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GASTRO INTESTINAL HYPERPERMEABILITY: A REVIEW

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ABSTRACT

Objective: To present a wide overview of the recent developments in the understanding of the aetiopathogenesis of gastrointestinal hyperpermeability.

Data sources: Medline, from 1985, was sourced for relevant articles. Review articles were included in order to minimise the number of references in the reference list.

Study selection: Results from experiments and observations on humans and other mammalian species were studied.

Data synthesis: The major mechanisms elucidated in the aetiopathogenesis of the gastrointestinal hyperpermeability were integrated and consolidated into a flow diagram and the major factors responsible for normal permeability presented for comparison.

Conclusion: The occurrence of increased gastrointestinal hyperpermeability is probably vastly underestimated. In addition to the hyperpermeability commonly associated with chronic gastro intestinal disorders, an increase in gastrointestinal permeability may occur in any condition of metabolic depletion, enterocyte ATP-depletion, stimulation of gastrointestinal pro-inflammatory cytokine production and disturbances of the normal gastrointestinal flora as with prolonged use of antibiotics.

INTRODUCTION

The main function of the gastrointestinal tract is to absorb nutrients efficiently. However, just as important to the host's survival is its seemingly contradictory role of preventing the transit of pathogens across the gastrointestinal wall. In this review, the factors involved in the maintenance of gastrointestinal barrier integrity are illustrated and the aetiopathogenesis of hyperpermeability is discussed. We conclude with a short summary of clinical tests and therapeutic options.

Mechanisms involved in maintenance of gastrointestinal barrier integrity: Bacteria and antigens in the lumen of the gut are separated from the enterocytes by both non-specific and immunologic barriers (Figure 1). The enterocyte layer can be crossed by either transcellular or paracellular routes. A major factor in the maintenance of normal permeability is the structural integrity of the enterocytes. Transcellular transport, including diffusion, as well as symport and antiport by means of carrier proteins and ion channels can occur. Many of these transcellular transport processes require ATP, and any factor, which depletes the energy stores, will generally increase gut permeability. If potential

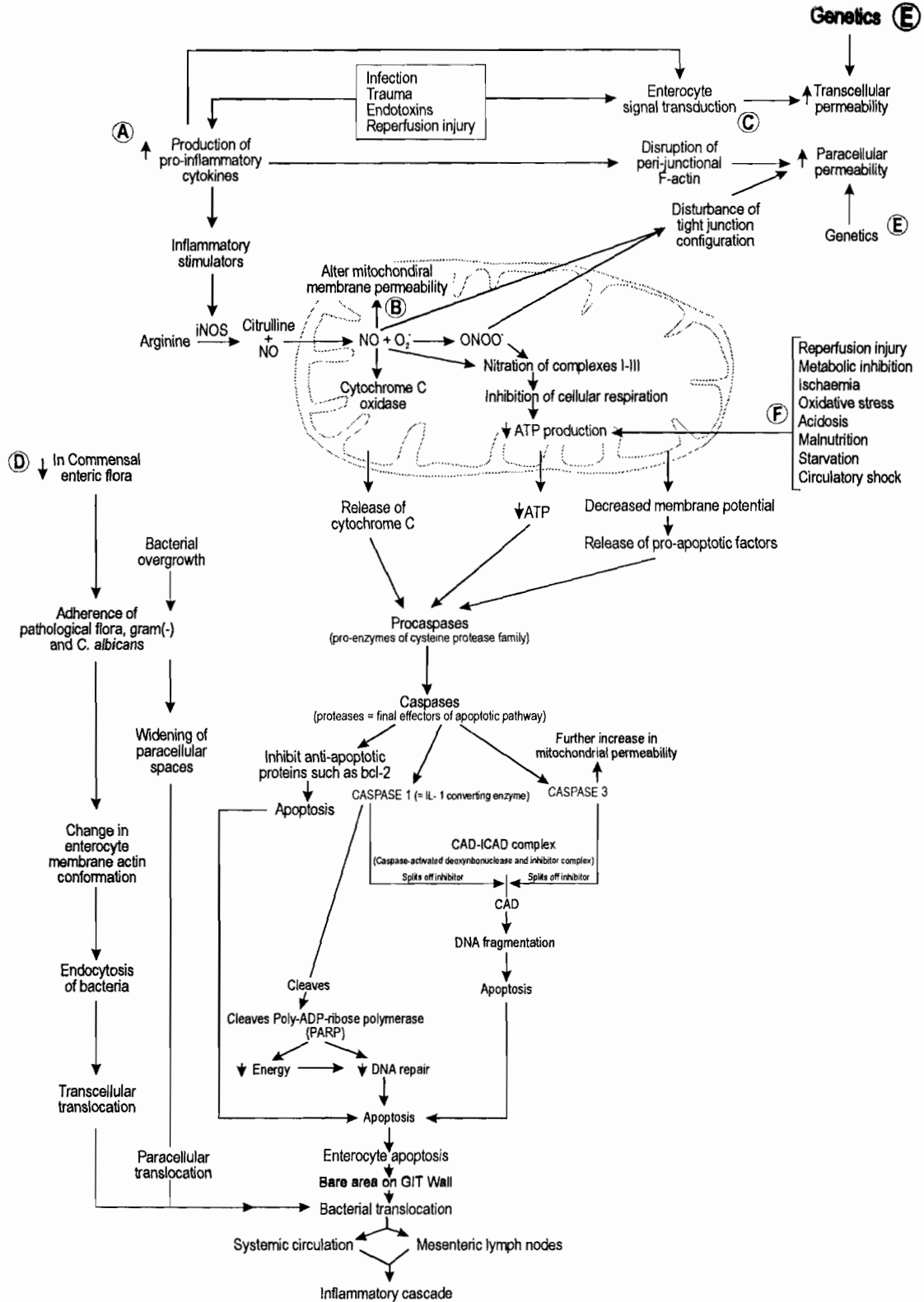
antigens cross into the enterocyte immune activation can occur, as enterocytes may act as antigen-presenting cells.

The paracellular space is enclosed by tight junctions, which consist of several strands formed by the fusion of outer membrane proteins of adjoining enterocytes(1-3). The number of strands determines the tightness of the junctions and acts as a rate-limiting factor regulating the selective permeability of the paracellular areas(1-4). Intracellular signals transmitted to the tight junctions normally regulate the permeability by inducing dynamic cytoskeletal re-arrangements. Any factor that either damages a component of the tight junctions or prohibits the necessary cytoskeletal re-arrangements will alter normal gut permeability.

The aetiopathogenesis of hyperpermeability of the gastrointestinal barrier: Various factors may lead to gastrointestinal hyperpermeability with a resultant increase in microbial translocation and overwhelming of the normal defense against translocated microbes and microbial products. A number of causes are known to result in a pathological increase in gut permeability (Table 1)(5,6).

Figure 1

Maintenance of normal gastrointestinal barrier integrity



Foreign antigens in the lumen of the gut are prevented from translocating by non-specific and immunologic factors. Passage through the gut wall has been described through transcellular or paracellular routes. Adapted from(30) and(43).

Table 1

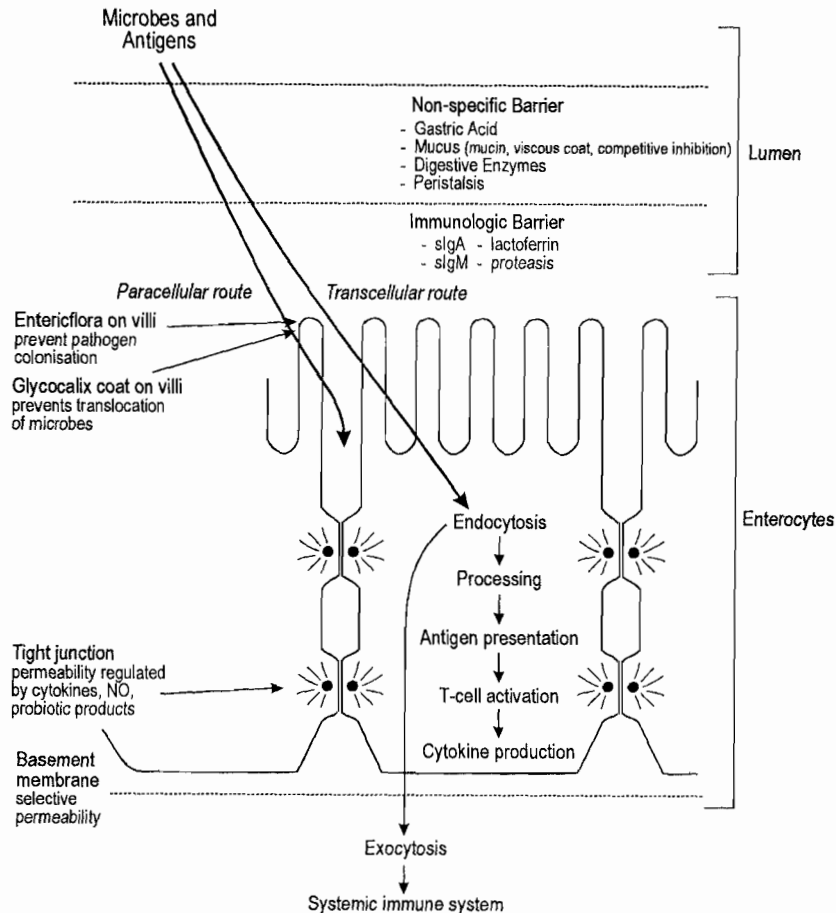
Potential causes of pathological increase in gut permeability

Bacterial overgrowth
Starvation and malnutrition
Thermal injury
Trauma
Biliary obstruction
Immunosuppression
Disturbance of the normal intestinal flora by antibiotics
Circulatory shock and reperfusion injury
Endotoxaemia
Metabolic depletion and acidosis
Inflammation and infection
Non-steroidal anti-inflammatory drugs
Sometimes in non-gut related surgery
Prolonged exercise
Travellers' diarrhoea
Ischaemia and oxidative stress

The major theories proposed for an increase in gastrointestinal barrier permeability illustrated in figure 2 are discussed in the following sections.

Figure 2

Factors involved in gastrointestinal hyperpermeability



Increased pro-inflammatory cytokine production (A) can stimulate the induction of nitric oxide synthase (iNOS) (B), which leads to NO and ONOO- production which may lead to stimulation of the caspases complex - with eventual apoptosis. Proinflammatory cytokines can also change paracellular and transcellular translocation (C). A decrease in normal intestinal flora (D) can also increase paracellular as well as transcellular bacterial translocation. Genetically determined increases (E) in transcellular and paracellular permeability may underlie some diseases. Various conditions (F) can cause apoptosis by a decrease in ATP production.

Nitric oxide: In the gut, as elsewhere in the body, NO is produced during conversion of arginine to citrulline under influence of the enzyme nitric oxide synthase (NOS). All three NOS isoforms may be involved in gut physiology and pathology: a) the neuronal derived nNOS (NOS1), expressed in the myenteric plexus, b) the endothelial eNOS (NOS3) which is involved in gut perfusion and c) inducible NOS or iNOS (NOS2) which is only present to any significant extent in the gut in the presence of inflammatory and other stimulatory influences(7-9). Under normal conditions, NO plays a gastrointestinal homeostatic role through the regulation of blood flow, regulation of gut permeability and in the defense against pathogenic organisms. Clinical evidence for the role of NO in gastrointestinal tract pathology of humans is derived from the finding of increases in iNOS activity in patients with inflammatory bowel disease(10) and in infants undergoing surgical resection for necrotising enterocolitis(11) where upregulation of iNOS was found in association with apoptotic areas. During sustained overproduction of NO, both NO and the subsequently produced peroxynitrite ($\text{NO} + \text{O}_2^- = \text{ONOO}^-$), can lead to deleterious effects such as dilation of intercellular tight junctions (12) and enterocyte apoptosis(12,13). One would logically presume that suppression of excessive NO production could be of benefit to patients. However, indications are that gastrointestinal mucosal injury caused by ischaemia and reperfusion(14), endotoxaemia(15), platelet activating factor activation(16) and endothelin(2) could be worsened by suppression of the endogenous NO production. In fact, on several occasions, it has been reported that stimulation of NO production ameliorates mucosal injury(17). Possible mechanisms involved in this protective effect of NO include the suppression of mast cell-derived inflammatory mediator-release, inhibition of leukocyte adhesion to endothelial walls(18,19), protection against certain bacterial toxins by inhibition of mast cells and neutrophils(20) and stimulation of intestinal mucosal recovery after mesenteric ischaemia(21). However, excessive NO production can contribute to gastrointestinal hyperpermeability.

Hypoxia, oxidative stress, reperfusion injury, metabolic inhibition and acidosis: Conditions which cause hypoxia of the gastrointestinal wall can affect permeability due to depletion of ATP, accumulation of reducing equivalents in the cells or by a number of reactions with free iron(2).

Oxidative stress, including the formation of the superoxide anion, hydrogen peroxide and the hydroxyl radical may lead to increased permeability of both enterocytes and endothelial cells through activation of phospholipase C-dependent hydrolysis, lipid peroxidation, perturbation of transport processes and disturbance of mitochondrial function with subsequent depletion of ATP stores and enterocyte actin-dependent cytoskeletal malfunctioning(2). This would lead to an increase in both the paracellular and transcellular transport processes. It could further contribute to the NO-induced

apoptosis, both by reducing the ATP stores and by providing the reactive oxygen species for peroxynitrite production.

The danger of reperfusion lies in the excessive formation of reactive oxygen species. Intestinal ischaemia and reperfusion aggravate barrier damage even further because it also activates the phagocytic system and, in combination with an increase in cytokine production, may lead to inflammatory damage as well as to NO induction. The final result is increased bacterial translocation(22).

Any factor that leads to metabolic inhibition can increase gut permeability by reducing the ATP stores, leading to enterocyte apoptosis with subsequent bare areas of increased permeability. With metabolic inhibition, general destabilization of the enterocyte tight junction cytoskeleton will further increase paracellular transport(23,24).

Several mechanisms underlying increased gut permeability are ascribed to acidosis. The most important actions are probably the fact that acidosis can, even in the absence of metabolic depletion, give rise to ATP depletion, and that the production of reactive oxygen species are enhanced in conditions of acidosis(25). The latter process may further be augmented by the release of iron from protein-bound stores(26). However, several protective mechanisms of acidosis against damage to the intestinal wall have also been described(2).

Cytokines and other inflammatory mediators: The gut is constantly exposed to intraluminal endotoxins as well as to inflammatory mediators from macrophages and lymphocytes and has been described as a cytokine liberating organ(27). Cytokines such as interleukin-1 and interferon-7 can induce the expression of iNOS mRNA leading to increased gut permeability by stimulating the production of NO(28).

Growth hormone and cholecystokinin: Though growth hormone has been shown to improve gut mucosal structure, it increases mucosal permeability to intestinal bacteria. A possible explanation for this apparent paradox may be that high growth hormone levels stimulate the release of somatostatin via negative feedback, which in turn inhibits cholecystokinin secretion by the enteroendocrine cells of the gut mucosa(29). Cholecystokinin modulates the function of secretory IgA, which is a major protective factor in preventing bacterial adherence and thus maintaining normal intestinal permeability. Gut resection or other damage to the gastrointestinal tract may result in a decreased number of enteroendocrine cells, less cholecystokinin and less secretory IgA. The final result is increased bacterial adhesion and an increase in bacterial translocation(29).

Disturbance in the normal gastrointestinal flora: Normal flora colonises the gut by binding to receptors on the luminal surface of enterocytes. Under normal conditions, the competition between the commensal and

the pathogenic organisms offers a kind of protection against pathogenic invasion. Antibiotic medication disturbs the normal commensal bacteria, allowing colonisation by pathogenic bacteria with subsequent increase in their translocation. Pathogenic bacteria will colonise the gut by first adhering to the glycoconjugates of the microvillous membrane, which are receptors for various physiological ligands. By pirating the receptors of natural agents, the pathogenic bacteria activate signal transduction pathways leading to a series of biochemical processes which eventually facilitate its translocation into the interstitium(30). This translocation is facilitated by a) the production of molecules which open the tight junctions and so increase paracellular translocation and b) modification of the enterocyte actin composition with subsequent translocation by means of endocytosis (transcellular translocation). Exo- and endotoxins can, in the same way, activate enterocyte receptor-mediated intracellular pathways resulting in the release of pro-inflammatory cytokines, inflammatory tissue destruction, as well as NO-induced apoptosis(12,13,30).

Tests for gut permeability: Intestinal permeability can be estimated by measuring the concentration of administered macromolecules or single sugars in the blood or urine (31-33). The types of molecules used as probes include ⁵¹chromium-labelled ethylenediaminetetra-acetate, the polyethylene glycols, horseradish peroxidase and proteins, and various sugars(31-33). ⁵¹Chromium-labelled ethylenediaminetetra-acetate is given orally and measured in the urine by gamma counter. The results obtained are considered a fair reflection of intestinal permeability, though the test has the disadvantages associated with radioactive substances. The polyethylene glycols represent a mixture of ethylene glycol polymers. It is a non-invasive test as the probe is taken by mouth and the test performed on urine. Analytical procedures (gas-liquid or high-performance liquid chromatography) and clinical interpretations of the results are complicated, which renders the ethylene glycol polymers unsuitable for clinical use in all but the more sophisticated laboratories. The horseradish peroxidases and proteins are not really biologically inert and may lead to complications such as immunological stimulation. Various sugars are used including the monosaccharides (D-mannitol, L-rhamnose and D-xylose) and the disaccharides (lactulose, cellobiose, sucrose and lactose).

Changes in intestinal permeability can also be evaluated by the simultaneous oral administration of two different water-soluble probe molecules that are neither digested nor metabolised. One is usually a relatively small molecule that permeates moderately well through normal mucosa, and the other is usually a larger molecule with minimal permeation of the normal mucosa. These molecules cross the epithelium, are taken up into the circulation, and are then excreted in the urine. The amounts absorbed depend on their physicochemical features as well as the intestinal mucosal integrity. Differential

absorption can then be ascertained by measuring the different concentrations of the probes excreted in the urine. The ratio of the two probes is an indication of intestinal permeability. The advantage of a differential absorption test, above that of using a single sugar, is that variables such as the volume of urine collected, the time interval between intake and testing will presumably influence both probes and the ratio will not be much affected. The mannitol and lactulose ratio is most frequently used. However, while it may be the best combination for the determination of gastrointestinal permeability, it is not suitable for rating the seriousness of conditions. Still, it remains a useful tool for screening for small intestinal disease, for evaluating the effectiveness of treatment and, perhaps, even as prognostic indicator(31,32). Some studies indicate that the combination of lactulose (L) and L-rhamnose (R) may provide satisfactory results(32,33). The evaluation on this combination was done using both urine and blood samples and the ratio of the two sugars determined. Blood samples used to test for intestinal permeability have proven to be a valid alternative to urine collection and testing, and blood specimens show a lower test failure rate(32). A ratio for lactulose to rhamnose of 5g/0.5g in solution is sufficient to discriminate between normal and increased permeability (32,33). A recent report shows the polyethylene glycol 4000 marker (PEG4000) to be suitable for testing both intestinal integrity and bacterial translocation rate (34).

Treatment: Two major interesting trends in the nutritional support of critically ill patients, have surfaced over the last couple of years. The first is the shift away from intravenous administered of nutrient to enteral feeding. The second is decreasing the quantity of nutrients while increasing the quality of the nutrient mix.

Enteral nutrition stimulates motility, mucus production and gastrointestinal hormone release. Maintenance of these processes plays a vital role in normal barrier function. It is suggested that enteral feeding helps to prevent starvation of the enterocytes during the hypermetabolic state of critical illness and helps to maintain normal immune function, while enhancing intestinal integrity and mucosal barrier function.

Substances included in the nutrient mix are amino acids (glutamine, arginine, ornithine), fatty acids (short chain *n*-3 polyunsaturated) and nucleotides (RNA)(35). Critically ill patients who receive these special nutrients are reported to have fewer complications, spend less time in hospital and have a decreased mortality rate(36) due to beneficial effects on their nutritional, metabolic and immune function. The correct enteral feeding mix was shown to modify hepatic Kupffer cell function, cytokine-release and acute phase protein production(35,37). However, some contradictions about the value of the individual nutrients exist. There are, for instance, indications that L-arginine, although in some way beneficial to mucosal barrier function, may also exacerbate inflammation and that the L-arginine-nitric oxide pathway

may be instrumental in a number of deleterious effects(35). Clinical evidence with regard to ornithine is insufficient for making any substantiated judgement. The feasibility of L-glutamine supplementation is widely supported. Enterocytes (as well as fibroblasts, lymphocytes and macrophages) use glutamine as their principal metabolic fuel, and are dependent on preformed glutamine as they have little glutamine synthetase activity. Commercially available total parenteral nutrition (TPN) solutions do not contain glutamine, and administration of these solutions has been shown to lead to small intestine villous atrophy, which can be reversed by glutamine addition(38,39). Glutamine supplementation by oral or intravenous routes during periods of catabolic stress has been shown to improve the function of the gut barrier, as well as various markers of immune function(38,39).

Biotherapeutics comprise the combination of prebiotics (substances which support the proliferation of probiotics) and probiotics (commensal bacterial strains of human origin)(40). Many functions have been ascribed to probiotics including competitive inhibition against pathogen colonisation and immune stimulation. The reader is referred to the symposium proceedings of the American Society for Nutritional Sciences Annual Meeting(41) for a fairly comprehensive overview on the implications of probiotic bacteria for human health and to the results of the ENDO project (European Commission-funded project on non-digestible oligosaccharides) for a consensus on prebiotics(42). The ENDO project, in short, provides evidence for the positive prebiotic effect (i.e. food-induced increases in the number or activity of bifidobacteria and lactic acid bacteria in the human intestine) of non-digestible oligosaccharides.

CONCLUSION

A wide variety of factors can lead to an increase in gastrointestinal permeability with subsequent microbial translocation (Figure 2). Increased barrier permeability might have an early role in the development of several immune-related gastrointestinal diseases(6) and a number of chronic conditions are commonly associated with gastrointestinal hyperpermeability, including Crohn's disease, ulcerative colitis, inflammatory bowel disease, acute liver failure and cirrhosis, ankylosing spondylitis, and coeliac disease.

Furthermore, microbial translocation may be the cause of sepsis in critically ill patients, which may result in multiple organ failure and death. The development of increased permeability is, however, not limited to such serious complications as any factor that leads to metabolic suppression, enterocyte ATP-depletion, stimulation of proinflammatory cytokines or a disturbance in the natural gastrointestinal flora can lead to gastrointestinal hyperpermeability. Biotherapeutics and the correct enteral nutrition are increasingly recognised to be of importance for the maintenance, as well as the recovery, of normal gut permeability.

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