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Review article

Pathophysiology of the Endothelium

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Abstract

The endothelium is a single layer of cells that lines the luminal surface of blood vessels. It is strategically situated to act as a direct interface between the components of circulating blood and local tissue. In the past, the endothelium was considered to be inert, described as a `layer of nucleated cellophane', with only non-reactive barrier properties. However, it is now becoming clear that endothelial cells actively and reactively regulate numerous local blood vessel functions, including barrier function, vascular tone, hemostasis, inflammation, immunity and the growth of new vessels.

When affected by infection, stress, hypertension or dyslipidemia, endothelial cells undergo changes resulting in ''dysfunction''. Endothelial dysfunction or activation contributes to a variety of disease states. This review will be describing the main functions of the endothelium -in an attempt to demonstrate its importance and complexity.

Keywords: *Endothelial cells; barrier function; vascular tone; hemostasis; inflammation; immunity; angiogenesis*

Introduction

The endothelium is a thin layer of cells that lines the interior surface of blood vessels (on the intima), forming a connection between circulating blood in the lumen and the rest of the vessel wall. The cells that form the endothelium are called endothelial cells (ECs) [1]. They in arteries and veins appear more continuous and thicker than those in capillaries which are fenestrated and thinner to allow for exchange of metabolites and gases [2].

EC is lined with a very fine and fragile layer called the glycocalyx. It consists of glycoproteins, glycosaminoglycans and proteoglycans. Glycosaminoglycans are including heparin sulfate, a cofactor for antithrombin III that amplifies its anti-thrombotic properties, and dermatan sulfate, which interacts with heparin cofactor II. Destruction of the glycocalyx leads to increased capillary permeability [3].

Physiological Functions of the Endothelium

Endothelium has many functions in vascular biology including endothelium transport function (barrier function), regulation of blood clotting (thrombosis and fibrinolysis), regulation of vascular tone (vasoconstriction and vasodilatation), inflammation, immunity (innate and acquired) and formation of new blood vessels (angiogenesis) [4].

Endothelium Transport Function (Figure 1)

An important function of the endothelium is to regulate the transport of liquid and solutes across the semi-permeable vascular endothelial barrier [5]. Materials can cross the endothelium via two routes: through the cell body (transcellular) or between the cells (paracellular or intercellular) [6].

Paracellular Permeability "Cell–Cell Junctions

Endothelial cells are joined to each other and form junctional complexes consisting of tight junctions and adherence junctions, which are the sites of Diffusional transport of solutes. Lipid soluble molecules, such as oxygen and carbon dioxide, readily pass through the lipid components of endothelial cell membranes. Water-soluble molecules, however, must diffuse through water-filled pathways formed in the

capillary wall between adjacent endothelial cells. These pathways, known as pores, are not cylindrical holes but complex passageways formed by irregular tight junctions [7].

Within multicellular organisms, several organs are relatively independent of whole body homeostasis and are wrapped by endothelial cell sheets. A clear example is the blood-brain barrier, made of highly specialized ECs whose tight junctions protect the central nervous system [8].

Transcellular Permeability

Most pores permit only molecules with a radius < 3 to 6 nm to pass through the vessel wall. These small pores only allow water, inorganic ions, glucose, amino acids, and similar small, water soluble solutes to pass; they exclude large molecules, such as serum albumin and globular proteins as well as blood cell components [9].

An alternative pathway for water-soluble molecules through the capillary wall is via vesicular transcytosis, which is a combination of endocytosis, vesicular transport across the cell, and exocytosis [10]. Vesicle-mediated transcytosis occurs when plasma proteins bind to albumin glycoprotein (gp60) receptors on the endothelial cell surface [11]. Plasma proteins are concentrated in caveolae "cave-like", which then undergo endocytosis and form vesicles. Vesicle attaches to microtubules in the cell's cytoskeleton and is moved across the cell by a process known as vesicular transport [5]. This process can be completed by individual vesicles transport from the apical to basolateral membrane of an endothelial cell, or by connected vesicles called Vesiculo-Vacuolar organelles (VVOs) that form channel-like [12]. At the opposite side of the epithelium, the contents of the vesicle are expelled into the interstitial fluid by exocytosis. Transcytosis makes it possible for large proteins to move across a cell membrane and remain intact [13]. It may be an important mechanism of transport of insulin, transferrin, and albumin [14]. The vesicle-mediated transport pathway is insensitive to transendothelial gradients of oncotic and hydrostatic pressures and the size of the transported molecule [5].

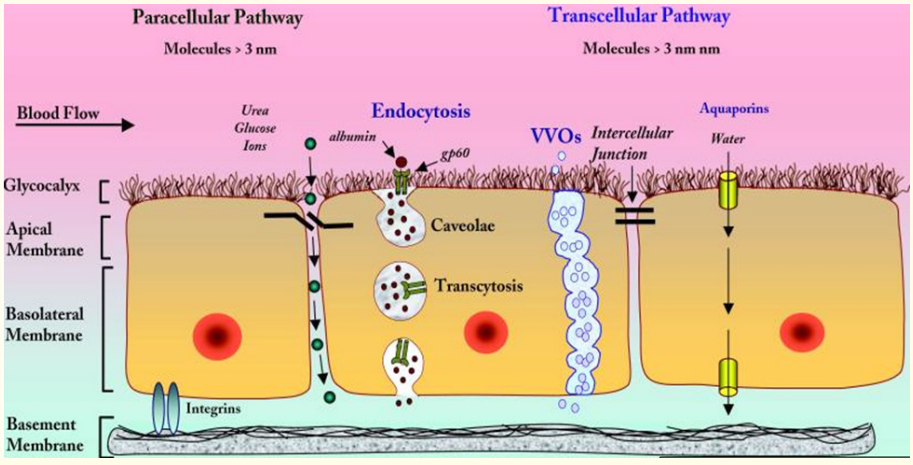

Figure 1: *Endothelium transport mechanisms. (Dolly and Asrar, 2006).*

Regulation of Hemostasis and Fibrinolysis

When a blood vessel is injured, the injury initiates a series of reactions, resulting in hemostasis. It occurs in three stages; vasoconstriction, platelet plug formation and coagulation of blood. ECs play a role in each step of this process [15].

Prothrombic Activity of Endothelial Cells

ECs play an important role in the vasoconstriction by release of endothelin and thromboxane A2 [16]. Vasoconstriction is followed by platelet adhesion to the vascular wall. ECs synthesize von Willebrand factor (VWF), fibronectin and thrombospondin. VWF forms bridges between the sub-endothelial structures and a specific receptor in platelet membrane. It also, serves as a 'glue' linking platelets to the subendothelial matrix and to other platelets [17]. Endothelial fibronectin cross-links fibrin monomers, and endothelial thrombospondin reduces local fibrinolysis and promotes platelet aggregation [15]. ECs synthesize factor V. They have, also, specific binding sites for factors IX/IXa and factor Xa. These binding sites localize the coagulation process to the endothelial surface and suppress their circulation [16].

The most important role of the endothelium in coagulation mechanism is the release of tissue factor (TF). Expression of TF on their surface results in the initiation of the extrinsic pathway of blood coagulation. TF binds and activates factor VII. The TF-VIIa complex in turn mainly activates factor X [17]. The contact between coagulation factors and the vascular wall occurs either via non-specific physical interactions or through specific receptors synthesized by ECs [9].

Antithrombotic Activity of Endothelial Cells

The endothelium normally provides a non-thrombogenic surface because it is very smooth and contains, for example, heparan sulfate which acts as a cofactor for activating antithrombin, a protease that inactivates several factors in the coagulation cascade. Presence of -ve charged protein layer - glycocalyx- in endothelium repels clotting factors and platelets. While, the most important anticoagulant activity of the endothelium is the prevention of formation of thrombin. Also the endothelium produces TF pathway inhibitor, which binds to activated factor X and then inhibits the tissue factor-activated factor VII complex [16].

Endothelium also opposes primary hemostasis by liberating vasodilator substances [prostacyclin (PGI2), NO] and ADPases that catabolize ADP, one of the most potent platelet activators [17]. PGI2 is a powerful inhibitor of platelet aggregation. It also inhibits binding of factor VIII/vWf and fibrinogen to their specific receptors on platelet membrane surface [16].

Role of ECs in Fibrinolysis

Endothelial cells produce thrombomodulin, a thrombin-binding protein, on their surfaces. TM combines with thrombin to form thrombomodulin- thrombin complex. This complex convert's protein C to activated protein C. Protein C when activated, it inhibits both of active factor V&VIII (in presence of protein S) and tissue plasminogen activator inhibitors. In presence of urokinase plasminogen activator (u-PA), both steps will convert plasminogen to plasmin. Plasmin breakdown fibrin clot into fibrin degeneration (excreted in urine). The absence of protein C leads to uncontrolled intravascular clotting and death in infancy [9]. Also thrombomodulin has its own intrinsic anticoagulant activity since several works have demonstrated its ability to bind and directly inhibit activated factor X [15].

Regulation of Vascular Tone

The endothelium releases various vasoactive factors. These can be vasodilatory factors such as nitric oxide; endothelium derived hyperpolarizing factor (EDHF) and PGI_2 or vasoconstrictive factors such as thromboxane (TXA_2) and endothelin (ET) [18].

In a healthy individual, a delicate balance between vasoconstriction and vasodilation is maintained by ET and other vasoconstrictors on the one hand and nitric oxide, PGI_2 and other vasodilators on the other [9].

Nitric Oxide (NO)

Nitric oxide, known as the 'endothelium-derived relaxing factor', is biosynthesized endogenously from L-arginine, oxygen, and nicotinamide adenine dinucleotide phosphate-oxidase (NADPH) by various nitric oxide synthase (NOS) enzymes [19]. There are four types of NOS; neuronal isoform (nNOS) which produces NO to act as a neuronal messenger that regulates synaptic neurotransmitter release, macrophage or inducible isoform (iNOS) which is only expressed in cells that have been exposed to inflammatory mediators or other injurious stimuli that activate the macrophages, endothelial NOS (eNOS) which produces NO in the vasculature and mitochondrial NOS

(mtNOS) [20]. The most important stimuli are physical factors such as shear stress and pulsatil stretching of the vessel wall as well as circulating and locally released vasoactive substances [20].

NOS can be inhibited by N-substituted analogues of L-arginine, such as N-methyl-L-arginine, N-nitro L-arginine, and N-amino-L-arginine [21]. Endothelial iNOS may be induced in both physiological and pathological conditions. Known physiological settings include pregnancy, treatment with estradiol, shear stress, chronic exercise or receptor agonists (such as bradykinine and vascular endothelial growth factor) [22]. In activated ECs, iNOS can be induced producing much higher levels of NO than those present under physiological conditions and that is implicated in the pathogenesis of a wide variety of diseases involving endothelium [23].

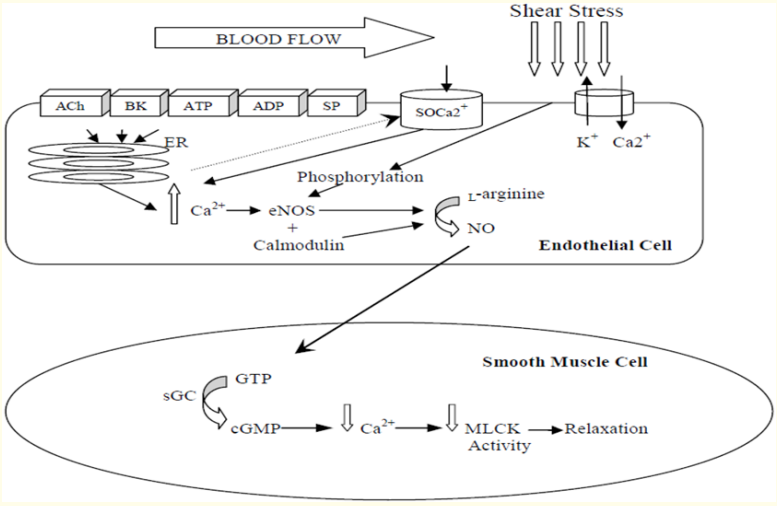

Figure 2: *Endothelial NO production and it actions in the vascular smooth muscle cell.*
Ach: acetylcholine; BK: bradykinin; ATP: adenosine triphosphate; ADP: adenosine diphosphate; SP: substance P; SOCa^{2+}: store-operated Ca^{2+} channel; ER: endoplasmic reticulum; NO: nitric oxide; sGC: soluble guanylyl cyclase; cGMP: cyclic guanosine-3', 5-monophosphate; MLCK: myosin light chain kinase.
**When Ca^{2+} stores of the endoplasmic reticulum are depleted a signal is sent to SOCa^{2+} channel which allows extracellular Ca^{2+} into the endothelial cell [2] (Sandoo et al., 2010).*

It appears to be the most important in terms of regulating blood flow under normal physiological conditions [24]. NO is involved in what is termed flow-dependent vasodilation which is particularly important as a mechanism for increasing coronary blood flow when cardiac activity and metabolism are increased [2].

Vasodilatation caused by NO begins with stimulation of the soluble guanylate cyclase and subsequent formation of cyclic-GMP. Cyclic-GMP activates protein kinase C, which causes reuptake of Ca^{2+} and the opening of calcium-activated potassium channels. The fall in concentration of Ca^{2+} ensures that the myosin light-chain kinase can no longer phosphorylate the myosin molecule, thereby stopping the crossbridge cycle and leading to relaxation of the smooth muscle cell [25] (Figure 2).

NO have important role in immune system, nervous system, inflammation and blood flow [26]. It contributes to vessel hemostasis by inhibiting vasoconstriction, platelet aggregation, and leukocyte adhesion to the endothelium. Humans with atherosclerosis, diabetes, or hypertension often show impaired NO pathways [20]. A high salt intake was demonstrated to attenuate NO production in patients with essential hypertension [27].

Endothelium-Derived Hyperpolarising Factor (EDHF)

It is a vasodilator substance which hyperpolarizes the underlying smooth muscle (Sandoo et al., 2010). EDHF is released when ECs are activated by agonists such as bradykinin and acetylcholine (ACh) [28]. The nature of EDHF is still unknown, but studies indicate that potassium ions, hydrogen peroxide, or epoxyeicosatrienoic acids-products of arachidonic acid metabolism- are EDHF [29]. Another, possibly most convincing hypothesis, relates origin of EDHF with phospholipase A_2 (PLA_2). Arachidonic acid, released by activating PLA_2, plays a role in EDHF formation [24].

Prostacyclin and Thromboxane A_2: Endothelial cells are able to produce various prostaglandin molecules. PGI_2 and TXA_2 are the major products from the endothelium. They are formed from arachidonic acid in presence of the cyclo-oxygenase (COX) enzyme, of which there are two isoforms COX-1 and COX-2 [30]. COX-1 is expressed continuously in endothelial cells, whereas COX-2 is only expressed when the endothelium is damaged and exposed to inflammatory cytokines [2]. Prostacyclin is a potent vasodilator in addition to serving as an inhibitor of platelet aggregation. PGI_2 is a potent inhibitor of platelet aggregation. However, it is less potent as an inhibitor of platelet adhesion. Normal (uninjured) endothelium releases PGI_2 and NO which inhibit platelet aggregation. So, platelet plug is confined to site of injury [20].

The balance in the activity of PGI_2 and TxA_2 helps to maintain homeostasis in the healthy vessel. The importance of this balance becomes evident when using selective COX-2 inhibitors to reduce inflammation, which decreases the production of PGI_2 without affecting the production of TXA_2 [31]. Thus, by administrating COX-2 inhibitors, TXA_2 will cause vasoconstriction and platelet aggregation which is unopposed by PGI_2, increasing the risk for cardiac events [18].

Acetylsalicylic acid (aspirin) in small dose prevents platelet plug formation. It acts by inhibiting the cyclooxygenase enzymes that promote synthesis of the platelet activator TXA_2. The aspirin prevents clots formation by blocking platelet aggregation. Higher doses of aspirin are actually contraindicated in patients prone to thromboembolism. At higher doses, aspirin also reduces synthesis of PGI_2. PGI_2 normally inhibits platelet aggregation [32].

Endothelin (ET)

The vasoconstrictor ET is also produced by endothelial cells, with marked effects on vascular tone. It is the most potent vasoconstrictor substance [9].

Endothelin is synthesized from an intracellular precursor by endothelin-converting enzyme (ECE) found on the endothelial cell membrane [33]. ET-1 formation and release by ECs is stimulated by angiotensin II, vasopressin, thrombin, cytokines, reactive oxygen species, and shearing forces acting on the vascular endothelium. ET-1 release is inhibited by nitric oxide, as well as by PGI_2 and atrial natriuretic peptide [34].

It also has the following functions; stimulation of proliferation of vascular smooth muscle cells and fibroblasts, contraction of airway and intestinal smooth muscle, positive inotropic and chronotropic effects on the myocardium, stimulation of atrial natriuretic peptide secretion from atrial cardiocytes, and modulation of norepinephrine release from sympathetic terminals [9].

Role of Endothelium in Inflammation

ECs are able to amplify the inflammatory response by three main mechanisms: Adhesion molecule expression, cytokine production, and angiogenesis [9]. When there is a need to quickly eliminate a pathogen or to clean a necrotic area, ECs express adhesion molecules that regulate leukocyte trafficking between the circulating blood and the surrounding tissue [21]. Activation of leukocytes is mediated by chemoattractant molecules, such as chemokines and platelet activating factor (PAF) [17].

Leukocyte recruitment to inflammatory sites involves a cascade of interactions between leucocytes and endothelial cells (Figure 3) [17]. In the first step, called margination, neutrophils are recruited and come into contact with the endothelial surface and form bridges with endothelial cells at the inflamed sites. These bridges are made of selectins, P-selectins and E-selectins on endothelial cells and L-selectins on neutrophils. In the second step, called rolling, the neutrophils move along the endothelium like tumbleweed since the bridges are initially loose and form and detach continuously. Eventually, a more firm connection is built between integrins (α4 integrins) on neutrophils and intracellular and vascular adhesion molecules on the endothelial side. In the third step called diapedesis, or transmigration, neutrophils actively migrate through the blood vessel basement membrane into the tissue, with the aid of the firm connection but without damaging the endothelial cells [16]. Later, lymphocytes follow as well. The appearance and increase of the non-granular cells monocytes and lymphocytes mark the transition into chronic inflammation [17].

Integrins are transmembrane receptors that are the bridges for cell-cell and cell-extracellular matrix. Adhesion is mediated by interaction of leucocyte β2 and α4 integrins with members of the immunoglobulin superfamily (intercellular adhesion molecule-1 (ICAM-1) /vascular cell adhesion molecule-1 (VCAM-1), monocyte chemotactic protein-1 (MCP-1), platelet–endothelial cell adhesion molecule-1 (PECAM-1) [16].

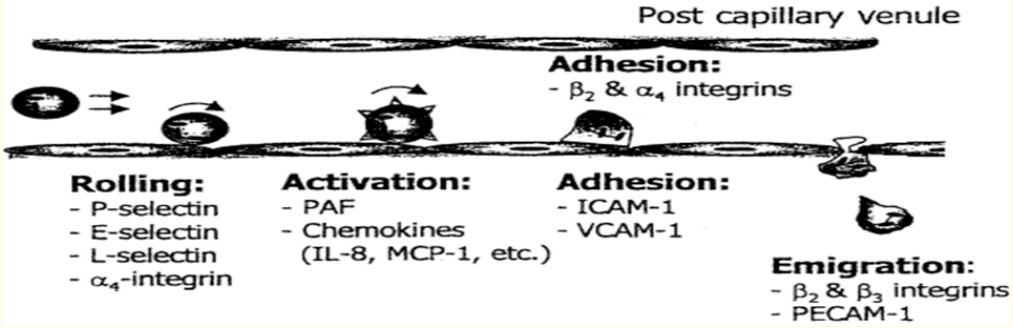

Figure 3: *Leukocyte endothelial cell interaction. Platelet activating factor (PAF), intercellular adhesion molecule-1 (ICAM-1), vascular cell adhesion molecule-1 (VCAM-1), monocyte chemotactic protein-1 (MCP-1), and platelet-endothelial cell adhesive molecule-1 (PECAM-1) [17]. (Kharbanda and Deanfield, 2001).*

Role of Endothelium in Immunity

In addition to its participation in the innate immune response, the endothelium participates in the adaptive response involving T lymphocytes. ECs in culture express major histocompatibility complex class I (MHC I) and can present antigen to (cluster of differentiation 8) CD8 cytotoxic T lymphocytes. Furthermore, after stimulation by interferon gamma, they express MHC class II and can interact with CD4 lymphocytes [9]. ECs can also express lymphocyte function-associated antigen 3 (LFA-3 = CD58) or B7.2 protein (CD86) costimulation molecules at their surface, molecules common to other antigen-presenting cells (dendritic cells, macrophages, B lymphocytes) [35]. Moreover, it is accepted that the ECs are involved in the pathophysiology of graft rejection [16].

Formation of New Blood Vessels (Angiogenesis)

Angiogenesis is the physiological process through which new blood vessels form from pre-existing vessels [36]. Angiogenesis is responsible for most, if not all, blood vessel growth during development and in disease. It is a normal and vital process in growth and development, as well as in wound healing and in the formation of granulation tissue [37]. However, it is also a fundamental step in the transition of tumors from a benign state to a malignant one, leading to the use of angiogenesis inhibitors in the treatment of cancer [36].

Chemical stimulation of angiogenesis is performed by various angiogenic proteins, including several growth factors such as vascular endothelial growth factor (VEGF), fibroblast growth factor (FGF) and platelet-derived growth factor (PDGF) [37]. Also, VEGF contributes to the inflammatory response through stimulation of the release of adhesion molecules, metalloproteinase and nitric oxide [9]. There are two types of FGF-1 and FGF-2. FGF-2 is a more potent angiogenic factor than VEGF or PDGF, however, it is less potent than FGF-1 [37]. As well as stimulating blood vessel growth, FGF-1 and FGF-2 are important players in wound healing. They stimulate the proliferation of fibroblasts and ECs that give rise to angiogenesis and developing granulation tissue, both increase blood supply and fill up a wound space/cavity early in the wound-healing process [36].

Pathophysiology of Endothelial Dysfunction

The traditional risk factors for coronary heart disease, which include hypercholesterolemia, hypertension, cigarette smoking, diabetes mellitus, and high-fat diet, have all been associated with impairments in endothelial function. Age, hypertension, atherosclerosis, and dyslipidemia may provoke endothelial dysfunction. [38]. Impaired endothelium function may promote the development of atherosclerosis through its effects on vasoregulation, platelet and monocyte adhesion, vascular smooth muscle cell growth, and coagulation [39].

Dyslipidemia

It is known that hypercholesterolemia stimulates the angiotensin II-type 1 receptor and impairs the synthesis of NO. Angiotensin II causes vasoconstriction [38]. Hypercholesterolemia increases the generation of asymmetric dimethylarginine (ADMA) which is an endogenous competitive inhibitor of NO synthesis [39]. Impairment of the NO synthesis increases the release of ROS, the expression of LDL receptor and the release of pro-inflammatory cytokines [40]. Moreover, hypertriglyceridemia favors an inflammatory state and increased levels of pro-inflammatory cytokines such as TNF-a and IL-6 [41].

An increase of low-density lipoproteins (LDL) and cholesterol in blood plasma are two main factors in development of atherosclerosis [38]. Decreased production of NO increases aggregation and adhesion of the endothelium to thrombocytes and leucocytes what in turn stimulates formation of atherosclerosis [24].

Tumor Necrosis Factor-α (TNF-α)

TNF-α impairs the synthesis of NO by inhibition of eNOS [42]. It may lead to increased cellular adhesion molecule (CAM) expression and decreases degradation of ADMA, an endogenous inhibitor of NOS [43]. Infusion of TNF-α causes impaired endothelium-dependent vasodilatation [44]. Mice deficient in TNF-α develop less atherosclerosis than those with intact TNF-α expression [45]. An anti-TNF-α agent has beneficial effects on endothelial function in patients with chronic inflammatory diseases [46].

Oxidative Stress

Increased oxidative stress may be another mechanism by which ED contributes to diseases. Increased ROS production in ED diseases is caused by increased of inflammatory cytokines as TNF-α. and TNF-α increases activity of the NADPH oxidases (NOX), which catalyze the transfer of electrons on to molecular oxygen to generate superoxide by neutrophils and endothelial cells [47]. ROS increases both of superoxide generation and surface expression of CAMs and decreases NO production [48].

Hypertension

In hypertension, the delicate balance between vasodilators and vasoconstrictors produced by the endothelium is disrupted, Synthesis of vasoconstrictors such as ET-1 is increased and vasodilators like NO is decreased which contribute to high blood pressure [33].

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