RESEARCH ARTICLE

Biophysical model of active transport through vesicles

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Abstract

The transport phenomena in the human organism mean the variation in time and space of generalized forces when they generate flows for which conservation laws apply. The more specific transport mechanisms are the following; ion pump, transporting molecules, ionic channels and active transport through vesicles. The process of cytopempsis, or the process of transport via the vesicles, is also a vesicular process, initiated in the membrane area. The vesicle in this case constitutes a means of transport of the substances through cell, the contents of the vesicle as a whole being transported through the cytoplasm, and then released. *Endocytosis* consists of the transport of substances from the extracellular environment to the interior of the cell with the help of a vesicle formed by the cell membrane. *Exocytosis* is the process by which the fusion of some vesicles of the cytoplasm with the cell membrane is produced being followed by the expulsion of the vesicle content outside the cell. The pattern of the pinocytosis considers its static character, which is the probability to form vesicles. This mathematical model is applicable from all active transport through vesicles.

Keywords: transport phenomena, pinocytosis, endocytosis, phagocitosis, mathematical model

Introduction

The transport phenomena in the human organism mean the variation in time and space of generalized forces when they generate flows for which conservation laws apply (J. Vincze 1970 [1]).

This general and strongly scientific definition of the transportation phenomena has two major merits: 1) particular forms of transportation can be deducted from it (mass transport – diffusion; energy transport – thermal conductibility; impulse transport – viscosity; electric charge transport – electric conductibility, crossed effects and other); 2.) it allows a quantitative characterization of the product exchange, which was impossible based on the previous definitions.

If W – the amount of the transported parameter, for which the conservation law is valid; K – a constant dependent on the type of transportation and the nature of the transported parameter; grad a – the generalized force, then the amount of the parameter (flow) transported through the surface dS in the dt time frame will be given by the relation:

$$W = K \int_{t_1}^{t_2} \iiint_{S(x,y,z)} grad\ a\, dS\, dt$$

If the transportation takes place only after a direction **x**, then we obtain the formula:

$$W = K \int_{t_1}^{t_2} \int_{x_1}^{x_2} \text{grad } a_x \, dx \, dt$$

The differential form is the following:

$$\partial W = K \cdot \frac{\partial a}{\partial x} \cdot \Delta S \cdot \Delta t$$

Making the proper replacements in the relation above, we obtain the classical laws which describe particular, simple transportation phenomena.

With non stationary transportation we understand those transportations where the value of the flow is modified in time from one point to the other. Making the right replacements in the relationship above we obtain the classical laws which describe the simple non stationary transportation phenomena.

The specific transport mechanisms

The more specific transport mechanisms are following: ion pump, transporting molecules, ionic channels [2] and active transport through vesicles.

Exceptionally, the macromolecules are transported directly through the membrane. In the human body, this type of transfer is observed in the case of the membrane of the endoplasmic reticulum for the proteins synthesized at the level of this structure; in

most cases, they are transported through the vesicles.

In 1883, Mecinikov described the process through which leukocytes embed particles, microbial germs for example. This process was called by Mecinikov phagocytosis.

Aschoff and Landau demonstrated in 1924 that if electronegative dyes are injected intravenously to animals, the size of which is several tens or hundreds of Å, these will be captured by cells of the macrophage group, which they grouped in a system called reticuloendothelial (SRE). Thus, SRE is made up of cells that retain inside electronegative colloidal substances injected into the circulation.

Other processes that take place in the membrane have also been described: the process of rofeocytosis and the process of cytopempsis.

The process of cytopempsis, or the process of transport via the vesicles, is also a vesicular process, initiated in the membrane area. The vesicle in this case constitutes a means of transport of the substances through cell, the contents of the vesicle as a whole being transported through the cytoplasm, and then released. This process represents a differentiation of transport for the endothelial cells of blood capillaries. The cells that make up the capillary endothelium have the property, as researches have shown since 1953 (Palade), to capture from the lumen of the capillary and to transport from the luminal internal part of the endothelial cells, through vesicles, blood content to the basal membrane, in the interstitial fluid. [3]

The process of rofeocytosis is a process in which nutrient substances penetrate deeply into the cell surface, having a role in cellular metabolism. This process was described with electron microscope for iron penetration into erythroblasts.

All these modes of transport, described as different cellular processes by the authors who observed them, have a unique lysosomal mechanism emphasized by Duve (1966). The lysosomal apparatus functions in connection with the membranes of the endoplasmic reticulum and the cell membrane from which it forms the walls of the vesicles in which the intra- or extra-cellular particles are incorporated. [4] Lysosomes are formations that are found inside the cell in the form of corpuscles, originating in the endoplasmic reticulum (Novikoff), the system of channels that meet with cisterns and vesicles, described by Porter and Palade. They are of two types: rough, constituting the ergastoplasm and smooth.

Proteins synthesized in ergastoplasma are transported along the channels of the endoplasmic reticulum and stored in dense bodies, which therefore contain proteins synthesized in the cell, having enzymatic functions of hydrolases (proteases, acid phosphatases, etc.). The enzymes stored here are then transported in the form of vesicles to Golgi's reticular apparatus, in which the lysosomes would form.

In the phagocytosis process, the lysosomes bind with the endocytic vesicle, which at the membrane level has captured content from the external living environment of the cell. From this fusion a phagocytic vesicle is created, in which the foreign embedded corpuscles are eventually digested with the participation of lysosomes. [5]

Endocytosis and exocytosis

Endocytosis consists of the transport of substances from the extracellular environment to the interior of the cell with the help of a vesicle formed by the cell membrane. Depending on the nature of the transported substance, endocytosis occurs as:
– phagocytosis - process present in the whole animal scale, in humans, with special importance in the non-specific defense processes, but also in the removal of old cells. It consists of the introduction of particles through vesicles from the extracellular environment into the cell;
– pinocytosis - consists in the introduction of liquid content through the vesicles into the cell (pinein = to drink) from the extracellular environment. Pinocytosis can be performed independently by receptors and mediated by receptors.

Exocytosis is the process by which the fusion of some vesicles of the cytoplasm with the cell membrane is produced being followed by the expulsion of the vesicle content outside the cell. Exocytosis processes are described in the case of secretory cells and at the terminal button level in the case of synaptic transmission.

Transcytosis is the process of transporting substances through the vesicles that, formed at the apical or basal pole of the cell, pass through the cytoplasm as such and release the contents at the opposite pole of the cell. This process is more active at the level of endothelial cells of the structure of the blood capillaries. [6]

In humans, phagocytosis plays an important role in the defense processes of the body as this way the bacteria, parasites, foreign substances, cellular debris, degenerated, old and malignant cells are embedded, and then destroyed. Cells capable of phagocytosis are called phagocytes. In humans, they are of two types: macrophages and neutrophils. Both result from precursor cells in the bone marrow, then circulate in the blood for several days (where macrophages are represented by monocytes, and polymorphomeleal leukocytes with neutrophilic granulations make up about 60% of the white blood cells), after which they move from the vessels into the tissues where they exercise the phagocytic function. [7] Macrophages are also called histiocytes. Phagocytes form in the body an eventual phagocytic system. This system has mobile components (circulating cells and especially neutrophils) and sessile components, represented by macrophages. These are found in the spleen, lymph nodes, liver (Kupffer cells), blood vessels, pulmonary alveoli, pleura, peritoneum.

However, the role of phagocytosis and the reticulohistiocitary or reticulo-endothelial system in the defense processes of the body is extremely important. The embedding of microbes by phagocytosis and their subsequent destruction by lysosomes is the last stage of the fight of the organism against infections. Antibiotics do not kill microbes, but only prevent their multiplication. Microbes are killed after phagocytosis. Usually the microbes colonize the interstitial space of a tissue. Neutrophils and other phagocytes are attracted to these sites by chemotactic signals emitted or induced by microbes. Phagocytes attach to the wall of small vessels that irrigate the affected tissue. They then pass through the vessel wall (the post-capillary venule) and move to the site of infection, where they are embedded, and

thus destroy the bacteria.

The phases of phagocitosis

Several phases of phagocytosis can be distinguished:

a) Chemotactism is the directed movement of phagocytes to the site of infection, as signals serving bacterial components (proteins or lipids) or those of the body: e.g. serum proteins that amplify the immune response.

b) The recognition and attachment of phagocyte particles is achieved through the receptors of the phagocyte plasmalemma that recognize ligands from the particle surface. In the case of phagocytosis of senescent or malignant cells, carbohydrate groups in the plasmalemma are recognized. In the case of bacteria, it is often necessary to cover them with antibodies and/or complement, so they can be phagocytosed.

c) Embedding is made by the phagocyte emitting extensions called pseudopods, which surround the particle and then close it completely in a vesicle internalized in the cytoplasm called phagosome. The embedding process was compared to closing a zipper.

d) The killing of phagocyte cells and their digestion is done after the fusion of the phagosome with a primary lysosome and the formation of the phagolysosome.

A probabilistic model

Endocytosis is a special type of biological transport, which refers to the particles of colloidal dimensions being engulfed by the cells. We know two forms of endocytosis: phagocytosis and pinocytosis. Phagocytosis means the transport of solid particles while the pinocytosis is the transportations of the big drops of liquid. Considering that in biology it is difficult to set apart the two aggregation states, lately the notion of endocytosis has been used preferentially.

The pinocytosis phenomenon is found in many types of cells, such as the macrophages, the cells of the capillary endothelium, the cells of the sheath of Schwann etc.

It is supposed that pinocytosis is the most diffused active mechanism of macromolecule transport, a hypothesis confirmed by the research made with the electronic microscope.

The pattern of the pinocytosis considers its static character, that is the probability to form vesicles. [8] The pinocytosis and phagocytosis phenomena – from mathematical point of wiev – are a probalistic manifestation. Likelihood of an event, measured by the ratio of the favourable cases – appearance of vesicles. The model of pinocytosis takes into consideration its statistical character, i.e. the probability of the formation of vesicle. The statistical model described for pinocytosis is equally applicable to phagocytosis as well, without any constrains.

Let us take **M** as the number of vesicles on the **dS** surface of the cellular membrane, $\mathbf{c_e}$ and $\mathbf{c_i}$ the concentration of the substance to be transported in the extra and intracellular environment, in which case the variation of the possibility to form the $\mathbf{n^{th}}$ vesicle according to the type is given by the equation:

$$\mathbf{K} \cdot \frac{dP_n(t)}{dt} = \mathbf{c_k} \cdot (\mathbf{P_{n-1}(t) - P_n(t)}) + \mathbf{c_b} \cdot (\mathbf{P_{n+1}(t) - P_n(t)}) ;$$

$1 \leq n \leq M$

The $\mathbf{n}^{th}$ vesicle can appear in two types: either there are **n-1** vesicles and after the binding of a molecule to be transported on the surface of the membrane the **n**th vesicle is formed, or the membrane contains **n+1** vesicles, of which one detaches towards the cytoplasm and so **n** vesicles remain.

From the probabilistic point of view, the two methods are independent events, that is, why their sum appears in the formula. Since the probability of formation and detachment of the vesicles depends on the concentrations of the substances on both sides of the membrane, the concentrations are proportionality factors. **K** is a one-dimensional constant. Thus, we obtained a system of differential first degree equations, from which - knowing the limit conditions - we obtain the following expression for **n** = 1:

$$\mathbf{K} \cdot \frac{dP_1(t)}{dt} = \mathbf{c_k \cdot (P_0(t) - P_1(t)) + c_b \cdot (P_2(t) - P_1(t))};$$

We cannot talk about pinocytosis unless there is at least one vesicle, that is why $\mathbf{c_e.P_0\,(t) = 0}$, and $\mathbf{c_i.P_1(t)}$ does not make sense. For this reason, when n = 1 these two terms disappear.
For the limit condition **n = M** we will have:

$$\mathbf{K} \cdot \frac{dP_M(t)}{dt} = \mathbf{c_k \cdot (P_{M-1}(t) - P_M(t)) + c_b \cdot (P_{M+1}(t) - P_M(t))};$$

Expression in which two terms are null, considering the maximum number of vesicles. Although it is extremely difficult to follow the dynamics of pinocytosis experimentally, this pattern gives us the dynamic description of the process as well as the deduction of a few conclusions. The apparition of the pinocytic vesicles presents a statistic distribution. In the same way, the equation above allows the characterization of the macromolecules in the cells in the interstitial liquid, that is exocytosis, a process which is found in all the secretive and excretory cells.

These deductions have been confirmed by Palade's research under the electronic microscope (1969), according to which the vesicles cannot be interpreted as sections passing through the pores of the membranes. From the mathematical point of view one can draw the same conclusion, since the life span of these vesicles is only a few seconds. [9]

The energetic level

The mathematic pattern of the pinocytosis can be used without restrictions in the case of phagocytosis as well. Since endocytosis is a specific active mechanism, we can suppose that it also needs energy, probably supplied by ATP as well. [10] The energy of the macroscopic bond is probably consumed for the regeneration of the membrane structure, respectively the formation of new membrane units.

In killing bacteria, however, the oxidase in the plasmalemma takes up an important role, which reaches, through the phagocytosis, the phagosome membrane. Oxidase catalyzes

the following reaction:

$2O_2 + NADPH \rightarrow 2O_2^- + NADP^- + H^+$

The superoxide anion (O_2^-) is generated inside the phagolysome, and is spontaneously converted into H_2O_2 oxygenated water. Then, from the reaction O_2^- with H_2O_2 results the free hydroxyl radical (OH^-) and singlet oxygen ($^1O_2$) that are highly reactive and toxic species that kill the microbes. [11] Due to the reaction catalyzed by the oxidase, there is a sharp increase in the oxygen consumption of the phaygocyte at the time of their activation under the action of chemotactic factors from the outbreak of infection.

The receptor theory

There are two types of pinocytosis: receptor-independent and receptor-dependent (or mediated).

Receptor-independent pinocytosis is also called fluid phase endocytosis and is found in many cells. For example, the macrophage ingests within 30 minutes the equivalent of the entire plasmalemma, while fibroblasts ingest one third of the plasmalemma, and large quantities of macromolecules are introduced into the cell. As in this interval the volume of the cell is kept constant, it means that, simultaneously with the endocytosis, an equivalent amount of plasmalemma materials is added. Thus, part of the endocytosis vesicles return to the plasmalemma, and the components of the plasmalemma are thus recirculated. The recycling of membranes consists in the fusion with plasmalemma of some endocytosis vesicles, the membrane of which has not been digested in lysosomes.

Receptor-dependent pinocytosis or receptor-mediated endocytosis is performed with the help of plasmalemma receptors that recognize specific macromolecules in the extracellular fluid. The binding of the receptor ligand induces the diffusion of the receptor-ligand complex in special areas of the plasmalemma, which appear as coated pits dressed, covered, on the cytoplasmic face of special proteins. Coated pits, also called caveolea, then become vesicles of endocytosis, which maintain for some time after internalizing their clothing or covering, and are thus covered with a network of proteins. The most well characterized protein is the clathrin, with a molecular weight of 180,000 daltons and with sequences conserved during evolution. Three clathrin molecules together with three smaller polypeptides join together in a characteristic structure. From this basic unit, the clathrin network is formed, like a net that dresses the coated pits or the vesicles. Sometimes the clathrin network dresses only the coated pit, so that when the vesicle is formed it will slip through the mesh of the network, becoming a uncoated endocytosis vesicle, called receptosome*;* to differentiate it from pynosome (the vesicle of the receptor-independent endocytosis). [12]

Receptor-mediated endocytosis achieves a concentration of certain ligands in endocytosis vesicles, which is an important difference compared to receptor-independent pinocytosis, in which the concentration of the substances in the vesicles is equal to that of the extracellular fluid. An important process that occurs through receptor-mediated endocytosis is the capturing of cholesterol into animal cells.

Conclusion

A particular form of vesicle transport is represented by the transcytosis, which carries out the transport of macromolecules through the capillary endothelial cells. Palade (1953) was the first to describe by electron microscopy in cytoplasm the endothelial cells vesicles that cross cells from one side to the other; he suggested that these vesicles play an important role in transporting plasma macromoles outside the vascular bed.

Transcytosis transport may occur either through independent vesicles moving through the endothelial cell from the luminal face (exposed to the interior of the vessel) to the interstitial face (exposed to the interstitial fluid), or more rarely the vesicles merge into a channel that crosses the cell. Transcytosis carries out exchanges between plasma and the interstitial fluid. In the transport of macromolecules, not only the size, but also the electrical charge and the chemical properties of the molecule are important. Thus, the vascular endothelium has an uneven distribution of electrical charges, with negatively charged microdomains. As proteins are also negatively charged, the preferred route of entry will be through the transcytosis vesicles. The chemical properties of the macromolecules to be transported are also important, the vascular endothelium (and probably other plasmalemmas) can discriminate proteins based on the chemical characteristics of the molecule. Proteins adsorbed on the surface of the plasmalemma are likely to be involved in this discrimination.

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