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Topical Review

Metabolic Regulation of Glucose Transport

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Introduction

Facilitated transport of glucose across plasma membranes, a passive Na^+-independent process present in all mammalian cells, is mediated by a family of homologous glycoprotein molecules that exhibit characteristic kinetic properties and are expressed in a tissue-specific manner. Because glucose is a universal energy-producing substrate, the regulation of its transport into cells is of fundamental importance in cellular homeostasis. This review is primarily focused on regulatory pathways that modulate the rate of glucose transport in response to alterations in cellular metabolism, with specific reference to conditions associated with increased demand for glucose utilization. Mechanisms mediating the regulation of glucose transport in response to a variety of other stimuli are also briefly considered.

Glucose Transport Is Enhanced under Many Conditions and by Many Stimuli

Table 1 summarizes a number of conditions and stimuli that are known to increase the rate of glucose transport. In several instances the stimulatory effect of a particular agent has been shown to occur in a variety of tissues and cell types, and in some cases the mediating mechanisms have been well defined. In Table 1 the enhancement of glucose transport is classified according to whether the stimulation is in response to increased demand for glucose (Group I), or occurs instead in the absence of an obvious increase in the demand for glucose (Group II). It should be emphasized that the signals leading to enhanced glucose transport in response to the conditions and agents comprising Group I are unknown, and it is possible that a rapid decrease in the intracellular concentration of glucose itself or of one of its metabolites may serve as the trigger [66, 73, 98, 105]. If this is the case, then such a change may likewise occur in response to one or more of the agents or conditions listed under Group II (Table 1) despite the fact that these transport responses are not obviously associated with increased metabolic demand for glucose. In addition, although the transport response to many of the conditions and agents listed under Group I is associated with higher rates of glucose metabolism, an increased demand for glucose *per se* may not be the trigger, and the response in some of the instances may well be part of a more general cellular response to severe perturbations such as those associated with cell injury [78, 102, 106].

Modulation of Glucose Transport Rate Commonly Occurs through Changes in Plasma Membrane "Permeability" to Glucose

Figure 1 is a cartoon depicting some of the cellular steps in the uptake and metabolism of glucose. The net rate of transport of glucose into cells (J) is a product of the permeability characteristics of the plasma membrane to glucose (P) and the glucose concentration difference across the plasma membrane (ΔC); i.e., $J = P \times \Delta C$. In the majority of mammalian cells, glucose, upon entry into cells, is rapidly phosphorylated to glucose-6 phosphate (G-6-P), such that the intracellular concentration of non-phosphorylated glucose is only a small fraction (usually less than 10%) of the glucose concentration in

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Table 1. Conditions and stimuli associated with a stimulation of facilitated glucose transport

I. In response to increased demand for glucose:
a. exercise
b. repeated electrical stimulation
c. transformation and associated "aerobic glycolysis"
d. exposure to thyroid hormone
e. glucose deprivation
f. hypoxia
g. inhibition of oxidative phosphorylation
h. exposure to alkaline pH
II. In the absence of a primary increase in the demand for glucose:
a. exposure to insulin
b. exposure to serum and growth factors
c. activation of protein kinase C
d. exposure to adenosine
e. exposure to cholera toxin
f. exposure to group IIb metals (Cd, Zn, Hg)
g. increased intracellular calcium concentration
h. inhibition of protein synthesis

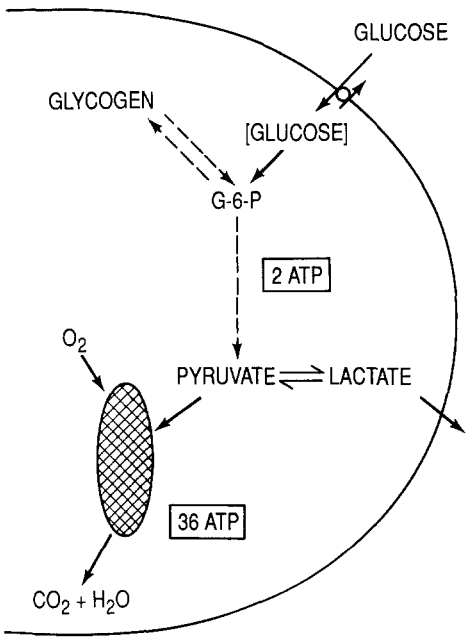

Fig. 1. Cellular steps in the uptake and metabolism of glucose.

the external medium. In such "low-glucose" cells the rate of glucose metabolism is limited by the rate of glucose transport [22, 29, 66]. Because the concentration of intracellular glucose in these cells is already low, a decrement in internal glucose concentration will not significantly increase ΔC, and hence the net rate of transport will not increase. A significant enhancement of glucose transport in such cells can thus take place only if there is a comparable increase in P. In contrast, in a few cell types such as hepatocytes and human erythrocytes which contain a very high number of glucose transporters in their plasma membranes, the rate of glucose entry is greater than that of glucose phosphorylation (and metabolism) and intracellular glucose concentration approximates the concentration of glucose in the external medium [12, 22, 29]. In such "high-glucose" cells, glucose metabolism rather than transport is rate-limiting for glucose utilization [22]. In these latter cell types, moreover, a modest decrease in intracellular glucose concentration—for example brought about by increased metabolism of glucose—could lead to a large fractional increase in the value of ΔC, and hence greatly augment the net rate of glucose transport. Finally, it is worth noting that a rise in external glucose concentration (as is commonly seen in diabetes) can lead to near-proportional increases in the rate of glucose entry into cells expressing the high K_m isoform of the transporter, GLUT2 (*see below*).

Complete oxidation of glucose to CO_2 and H_2O in mitochondria yields 18 times more ATP per mole of glucose than the partial metabolism of glucose to two moles of lactate (Fig. 1). Inhibition of mitochondrial oxidative phosphorylation moreover leads to a marked increase in the demand for ATP synthesis from metabolism of glucose to lactic acid, and it has been shown that the facilitation of glycolysis under these conditions is in large part due to an activation of phosphofructokinase, the rate-limiting enzyme in the glycolytic pathway [59, 76]. (The tonic suppression of glycolysis under aerobic conditions has been recognized for over 100 years and has been termed the "Pasteur effect" [77]). An augmented demand for enhanced glycolysis is also brought about by a variety of other stimuli such as a reduction in intracellular hydrogen ion concentration, contractile work, and a number of hypermetabolic conditions [66, 68]. As noted above, however, in the majority of cells and tissues intracellular glucose concentrations are low, and glucose transport itself is rate-limiting for glucose utilization [22]. Because in many instances glycogen stores are also minimal, it follows that, in such "low-glucose" cells, a sustained enhancement of glycolysis requires a corresponding stimulation of glucose transport. The central focus of this review is on mechanisms mediating the increase in plasma membrane "permeability" to glucose, P, that lead to this stimulation of glucose transport.

Glucose Transporter (GLUT) Isoforms Are Members of a Family of Homologous Glycoprotein Molecules

Recent biochemical and molecular-biological evidence indicates that facilitated glucose transport is mediated by at least five homologous transporter

isoforms (GLUTs) that are differentially expressed in various tissues [69, 79, 81]. These transporters are all believed to possess a similar two- and three-dimensional membrane topography that includes 12 trans-membrane helices [79]. In addition, mammalian glucose transporters exhibit a high degree of sequence-homology to transporters found in other species including cyanobacteria, *Escherichia coli*, yeast, algae, protozoa, and plants [38, 61]. A widely distributed GLUT isoform, GLUT1, is expressed at high levels in rat brain (blood-brain barrier), human erythrocytes, and placenta, and is present at lower levels in virtually all tissues [21, 69, 75, 79, 107]; this 492-amino-acid polypeptide is highly hydrophobic (~50% of the polypeptide lies within the lipid bilayer), and the rat and human forms show 98% homology [8, 70]. This isoform is expressed in HepG2 cells (a human hepatocarcinoma cell line) and is the predominant isoform expressed in a variety of other cell culture systems [70, 81]. GLUT1 as it exists in plasma membranes has been reported to be a multimeric protein with the functional form being a homo-tetramer [41, 80]. Glucose transport mediated by GLUT1 exhibits a K_m of 2–5 mM for glucose (the latter value approximating the glucose concentration in the blood) and shows a K_I of $\sim 1 \times 10^{-7}$ M for cytochalasin B [69, 79, 81]. A second isoform (GLUT2), bearing 55% homology to GLUT1, is expressed in liver, intestine, pancreatic β-cells, and kidney [69, 81, 94]. Transport mediated by GLUT2 exhibits a lower affinity for glucose (K_m of 25–50 mM for glucose) and shows a K_I of $\sim 1 \times 10^{-6}$ M for cytochalasin B [1, 69]. The physiological role of this second isoform in the regulation of glucose-stimulated insulin release from β-cells is currently under active investigation [69, 79]. A third form of the transporter (GLUT3), bearing 64% homology to GLUT1, is expressed in a variety of tissues in the human, while its expression in the rat appears to be restricted to the brain [71]. The ubiquitous distribution of this isoform in many human tissues (along with that of GLUT1) suggests that GLUT3 has an important role in the basal transport of glucose [79]. A fourth form of transporter, GLUT4, bears 65% homology to GLUT1 and is specifically expressed at high levels in tissues in which the rate of glucose transport is markedly augmented by insulin; such tissues include cardiac and skeletal muscle and adipose tissue [69, 81]. This isoform was initially referred to as the insulin-regulatable glucose transporter (IRGT). A fifth isoform (GLUT5), having only 42% homology with GLUT1, has a more limited tissue distribution [48] and may function as a fructose rather than a glucose transporter [11]. Still other isoforms of the transporters may well be identified in the future.

The determinants of the expression of the different GLUT isoforms in various tissues are at present not well understood, and it is important to point out that more than one isoform may be expressed in a single tissue or cell. Examples include the expression of GLUT1 and GLUT4 in heart, skeletal muscle, adipocytes, 3T3-L1 adipocytes, and L6 myotubes [69, 79], and the expression of GLUT1, GLUT3, and GLUT5 in Caco-2 cells (a human colonic adenocarcinoma cell line), where each isoform exhibits a distinct subcellular distribution [35].

Stimulation of Glucose Transport Reflects an Increase in Transport V_{max} rather than a Decrease in Transporter K_m for Glucose

Modulation of the rate of glucose transport through changes in the affinity of the transporter for glucose (K_m) has been proposed in the past, especially with respect to insulin action [18, 60, 104]. The importance of this mechanism, however, has not been studied in a systematic fashion, and in recent years most studies have suggested that mechanisms leading to large changes in the V_{max} of transport are of greater physiological importance [68, 69, 79]. Potential mechanisms mediating the enhancement of glucose transport V_{max} are listed in Table 2 and fall into two major categories: (*A*) mechanisms associated with an increase in the number of glucose transporters in the plasma membrane, and (*B*) mechanisms associated with no change in the number of glucose transporters in the plasma membrane.

Increased cell transporter content—usually reflecting increased synthesis of transporters—must necessarily be a relatively slow response because of the long time periods (hours) required for pretranslational and translational events to take place and for whole-cell GLUT content to attain an elevated new steady state; the increase in cell GLUT content could then lead to its increased abundance in the plasma membrane. Increased cell and plasma membrane GLUT content (Mechanism A.1 in Table 2) has been described in skeletal muscle after exercise training and following chronic repeated electrical stimulation [19, 43, 50, 85], although an increase in the intrinsic activity of plasma membrane transporters has additionally been reported [19, 49, 50]. Transformation, a process associated with an increased metabolism of glucose to lactate despite the presence of oxygen (a condition termed "aerobic glycolysis" [100], is attended by increases in glucose transport rate and transporter abundance [24, 46, 88, 106]; here the increase in GLUT content is mediated by enhanced GLUT biosynthesis resulting from enhanced transcription and translation of GLUT mRNA [106] as well as by decreased glucose trans-

Table 2. Potential mechanisms mediating the stimulation of glucose transport characterized by increased V_{max} of transport

A. Increased plasma membrane glucose transporter abundance due to:
1. Increased cell transporter content
2. Translocation from intracellular sites
B. Unchanged plasma membrane glucose transporter abundance:
1. Activation of pre-existing nonfunctional transporters
(a) Acquisition of glucose binding ability
(b) Acquisition of transport capability
2. Enhancement of the activity of pre-existing functional transporters

porter degradation [88]. Exposure to serum and specific growth factors (such as platelet-derived growth factor) and stimulation of protein kinase C by phorbol esters result in a dramatic enhancement of glucose transport and of GLUT1 gene transcription [3, 34, 42, 51, 57, 74, 86]. In addition, activation of protein kinase C by phorbol esters or tumor necrosis factor-α leads to a decrease in the degradation of GLUT1 mRNA, a phenomenon thought to be related to the presence of several AUUUA motifs in the 3′-untranslated region of GLUT1 mRNA [91]. The hypermetabolic state induced by thyroid hormone is likewise associated with a stimulation of glucose transport [10, 55, 87, 101]. Although the early effect of T_3 on glucose transport in chick cardiac myocytes has been reported to be independent of protein synthesis [32], other reports indicate a more delayed stimulation of glucose transport occurring after several hours of exposure to the hormone that is attended by proportional [101] or only slight [55] increases in whole-cell transporter content. A reduction of external glucose concentration below critical levels (<0.5 mM) also results in a stimulation of glucose transport that occurs after several hours [39, 54, 62, 73, 95, 99] and is often, but not always [52], associated with increased cell and plasma membrane GLUT content. Although in many instances the induction of glucose transporters in response to glucose deprivation is mediated pretranslationally [54, 62, 95], in some reports the increase in cell GLUT content has not been associated with an increase in the level of its encoding mRNA [40]. It is worth noting that prolonged hyperglycemic states are associated with decreased transport of glucose across the blood-brain barrier [31], whereas an increased rate of transport has been reported in hypoglycemic states [63]. Because glucose is the major energy substrate of the brain, the importance of these latter regulatory phenomena for normal brain metabolism is evident. Decreased GLUT4 content has been reported in diabetes in the rat [9, 26, 28] as well as in morbid obesity (but not in diabetes) in human subjects [25].

Hypoxia, inhibition of oxidative phosphorylation, and exposure to alkaline pH have all been shown to increase the rate of glucose transport in a variety of experimental models [34, 44, 47, 64, 82, 89, 96, 103, 105]. As discussed earlier, because these stimuli greatly augment the demand for glucose utilization, a corresponding increase in the availability of glucose through enhanced transport must occur rapidly if cells are to survive these metabolic insults. Such conditions and stimuli occur frequently in nature, suggesting that the associated glucose transport response may have been present early on and been preserved during the course of evolution. Consistent with this conjecture is the finding that the stimulation of glucose transport in response to inhibition of oxidative phosphorylation occurs in yeast, in avian erythrocytes, and in a variety of mammalian cells and tissues including cardiac and skeletal muscle [53, 66, 67, 103, 108]. In studies on the response of Clone 9 cells (a rat liver cell line) to inhibition of oxidative phosphorylation, it has recently been shown that the stimulation of glucose transport occurs in two phases: an early phase (0–2 hr) during which no change in cell GLUT1 content is observed, and a late phase (8–24 hr) that is associated with increased cell and plasma membrane GLUT1 abundance and is preceded by increased levels of GLUT1 mRNA [89]. Similar inductions of mRNA and GLUT protein have also been reported in bovine aortic endothelial cells and in L6 myotubes exposed to hypoxia for prolonged periods [5, 58]. These studies demonstrate that, in contrast to the response occurring soon after the initiation of the stimulus (to be discussed below), the late-phase response is associated with an increase in cell GLUT content that is mediated by transcriptional and/or post-transcriptional regulatory mechanisms [5, 58, 89].

Acute Stimulation of Glucose Transport Is Mediated by Both Translocation and Activation Mechanisms

The stimulation of glucose transport in response to conditions and stimuli *not* associated with increased cell GLUT content is of a more acute nature, although the transport response in some instances (e.g., following exposure to inhibitors of protein synthesis) is somewhat delayed and occurs only after several hours. The most widely studied example of rapid regulation of glucose transport is the response to insulin. Following the initial demonstration in 1980 by Cushman and Wardzala of an increased

number of cytochalasin B binding sites in plasma membrane fractions of adipocytes exposed to insulin [17], and by Suzuki and Kono of increased glucose transport in plasma membrane vesicles isolated from adipocytes treated with insulin [93], a large body of evidence has now accumulated demonstrating that exposure to insulin results in a translocation of transporters (predominantly GLUT4) from an as yet not fully defined intracellular site(s) to the plasma membrane [45, 69, 79, 81] (Mechanism A.2 in Table 2). In addition, recent evidence indicates that under basal conditions GLUT4 in adipocytes circulates rapidly (with a half-time of ~2 min) between the plasma membrane and a much larger pool of intracellular sites (the latter accounting for 94% of the total sites), and that insulin increases the rate of insertion and decreases the rate of removal of GLUT4 from the plasma membrane [45]. The rapid circulation of GLUT sites has been verified in 3T3-L1 cells under basal conditions, but only an insulin-induced increase in the rate of GLUT4 insertion (as opposed to a change in its rate of removal) could be demonstrated [109]. The mechanisms by which insulin induces these changes in rates of insertion and withdrawal from the plasma membrane are currently unknown. Despite the documented importance of translocation of GLUTs to the plasma membrane in mediating the stimulatory effects of insulin on glucose transport, many studies suggest the possibility that, in addition to the dominant role of translocation, insulin induces an activation of glucose transporters [45, 72, 92]. This inference has been derived from the finding that in a number of instances the fractional increment in plasma membrane GLUT sites in response to insulin is less (by two- to threefold) than the fractional increase in the rate of glucose transport, suggesting that the catalytic rate of individual transporters is augmented [30, 45, 92]. It should be noted, however, that such comparisons are based on the assumption that estimations of the number of GLUTs in the plasma membrane fraction are accurate, a premise that has been difficult to establish unequivocally because of uncertainties about the purity of plasma membranes obtained by currently available cell fractionation procedures. Other evidence suggesting the role of transporter activation in insulin action has been provided by Gibbs et al. [30]; in detailed time course studies on the effect of insulin on 3T3-L1 adipocytes these latter workers have shown that an increase in plasma membrane GLUT sites precedes the increase in glucose transport rate.

Activation of glucose transporters pre-existing in the plasma membrane may result from one or more mechanisms that convert either inactive or "hypoactive" transporter molecules into active forms (Mechanisms under B in Table 2). The transport of glucose by a transporter can conceptually be separated into at least two steps: the interaction of glucose with the glucose-binding site of the transporter, and the subsequent translocation of glucose across the plasma membrane. Hence, an activation of previously inactive transporters could involve acquisition of either glucose binding activity or of "transport capability" (Mechanism B.1). It is also possible that the catalytic turnover rate of previously active sites can be increased and lead to an enhancement of glucose transport rate (Mechanism B.2). Although the molecular mechanisms underlying such activation steps remain to be identified, it is possible that protein-protein interactions (between identical GLUT subunits or between GLUT and other regulatory proteins), covalent modifications of GLUTs, or alterations in the lipid microenvironment of GLUTs within the plasma membrane play a role in transporter activation. An important example of modulation of glucose transporter activity has been provided in studies demonstrating that phosphorylation of GLUT4 is attended by a decrease in its intrinsic activity [84]. Earlier studies had demonstrated that increased phosphorylation of GLUT4 sites resulting from exposure to okadaic acid (an inhibitor of type I and IIa phosphatases) was associated with a modest stimulation of glucose transport and increased plasma membrane GLUT4 sites [56]. In addition it was shown that okadaic acid partially inhibited the ability of insulin to stimulate glucose transport, and that exposure to insulin led to a dephosphorylation of GLUT4 sites [56]. The observations that phosphorylation of GLUT4 leads to a decrease in its catalytic activity [84], and that exposure to insulin is associated with a dephosphorylation of GLUT4 sites [56, 84], provide an explanation for the enhancement of the intrinsic activity of GLUT4 by insulin.

It has been proposed that an activation of glucose transporters pre-existing in the plasma membrane is the dominant mechanism mediating the stimulation of glucose transport induced by group IIb metal ions, by inhibitors of protein synthesis, and in the early response to inhibition of oxidative phosphorylation. In these instances the conclusion that there is transporter activation has been based upon the finding of a large increase in the rate of glucose transport despite little if any increase in the number of plasma membrane GLUT sites. Group IIb metal ions have been found to enhance the rate of glucose transport in rat adipocytes and in both 3T3-L1 fibroblasts and adipocytes [23, 36]. In detailed studies on the mechanism underlying this stimulation it was shown that the increase in transport induced by Cd^{2+} is not attended by any demonstra-

ble increase in plasma membrane GLUT content [36]. The stimulation of glucose transport in response to inhibition of protein synthesis has been documented in a number of studies using a variety of cell lines [2, 4, 14, 15, 27, 44]. The delayed onset of stimulation, usually occurring after 2–3 hr of exposure, and the lack of any increase in plasma membrane GLUT content [2, 15, 37] raise the possibility that, upon inhibition of protein synthesis, the cellular content of a rapidly turning-over protein having a negative modulating effect on GLUT function decreases, thereby removing a presumed tonic inhibition of GLUT transport function. Confirmation of the validity of this hypothesis awaits the demonstration of such a protein. The stimulation of glucose transport in rat heart in response to hypoxia is similarly associated with only a modest increase in the abundance of transporters in the plasma membrane [65, 67, 103]. Likewise, the early and marked stimulation of glucose transport in Clone 9 cells in response to inhibition of oxidative phosphorylation is associated with only a slight, if any, increase in plasma membrane transporter content [89, 90], strongly suggesting that the enhanced rate of transport under such conditions is mediated by activation of transporters pre-existing in the plasma membrane. Because the glucose transport response to inhibition of oxidative phosphorylation or hypoxia has been well documented in avian erythrocytes [66, 108], and because these "simple" cells, though nucleated, synthesize little if any RNA or protein [13], it is possible that these cells may prove particularly useful in future studies of mechanisms underlying GLUT activation.

The Signals Mediating the Stimulation of Glucose Transport in Response to Increased Metabolic Demand Remain Unknown

The signals mediating the enhancement of glucose transport in response to inhibition of oxidative phosphorylation, hypoxia, glucose starvation, incubation at alkaline pH, and other conditions associated with increased metabolic demand for glucose remain unknown. It is of interest to note, however, that the time course, the reversibility, and the magnitude of the transport response are similar in all of these conditions. This raises the possibility that these responses share a common signaling mechanism. Early studies focused on the possible role of changes in the concentration of specific intracellular metabolites (such as ATP and G-6-P) that might play a role in triggering the glucose transport response [105], but no specific chemical alteration was found that correlated well with the enhanced glucose transport. In other studies, on the regulation of glucose transport induced by glucose starvation, it was noted that both 2-deoxyglucose [98] and 3-*O*-methylglucose [52, 98] can prevent the glucose transport response and reverse the induced stimulation of transport. These findings, especially those involving 3-*O*-methylglucose, are difficult to understand because the latter glucose analogue, while readily transported by glucose transporters, is only minimally metabolized. It was concluded that the transport of glucose (or one of its analogues) is in itself sufficient to prevent the stimulation in response to glucose deprivation [98].

An increase in intracellular free calcium concentration has been reported to stimulate glucose transport in a wide variety of cell systems [6, 7, 16, 20, 33, 83, 97, 110] and has been proposed to play a mediating role in insulin action [33]. Elevations in cell calcium concentration would be expected to occur, at least transiently, in cells under metabolic stress especially if such conditions are associated with a decrease in cell ATP concentration. Decreased cell ATP itself would lead to a fall in calcium extrusion by Ca-ATPase and by the Na/Ca exchanger, the latter resulting from the rise in cell Na^+ concentration secondary to decreased Na,K-ATPase function. The stimulation of glucose transport is observed when intracellular calcium is increased by use of calcium ionophores or by serum and growth factors [7, 16, 20], and in skeletal muscle the increment in internal calcium concentration associated with enhanced glucose transport is less than that required to increase muscle tension [110]. In detailed studies using compounds that act to increase calcium release from muscle sarcoplasmic reticulum, it has been shown that small increments in cell calcium that are too low to result in any change in cell ATP or creatine phosphate, or in muscle tension, result in a dramatic stimulation of glucose transport, and that the effect is blocked by inhibitors of calcium release [110]. Whether an elevation in intracellular calcium is the proximate signal in the stimulation of glucose transport observed in response to different conditions associated with increased metabolic demand for glucose, and if so, what the mechanism(s) are by which an increase in intracellular calcium leads to a stimulation of glucose transport and activation of glucose transporters, remain to be elucidated.

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