Interface of malnutrition and periodontal diseases[1-3]

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ABSTRACT In response to periodontal pathogens neutrophils release oxidants, proteinases, and other destructive factors. The balance between these factors, the antioxidants, and endogenously synthesized antiproteinases determine the extent of periodontal damage. Malnutrition, particularly protein-energy malnutrition involving concomitant deficiencies of antioxidant nutrients, is characterized by impairment in production and cellular actions of the cytokines, diminished acute-phase protein response (APR) to infections, endocrinopathies, defective metabolism of drugs, and impaired response to stress. The APR plays a central role in promoting healing. Additionally, malnutrition elicits adverse alterations in the oral microbial ecology as well as in the volume and the antibacterial and physicochemical properties of saliva. Good dietary practices and optimal nutritional status are therefore important in mitigating the severity of inflammatory periodontal lesions but are likely of limited value if the stimuli from dental plaque are not removed. *Am J Clin Nutr* 1995;61(suppl):430S–6S

KEY WORDS Periodontal disease, malnutrition, diet, neutrophil dysfunction, antioxidants, acute-phase proteins

Introduction

Periodontal diseases (PDs) are chronic inflammatory conditions caused by specific microbial organisms, many of which derive their nutrient requirements from the host (1, 2). They are characterized by infiltration of leukocytes, particularly the polymorphonuclear neutrophils, loss of connective tissue, alveolar bone resorption, and formation of periodontal pockets. Dental plaque is considered to be the pathologic locus of PDs, and among the potential periodontal pathogens are the *Porphyromonas gingivalis*, *Prevotella intermedia*, *Fusobacterium nucleatum*, *Campylobacter rectus*, *Eikenella corrodens*, *Actinobacillus actinomycetemcomitans*, and the Spirochetes (3–5). The specific plaque hypothesis of PDs is however still in dispute (6). Saliva, diet, and nutrition play very significant roles in the formation and maturation of dental plaque (7, 8). The presence of increasing numbers of the putative periodontal pathogens appears to be an important stage in the genesis of PDs (4). These microorganisms harbor many virulent factors capable of promoting severe tissue destruction (5). Nonetheless, persuasive data support the current, widely held belief that the host's inflammatory responses to the potent microbial factors actually cause the local destruction of the periodontal tissues (1, 9, 10).

A recent report has examined the various risk factors for periodontitis and underscored the important role of neutrophil dysfunction (11). Juvenile periodontitis and gingivitis, particularly necrotizing ulcerative gingivitis, are more prevalent and occur in more severe forms in impoverished Third World communities than in affluent, developed countries (6, 8, 12–16). This is presumed to be solely due to the universally poor oral hygiene in such communities (16–18), an observation disputed by other investigators (6, 12, 19, 20). Studies in Africa (8, 14, 15), India (21, 22), and South America (23) indicate that contrary to observations in industrialized countries (24), necrotizing ulcerative gingivitis in the developing world affects primarily impoverished young children residing in unsanitary surroundings, and who are generally severely immunocompromised by malnutrition and various infections. Relevant to this observation is the well-documented observation of a complex web of interrelationships existing between poor sanitation (including poor oral hygiene), economic poverty, malnutrition, impaired immune function, infections, and many diseases with an inflammatory component (8, 25, 26).

In health, the host uses a variety of restraints and defenses to maintain what amounts to a mutual nonaggression pact with potential periodontal pathogens (27). The host's defense mechanisms have specific (the immune system) and nonspecific components. The latter are either passive [eg, anatomical barriers of the mucous membrane, and normal secretions of saliva and mucus (27, 28)] or active [production of phagocytic cells as well as synthesis of the acute-phase reactants, lysozyme, and the cytokines (29, 30)]. Depleted nutritional reserves in the tissues are associated with a lowering of immunity, progressive damage to mucosa, as well as a diminished resistance to colonization and invasion by pathogens (25). It is known for example, that host response to periodontal infection is influenced by the antecedent immune status such as preexisting low helper T-cell count or the ratio of CD_4+ (helper-inducer cells) to CD_8+ (suppressor-cytotoxic cells) (CD_4+:CD_8+) (5). This report therefore examines the potential contributions of malnutrition to the biological gradient and natural history of

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periodontal infections. An important underlying assumption of this review is that the immune dysregulation and/or depression that occurs in malnutrition promotes the vulnerability of the periodontium to inflammatory stimuli from plaque.

Pathogenesis of periodontal diseases: stages

The pathogenesis of PDs can be conveniently divided into the stages of microbial colonization, invasion, destruction, and healing (10). The last stage recognizes the fact that PD is episodic, with healing periods following periods of active tissue destruction. Examination of the role of diet and nutrition in the pathogenesis of PDs is best undertaken within the context of the four stages, and recognizes the possibilities that nutrient inadequacies may influence oral microbial ecology, the specific systems involved in the progression of periodontal lesions, and the repair process.

Malnutrition and oral microbial ecology

During the colonization stage, antibodies and other factors (secretory and serum-derived) normally impede bacterial attachment and adherence, and also promote maintenance of appropriate ecological balance among the numerous resident oral microbial organisms (10, 27). Diet, nutrition, and saliva play significant roles in the formation and maturation of dental plaque (7). Saliva is a complex secretion from the salivary glands, the constituents of which include proteins, glycoproteins, electrolytes, and small organic molecules, as well as compounds transported from blood (28, 31). The volume and the antibacterial and physicochemical properties of saliva are severely compromised in malnutrition (32–34). Malnourished humans show significant reduction in saliva contents of several proteins including secretory immunoglobulin A (35–37), a finding consistent with severe atrophy of the acinar cells as well as with prominent disorganization of the protein synthetic apparatus noted in both the parotid and submandibular salivary glands of protein-energy deficient laboratory animals (32, 38, 39). Activity of bacteria-agglutinating glycoprotein in saliva is decreased in malnutrition (33), and this may promote enhanced formation of dental plaque (40).

Studies in African children indicate that protein-energy deficient groups, in comparison with their age-matched, well-fed counterparts, have a high prevalence of various potentially pathogenic oral microorganisms, particularly the anaerobic microflora (**Table 1**). Anaerobic microorganisms most frequently isolated from malnourished children are *Prevotella melaninogenicus*, *Porphyromonas gingivalis*, *Prevotella oralis*, *Prevotella ruminicola*, *Actinomyces israelii*, *Fusobacterium sp*, and the Spirochetes (19). Increasing evidence points to the ability of the gram-negative anaerobic organisms to stimulate host defense mechanisms that in turn may be responsible for tissue destruction in PD (1, 5, 10).

Reasons for the differential overgrowth of potential periodontal pathogens in protein-energy malnutrition (PEM) are not clear. Normally present in saliva are small basic peptides, particularly those in which histidine, lysine, and arginine predominate, as well as the free amino acids and their metabolic products such as the polyamines, urea, and ammonia (28, 41–43). These small organic molecules are nutritionally important to many oral bacteria (7, 44, 45). The amino acid arginine is present in saliva in large amounts relative to the other dietary indispensable amino acids (46). Arginase activity (L-arginine aminohydrolase, EC 3.5.3.1) in the major salivary glands is among the highest in extrahepatic tissues (47–49). Studies in human (37) and experimental PEM (50) reveal significantly reduced arginase activity in salivary glands, a situation favoring increased availability of free arginine in salivary secretion. Many oral bacteria, some of which are dominant plaque microorganisms, utilize the arginine deaminase pathway, which catalyzes conversion of arginine into ornithine, polyamines, ammonia, and carbon dioxide, with the formation of 1 mol ATP/mol arginine consumed (51, 52). The increased availability of arginine from salivary glands in PEM would seem to favor elevation of plaque pH (7, 53, 54). Such changes in metabolism of salivary glands may contribute to the frequently reported overgrowth of potential periodontal pathogens in malnutrition (14, 19).

TABLE 1
Effect of malnutrition on oral microbial ecology[1]

Species and group	Well nourished	Malnourished
Oral microorganisms (% positive samples)		
Facultative and aerobic flora		
Gram-negative rods	80	77
Gram-positive cocci	100	100
Nonsporing anaerobes		
Gram-negative rods	20	100
Gram-positive rods	0	77
Gram-positive cocci	0	47
Spirochetes	0	88
Gram-positive bacteria (% positive isolates)		
Aerobic cocci		
Streptococcus mutans	60	77
Streptococcus mitis	60	88
Streptococcus salivarius	60	65
Anaerobic cocci		
Peptostreptococcus	0	41
Anaerobic rods		
Actinomyces israelii	0	76
Gram-negative rods (% positive isolates)		
Aerobic		
Pseudomonas aeruginosa	40	41
Klebsiella pneumoniae	40	35
Anaerobic		
Prevotella melaninogenicus	0	100
Porphyromonas gingivalis	0	76
Prevotella oralis	0	88
Fusobacterium sp	20	71

[1] Adapted from reference 19.

Microbial invasion and tissue destruction

For a clear evaluation of the potential role of diet and nutrition in the modulation of PDs, a brief review of the essential features of the pathogenesis of PD is necessary. Phagocytes, particularly the polymorphonuclear neutrophils (PMN), constitute the cellular hallmark of inflammatory response to the periodontal pathogens. In individuals with either intrinsic or acquired dysfunction of the PMN, periodontal tissues are broken down very rapidly, suggesting that the primary

role of the PMN in human periodontium is protective (10, 11, 55).

Intracellular killing of bacteria by PMN is mediated by *1*) reactive oxygen species, eg, hydrogen peroxide–myeloperoxidase system, *2*) oxygen-dependent lipid peroxidation, and *3*) oxygen-independent mechanisms that involve release of toxic granule components. The PMNs show not only antibacterial effects but also have the potential to damage periodontal tissues through release of lysosomal enzymes (5, 10, 56). Neutrophil granules contain a large family of more than twenty enzymes, but three proteolytic enzymes, namely, serine proteinase, elastase, and the two metalloproteinases (collagenase and gelatinase) have the greatest potential for tissue damage. Release of the reactive oxygen species and PMN granule contents to the extracellular environment may lead to local tissue destruction characteristic of PDs (1, 5, 9, 57). The lysosomal enzymes degrade both the fibrillar components and ground substance of the periodontal connective tissues.

Inflammation is the price the host pays for an effective and efficient defense system against periodontal pathogens. The inflammatory process also involves complex interactions between cells and potent soluble mediators known as cytokines. The latter constitute a diverse range of polypeptides produced by the phagocytes, T and B lymphocytes, fibroblasts, keratinocytes, and various endothelial cells. They include all the known interleukins (IL-1–12), interferons (IFN-α, -β, and -γ), tumor necrosis factors (TNF-α and -β), colony stimulating factors (G-CSF, M-CSF, and GM-CSF), the transforming growth factor β family, and other growth factors (30, 58, 59). Their actions include enhancement of the recruitment, proliferation, activation, and differentiation of white blood cells, fibroblasts, and osteoblasts, as well as mediating a wide range of metabolic changes. These changes include the acute-phase response, which plays a key role in promoting healing after tissue injury (30, 60). Cytokines interact extensively with the neutrophils and endothelial cells, and thereby may change the course of an inflammatory process (61, 62). The main members of the cytokine ''orchestra'' in bacterial infection are IL-1, IL-6, IL-8, TNF, and IFN-γ, as well as the counter-regulatory molecules TNF receptors and IL-1 receptor antagonist (IL-1Ra) (62). IL-1 in particular is of primary and strategic importance to the outcome of infections, particularly those caused by bacteria, because it is a potent inducer of synthesis of IL-8 (which is also called neutrophil activating protein-1) (63).

There are suggestions that increased concentrations of IL-1α, IL-1β, and TNF, particularly IL-1β in tissues from sites of PD, are important mediators of alveolar bone resorption (64). Additionally, IFN-γ, which inhibits the bone-resorbing action of IL-1β in vitro, is believed to play a role in preventing attachment loss in PD (1). These conclusions must however be considered provisional. The current concept is that tissue homeostatic mechanisms are regulated by cytokine cascades and networks rather than by individual cytokines (65). It is now known that the actions of combinations of cytokines in vivo are complex and not readily predictable from knowledge of the action of individual cytokines. For example, transforming growth factor-β (TGF-β) and platelet-derived growth factor (PDGF) each stimulates proliferation when applied individually to osteoblasts but inhibits growth of osteoblasts when applied together. Additionally, the ultimate effects of cytokines may vary depending on the preexisting potential of the target cell (65). For example, TGF-β induces cartilage cells to produce collagen early in development but this function is suppressed in mature cells (65). Finally, the reported suspected role of increased IL-1β production in PD (64) must be considered inconclusive because the production of a small amount of IL-1 concurrently with a large amount of the endogenous inhibitor IL-1Ra is a natural response in infectious diseases of bacterial origin (63). In healthy individuals, endotoxin induces 100-fold higher molar concentrations of the receptor antagonist IL-1Ra than of IL-1 (62, 66). Similarly, serum concentrations of TNF receptors are markedly increased during systemic gram-negative infection (62). Thus the destructive effects of a cytokine like IL-1β may be dependent mainly on inadequate production of the inhibitor IL-1Ra.

Nutrition and cytokine biology

With respect to cytokine biology, nutritional factors act at two main levels, namely, they influence synthesis and release of the cytokines and they affect the direct and indirect actions of cytokines on target tissues as well as influence the subsequent responses of these tissues (30, 67). PEM markedly reduces the ability to produce cytokine, hence the poor prognosis of inflammatory lesions in affected individuals (67–70). Experiments have demonstrated decreased circulatory IFN-γ in undernourished animals (71). Prominent reductions in IFN production and natural killer cell activity are also noted in vitamin A deficiency. IFN-γ is suspected to play a key role in inhibiting the bone-resorbing action of IL-1β, and indeed, active attachment loss in PDs is associated with reduced IFN-γ in the gingival crevicular fluid (1). PD is episodic with periods of healing alternating with periods of active tissue destruction (10). Factors responsible for the healing phase include IFN-γ, TGF-β, IL-4, and IL-1Ra (5), and synthesis of many of these factors is severely compromised in malnutrition (*see* references 30 and 67 for excellent reviews of dietary manipulation of the inflammatory response).

Malnutrition and the acute-phase protein response

The acute-phase response (APR), a nonspecific, beneficial reaction to tissue injury, includes fever, increased peripheral leukocyte count, hormonal changes, and alterations in the pattern of hepatic protein synthesis (60). APR is characterized by increased hepatic synthesis of various acute-phase proteins (APPs). The human APPs include C-reactive protein and serum amyloid A, which show up to a 1000-fold increase; α_1-acid glycoprotein, α_1-proteinase inhibitor, haptoglobin, and fibrinogen, which show a two- to fourfold increase; and ceruloplasmin (EC 1.16.3.1) and complement components C3 and C4, whose magnitude of increase is only 50% (60). Generally, the magnitude of the APR varies directly with the severity of the inflammatory state and the extent of tissue injury. These APPs enhance antioxidant defenses. C-reactive protein plays a role in complement activation (72), opsonization (73), and increasing platelet aggregation (74), whereas serum amyloid A is reported to inhibit the oxidative burst during inflammation (75). α_1-Proteinase inhibitor (α_1-AT) is believed to account for $\approx$90% of total proteinase inhibitory capacity of blood in humans.

Under normal conditions in well-fed individuals, the concentration of antigenic elastase (enzyme coupled with α_1-AT) in human gingival crevicular fluid is increased threefold after 3 wk of plaque accumulation, whereas the concentration of α_1-AT is elevated by a much higher margin (76). α_1-AT is particularly vulnerable to oxidative inactivation with consequent loss of elastase-inhibitory capacity, but this can be prevented by availability of adequate vitamin C (57). Although α_2-macroglobulin does not manifest acute-phase behavior in humans (60), synthesis of this inhibitor is reported in human gingiva (77). In a subsequent study, Giannopoulou et al (76) noted marked increases in gingival cervicular fluid concentrations of α_2-macroglobulin and functional elastase (enzyme coupled with α_2-macroglobulin) after 3 wk of dental plaque accumulation. α_2-Macroglobulin is synthesized by several cell types such as the fibroblasts, monocytes, macrophages, and hepatocytes, and along with related proteins, binds host or foreign peptides and particles, thereby serving as a humoral defense barrier (78).

It is now known that in children suffering from even mild malnutrition, the APR to infection is severely blunted, an observation that has serious prognostic implications in view of the central role of the APR in promoting tissue healing (79). Similarly, in rats fed a protein-deficient diet, there is a marked attenuation of the APR to trauma as evaluated by the circulating concentration of α_2-macroglobulin (a major APP and broad-spectrum protease inhibitor in rats) (80). The peak value of α_2-macroglobulin in the protein-deficient rats was only 44% of values in control animals and was delayed in time, attaining only 14% of control values after day 1 of trauma (81). α_2-Macroglobulin, with its unique internal cyclic thiol ester bond, interacts and captures virtually any proteinase whether self or foreign, suggesting a function as a "panproteinase inhibitor" (78). In the human, plasma α_2-macroglobulin varies from 1–5 g/L with a mean of 2 g/L (82). The findings of blunted APR in malnutrition are consistent with impaired synthesis of cytokines because cytokines in general, and IL-1 and IL-6 in particular, are believed to provide the principal stimulus for synthesis of the APPs (30, 67, 83, 84), although neurohumoral factors may also contribute (85).

Studies have examined the key nutrient requirements for increased synthesis of the APPs and other proteins whose syntheses increase during inflammation. Inflammatory disease may change the requirements for energy and for specific amino acids, particularly the metabolically interrelated glycine, serine, methionine, and cysteine (83). Responses to cytokines entail synthesis of substances rich in cysteine and glycine, for example, reduced glutathione, metallothionein, and some plasma proteins. The sulfur amino acids cysteine and methionine, which are usually very limiting in PEM, account for 32% of the total amino acid residues in human metallothionein. Similarly, during inflammation, there is a markedly increased requirement for the structurally simple, dietary nonessential amino acid glycine (which constitutes 34% of the amino acid residues in collagen). It is now known that in all periods of rapid growth, glycine may be the first limiting nutrient for protein deposition (86, 87). Recent studies have also shown that cysteine and glycine supplementation modulates the metabolic response to TNF-α in rats fed a low-protein diet (88).

Trace elements are present in several APPs, and Grimble (67) has carried out an extensive review of the modulatory effects of these micronutrients and the vitamins on the APPs and other proteins that undergo increased synthesis in response to cytokine production. For example, it has been shown that zinc deficiency impairs metallothionein synthesis in response to IL-1 and endotoxin (89).

Nutrition and neutrophil function

Secondary neutropenia can be caused by malnutrition, infections, and other conditions (61), and neutropenia intensifies the severity of bacterial infections. Early severe gingivitis and marked alveolar bone loss occur in neutrophil disorders such as cyclic neutropenia, agranulocytosis, and the Chediak-Higashi disease.

Through the use of a novel HPLC method that combines rapid separation with a highly sensitive coulometric electrochemical detection system, it is now known that ascorbic acid concentrations in human peripheral mononuclear leukocytes and perhaps in other cells could be as high as several millimolar, considerably higher than previously reported values (90). Human neutrophils contain 1.0–1.4 mmol ascorbic acid/L, found only in the reduced form, with $\geq$ 94% of the vitamin present unbound in the cytosol (91). Among the functions attributed to vitamin C in neutrophils are enhancement of chemotaxis, facilitation of the oxidative destruction of microorganisms, preservation of neutrophil integrity, and protection of host tissue by acting as a reducing agent to neutralize the bacterial products produced by neutrophils during the metabolic respiratory burst (91). In a study of bactericidal activity in neutrophils from scorbutic guinea pigs, Goldschmidt (92) noted that the neutrophils had 16 times less ascorbate than did the control leukocytes, killed only 12% of phagocytosed actinomycetes, and had no chemotactic responses in vitro.

The balance between the interactions of reactive oxygen metabolites, proteinases, and antiproteinases determines the ability of phagocytes to damage the periodontal tissues. The roles of nutrients either as antioxidants or as key components of antioxidant enzymes are well known (93). In protein deficiency, the antioxidative enzyme activities are significantly depressed with consequent enhancement of tissue lipid peroxidation (94). Marked tissue depletion of the key antioxidant nutrients (eg, zinc, α-tocopherol, β-carotene, and ascorbic acid) as well as of γ-glutamyl-cysteinyl-glycine occurs in human PEM and in other forms of malnutrition (93, 95). γ-Glutamyl-cysteinyl-glycine is normally present in cells at concentrations of 0.5–10 mmol/L, accounts for $>$ 90% of cellular nonprotein thiols, and serves as a major cellular antioxidant as well as an important modulator of T-cell activation (96). It is also required for synthesis of the leukotrienes, which are important mediators of inflammation. Tissue concentration of the precursor amino acid, cysteine, is also markedly decreased in PEM (97), and there is now good evidence that this sulfur amino acid functions in its own right as a cytokine that regulates the functional activity of lymphocytes (98).

Trace elements are present in several antioxidant enzymes. Studies have shown that the ability of rats to increase plasma ceruloplasmin concentrations, and copper-zinc superoxide dismutase in the lung, in response to the dual stress of endotoxin and high oxygen concentration, is impaired by copper deficiency (67).

Other relevant features of malnutrition

Most of the immunologic dysfunctions in human PEM and in deficiencies of specific essential nutrients are reminiscent of the changes usually encountered in individuals seropositive for human immunodeficiency virus (HIV) (25, 99). Severe PD is a common feature of HIV infection (100). Inverted ratios of helper to suppressor T cells is a universal finding in PEM (25). Genco (5) has underscored the observation that in PD lesions, the ratio of T helper to T suppressor cells is decreased more than in health or in the peripheral blood, and that the same phenomenon is seen in gingival tissues as well as in the peripheral circulation of patients with acquired immune deficiency syndrome (AIDS).

Also encountered in PEM and other forms of malnutrition are endocrine function adaptations usually involving decreased syntheses of insulin, somatomedin-C, estrogen, androgen, and triiodothyronine, with increased production of growth hormone, cortisol, and reverse triiodothyronine among others (101). Increased circulating concentrations of glucocorticoids suppresses immunological and inflammatory responses, wound healing, bone matrix formation, collagen synthesis, and mitotic activity of the gingival epithelium (102). Grimble (67) has reported that increased circulating glucocorticoids not only stimulate muscle proteolysis and production of APPs but also inhibit cytokine production by the macrophages. Decreased insulin production with increased blood glucose impairs cellular uptake of ascorbate, and may offer a partial explanation for the frequent association between severe PD and diabetes.

In malnutrition, particularly PEM and ascorbic acid deficiency states, there is a significant increase in tissue histamine concentrations (103, 104). Ascorbic acid has an important role in detoxifying histamine in various conditions of stress (105). When plasma vitamin C concentrations in humans fall much below 0.7 mg/100 mg, there is a prominent increase in blood histamine concentrations (104). Persistent high endogenous circulating concentrations of histamine in malnutrition will elicit hyperemia and increased capillary permeability (106), as well as diminished PMN chemotaxis (107). A study of the effects of controlled ascorbic acid depletion and supplementation on human periodontal health has revealed that although vitamin C depletion is not associated with severe PD, measures of gingival inflammation and bleeding vary with changes in plasma and leukocyte ascorbate concentrations (108).

General comments

Complex interactions exist between nutritional status of the host, infections, and many other diseases that have an inflammatory component. The most common types of PDs are inflammatory lesions elicited by specific pathogens in the dental plaque. Under normal conditions, inflammatory stimuli not only promote release of reactive free radicals but also bring about extensive metabolic changes that are modulated by potent soluble mediators known as the cytokines. Defective synthesis of these important mediators impairs the APR to infections even in the mildly malnourished, and the resulting diminished hepatic synthesis of the APPs militates against the healing process (79).

In malnutrition, there is usually a variable degree of tissue depletion of key nutrients including the major antioxidant nutrients. We now have in our hands sophisticated tools for probing the cellular and molecular effects of these various nutrients and how their deficiency or imbalance relates to the pathogenesis of PD.

The crucial role of certain specific plaque pathogens in the genesis of periodontal destruction is no longer in dispute. It is however equally necessary to recognize that like other bacterial infections, PDs are "...neither self-subsistent, circumscribed, autonomous organisms, nor entities which have forced their way into the body, nor parasites rooted on it but ... represent only the course of (physiological) phenomena under altered conditions" (109). Available evidence from the molecular and cellular effects of malnutrition suggests that nutrient deficiencies and imbalance have the potential to influence the biological gradient and natural history of periodontal infections. Good nutrition is therefore a useful adjunct in delaying and/or mitigating severe inflammatory periodontal lesions while at the same time promoting healing, but is of very limited value if the chronic inflammatory stimuli from dental plaque are not removed.

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