable for obvious technical reasons. Indeed, because the crypt stem cells appear to be the cells at the origin of colorectal cancer,^{97,98} the identification of the luminal compounds at the origin of mutations in these cells, as well as the threshold of concentrations above which genotoxic and cytotoxic effects are expected, would represent a central piece of information in the field. In the same line of thinking, a recent article has shown that the lifetime risk of cancers of different types is well correlated with the total number of divisions of the normal self-renewing cells.⁹⁹ Although this latter correlation allows to partly explain the relatively high frequency of colorectal cancer among other less frequent types of cancers, it does not provide a satisfactory explanation on the fact that groups of individuals migrating from a country with low incidence of colorectal cancer to a country with high incidence meet the incidence of the welcoming country¹⁰⁰; this suggests an impact of environment (and notably of the luminal environment of colonocytes) on the colorectal carcinogenesis process.

Another crucial point is related to the capacity of the bacterial metabolites to cross the mucus layers before entering the colonocytes. Most of *in vitro* studies have been performed with colonocytes directly exposed to bacterial metabolites. From that point of view, intraluminal colonic instillation of bacterial metabolites in anesthetized animal models represents an experimental design closer to the physiological situation.¹⁷

Finally, by better identifying the changes in the luminal environment that increase or decrease the risk of alteration in the normal process of colonic/rectal epithelium renewal and functions, it should become possible to intervene, particularly from a dietary point of view, for limiting the risk of pathology from inflammatory and neoplastic types in this anatomical region.

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